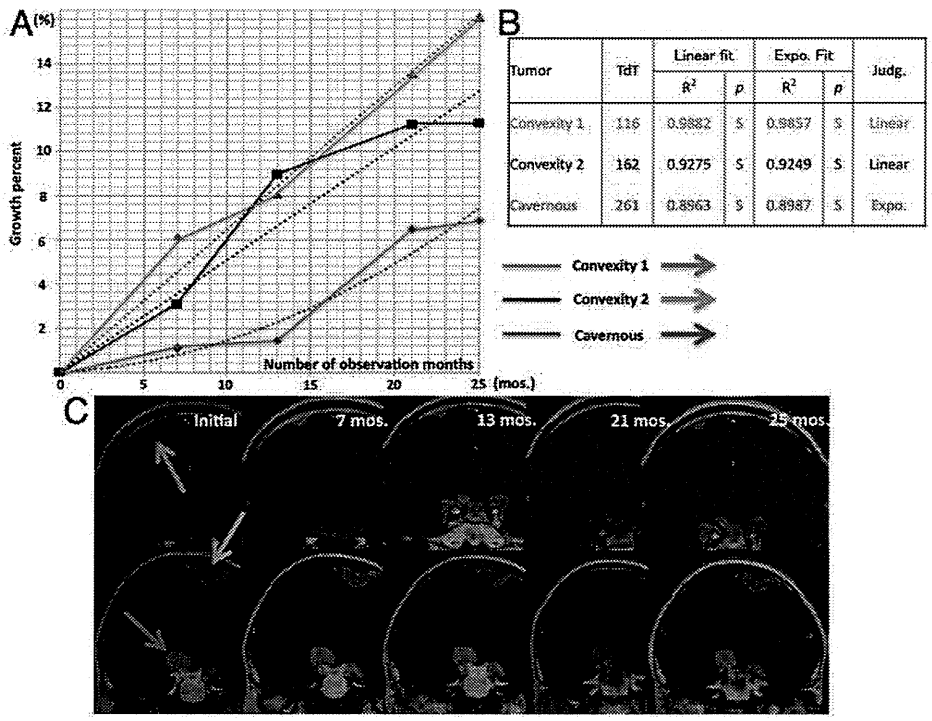

FIG. 2. Sequential volumetric data (A), the data for growth rates and results of regression analyses (B), and MR images (C) from a representative case of a 67-year-old man with multiple IDMs. Note that the percentage of growth of cavernous IDMs *(red line)* is smaller than those of convexity ones (*gray* and *blue lines*). Expo. = exponential; Judg. = judgment of growth pattern; p = p value; R^2 = coefficient of determination; S = statistically significant; TdT = tumor doubling time. Refer to the text for explanation of the dotted lines.

Kane et al.,[7] based on their statistical observation of 378 surgical cases, they reported that anatomical location is a risk factor for Grade II and III meningiomas.

All these reports, including ours, seem to suggest that non–skull base meningiomas have a more aggressive behavior. In 2003, we proposed that loss of 1p is significantly correlated with malignant progression of meningiomas by analyzing 72 tumors, including WHO Grade II and III tumors, with fluorescence in situ hybridization and loss of heterozygosity analyses.[13] This small study included 49 Grade I, 15 Grade II, and 8 Grade III tumors. Although the data were not shown in this paper, it was our impression that most Grade II and III tumors are found in a parasagittal (non–skull base) location. Therefore, we decided to categorize these tumors according to location, and we were able to obtain a statistically significant difference in the percentage of cells with 1p deletions (Table 8). We noted that skull base meningiomas harbored a significantly lower percentage of cells with 1p loss (20.31%) compared with non–skull base tumors (37.87%). This observation seems to further suggest that skull base tumors may indeed have fewer genetic aberrations and may have a less aggressive biological nature.

Regarding growth patterns, Nakasu et al.[15] and our group[6] reported that IDMs do not always grow exponentially and show various patterns of growth including a linear pattern. Based on the rule of proliferation kinetics, tumors with an exponential pattern of growth will maintain their cell-doubling time constant over time. In contrast, those with linear growth patterns will have a reduced cell-doubling time over time. In this study, 60% of skull base IDMs showed an exponential pattern of growth, whereas 33% of non–skull base IDMs showed an exponential pattern. However, these data must be cautiously interpreted because most of the tumors did fit both exponential and linear patterns statistically when regression analysis was performed. In fact, among the 63 tumors with either a linear or exponential growth pattern described in Table 4, only 1 tumor fit an exclusively linear pattern (data not shown). In other words, the data in Ta-

TABLE 4: Growth patterns of 71 growing IDMs

Pattern	No. of Cases (%)	
	Skull Base	Non–Skull Base
no trend	0 (0)	8 (14)
exponential	9 (60)	18 (32)
linear	6 (40)	30 (54)

TABLE 5: Summary of clinical courses of 113 IDMs

Course	No. of Cases (%)	
	Skull Base	Non–Skull Base
remained asymptomatic	37 (97.4)	69 (92.0)
underwent surgery	1 (2.6)	4 (5.3)
underwent radiotherapy	0	2 (2.7)

TABLE 6: Biological comparison between skull base and non–skull base symptomatic meningiomas

Variable	All	Skull Base	Non–Skull Base	p Value (statistic)*
no. of cases	210	94	116	
age in yrs				0.096 (unpaired t-test)
mean	57.2	55.5	58.6	
range	16–90			
sex ratio (F:M)	156:54	68:26	88:28	0.635 (Fisher)
MIB-1 index (%)				0.013 (unpaired t-test)†
mean	2.45	2.09	2.74	
range	0.1–11.1			

* The p values represent the differences between the skull base and non–skull base symptomatic meningiomas.
† Statistically significant.

ble 4 reflect the fact that we took the larger coefficient of determination (R^2) in each tumor. Therefore, we believe that it is still too early to conclude that skull base tumors are more likely to present with an exponential pattern of growth. Longer follow-up periods and more cases are still warranted.

We believe that our findings may contribute to the understanding of the natural history of IDMs. It may also impact the way we currently manage IDMs. Those with a skull base location can be observed with sequential follow-up MR images more safely and longer than those with non–skull base tumors, except when they are located at the medial sphenoidal region adjacent to the optic nerve. We may be able to recommend a longer interval of follow-up MR imaging in skull base IDMs than in non–skull base tumors after confirming that IDMs tend not to grow during the early phase of follow-up.

For IDMs that became symptomatic or for uncomplicated symptomatic meningiomas requiring intervention, the findings in this paper may also be useful. As has been reported before, non–skull base meningiomas are more amenable to total resection and have better recurrence-free survival rates,[11] whereas total resection of skull base meningiomas may be limited by adjacent critical structures including the brainstem and cranial nerves. Black and colleagues[1] reported a series of 100 patients with skull base meningiomas who were treated with a combination of aggressive surgery and conformal radiation therapy. The authors found that this approach yielded an acceptable functional status in 99% of patients. McGovern et al.[11] also reported that adjuvant radiation therapy for skull base meningiomas improved the recurrence-free survival rate of subtotally resected skull base tumors to levels similar to those that were completely resected. Consistent with their suggestion, and taking into consideration the findings in this paper that skull base meningiomas have less aggressive behavior, it seems that maximal surgical reduction limited by the preservation of patient performance status, and the addition of postoperative radiotherapy or radiosurgery,[2,5] intensity-modulated radiotherapy,[12,19] and proton therapy,[22] may be an acceptable option for these patients.

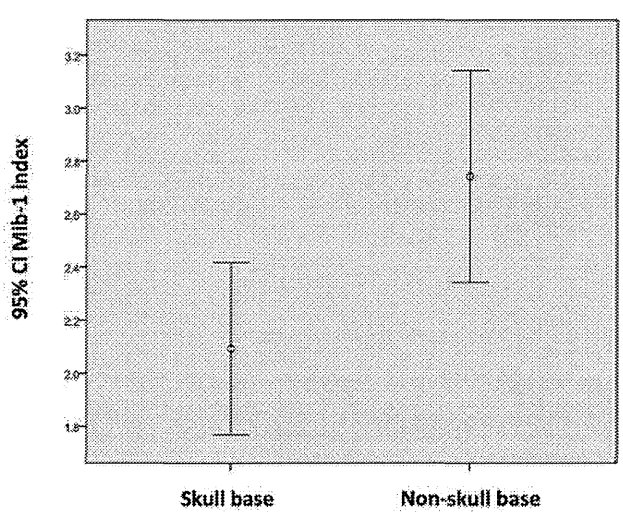

FIG. 3. Graph showing the MIB-1 index in surgical specimens from the skull base and non–skull base groups. The mean MIB-1 index for skull base tumors was markedly low (2.09%) compared with that for non–skull base tumors (2.74%; p = 0.013). *Circles* show the mean MIB-1 index, and *bars* show the 95% CI.

Conclusions

A sequential volumetric analysis of 113 IDMs revealed that skull base IDMs tended not to grow compared with non–skull base IDMs. Even when the skull base IDMs grow, the rate of growth is significantly lower than that for non–skull base tumors. Furthermore, a biological comparison of skull base and non–skull base sur-

TABLE 7: Relation of MIB-1 index to location and sex

Variable	Male	Female	p Value (unpaired t-test)
no. of cases	54	156	
mean age in yrs	59.59	56.42	0.13
mean MIB-1 (%)			
all	2.99	2.27	0.045*
skull base	2.82	1.82	0.021*
non–skull base	3.16	2.61	0.249

* Statistically significant.

TABLE 8: Loss of 1p by genetic analysis and location

Location	No.	Mean % Cells w/ 1p Loss*
Skull base	17	20.31
Non–skull base	55	37.87

* p = 0.019 (Mann-Whitney).

gically treated meningiomas in 210 consecutive patients showed that the mean MIB-1 index was significantly lower in skull base tumors. These findings may impact our understanding of the natural history of IDMs, as well as the strategies for management and treatment, not only of IDMs, but also of symptomatic meningiomas.

Disclosure

This work was supported in part by Grants-in-Aid No. 22103508 for Scientific Research of Computational Anatomy from the Ministry of Education, Science, Sports and Culture, Japan, to Naoya Hashimoto, M.D., Ph.D.

Author contributions to the study and manuscript preparation include the following. Conception and design: Hashimoto, Yoshimine. Acquisition of data: Hashimoto, Rabo, Okita, Kagawa, Fujimoto, Morii. Analysis and interpretation of data: Hashimoto, Kinoshita, Morii. Drafting the article: Hashimoto, Rabo. 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: Hashimoto. Statistical analysis: Hashimoto, Rabo. Administrative/technical/material support: Kagawa. Study supervision: Yoshimine.

Acknowledgment

The authors would also like to thank Ms. Mariko Kakinoki for her secretarial assistance.

References

1. Black PM, Villavicencio AT, Rhouddou C, Loeffler JS: Aggressive surgery and focal radiation in the management of meningiomas of the skull base: preservation of function with maintenance of local control. **Acta Neurochir (Wien) 143:** 555–562, 2001
2. Elia AE, Shih HA, Loeffler JS: Stereotactic radiation treatment for benign meningiomas. **Neurosurg Focus 23(4):**E5, 2007
3. Firsching RP, Fischer A, Peters R, Thun F, Klug N: Growth rate of incidental meningiomas. **J Neurosurg 73:**545–547, 1990
4. Go RS, Taylor BV, Kimmel DW: The natural history of asymptomatic meningiomas in Olmsted County, Minnesota. **Neurology 51:**1718–1720, 1998
5. Han JH, Kim DG, Chung HT, Park CK, Paek SH, Kim CY, et al: Gamma knife radiosurgery for skull base meningiomas: long-term radiologic and clinical outcome. **Int J Radiat Oncol Biol Phys 72:**1324–1332, 2008
6. Hashiba T, Hashimoto N, Izumoto S, Suzuki T, Kagawa N, Maruno M, et al: Serial volumetric assessment of the natural history and growth pattern of incidentally discovered meningiomas. Clinical article. **J Neurosurg 110:**675–684, 2009
7. Kane AJ, Sughrue ME, Rutkowski MJ, Shangari G, Fang S, McDermott MW, et al: Anatomic location is a risk factor for atypical and malignant meningiomas. **Cancer 117:**1272–1278, 2011
8. Kasuya H, Kubo O, Tanaka M, Amano K, Kato K, Hori T: Clinical and radiological features related to the growth potential of meningioma. **Neurosurg Rev 29:**293–297, 2006
9. Kuratsu J, Kochi M, Ushio Y: Incidence and clinical features of asymptomatic meningiomas. **J Neurosurg 92:**766–770, 2000
10. Mahmood A, Caccamo DV, Tomecek FJ, Malik GM: Atypical and malignant meningiomas: a clinicopathological review. **Neurosurgery 33:**955–963, 1993
11. McGovern SL, Aldape KD, Munsell MF, Mahajan A, DeMonte F, Woo SY: A comparison of World Health Organization tumor grades at recurrence in patients with non-skull base and skull base meningiomas. Clinical article. **J Neurosurg 112:** 925–933, 2010
12. Milker-Zabel S, Zabel-du Bois A, Huber P, Schlegel W, Debus J: Intensity-modulated radiotherapy for complex-shaped meningioma of the skull base: long-term experience of a single institution. **Int J Radiat Oncol Biol Phys 68:**858–863, 2007
13. Murakami M, Hashimoto N, Takahashi Y, Hosokawa Y, Inazawa J, Mineura K: A consistent region of deletion on 1p36 in meningiomas: identification and relation to malignant progression. **Cancer Genet Cytogenet 140:**99–106, 2003
14. Nakamura M, Roser F, Michel J, Jacobs C, Samii M: The natural history of incidental meningiomas. **Neurosurgery 53:** 62–71, 2003
15. Nakasu S, Fukami T, Nakajima M, Watanabe K, Ichikawa M, Matsuda M: Growth pattern changes of meningiomas: long-term analysis. **Neurosurgery 56:**946–955, 2005
16. Olivero WC, Lister JR, Elwood PW: The natural history and growth rate of asymptomatic meningiomas: a review of 60 patients. **J Neurosurg 83:**222–224, 1995
17. Onizuka M, Suyama K, Shibayama A, Hiura T, Horie N, Miyazaki H: Asymptomatic brain tumor detected at brain checkup. **Neurol Med Chir (Tokyo) 41:**431–435, 2001
18. Perry A, Louis DN, Scheithauer BW, Budka H, von Deimling A: Meningiomas, in Louis DN, Ohgaki H, Wiestler OD, et al (eds): **WHO Classification of Tumours of the Central Nervous System, ed 4.** Lyon: IARC press, 2007, pp 164–172
19. Pirzkall A, Debus J, Haering P, Rhein B, Grosser KH, Höss A, et al: Intensity modulated radiotherapy (IMRT) for recurrent, residual, or untreated skull-base meningiomas: preliminary clinical experience. **Int J Radiat Oncol Biol Phys 55:**362–372, 2003
20. Sade B, Chahlavi A, Krishnaney A, Nagel S, Choi E, Lee JH: World Health Organization Grades II and III meningiomas are rare in the cranial base and spine. **Neurosurgery 61:**1194–1198, 2007
21. Sughrue ME, Rutkowski MJ, Aranda D, Barani IJ, McDermott MW, Parsa AT: Treatment decision making based on the published natural history and growth rate of small meningiomas. A review and meta-analysis. **J Neurosurg 113:**1036–1042, 2010
22. Vernimmen FJ, Harris JK, Wilson JA, Melvill R, Smit BJ, Slabbert JP: Stereotactic proton beam therapy of skull base meningiomas. **Int J Radiat Oncol Biol Phys 49:**99–105, 2001
23. Yano S, Kuratsu J: Indications for surgery in patients with asymptomatic meningiomas based on an extensive experience. **J Neurosurg 105:**538–543, 2006
24. Yoneoka Y, Fujii Y, Tanaka R: Growth of incidental meningiomas. **Acta Neurochir (Wien) 142:**507–511, 2000

Manuscript submitted June 9, 2011.
Accepted November 2, 2011.
Please include this information when citing this paper: published online December 16, 2011; DOI: 10.3171/2011.11.JNS11999.

Address correspondence to: Naoya Hashimoto, M.D., Ph.D., Department of Neurosurgery, Osaka University Graduate School of Medicine, Yamada-oka 2-2, Suita City, Osaka 565-0871, Japan. email: hashimotonaoya@me.com.

Neurol Med Chir (Tokyo) 52, 539～547, 2012

The 70th Annual Meeting Special Topics — Part II: Multidisciplinary Treatment for High Grade Gliomas

Cilengitide Treatment for Malignant Glioma: Current Status and Future Direction

Kazuhiko KUROZUMI,[1] Tomotsugu ICHIKAWA,[1] Manabu ONISHI,[1] Kentaro FUJII,[1] and Isao DATE[1]

[1]*Department of Neurological Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Okayama*

Abstract

Malignant glioma is the most common primary brain tumor and accounts for the majority of diagnoses. Treatment has involved a combination of surgery, radiation, and chemotherapy, yet these modalities rarely extend the life of the patient to more than one year from diagnosis. Integrins are expressed in tumor cells and tumor endothelial cells, and are important in angiogenesis and invasion in glioma. $\alpha v\beta 3$ and $\alpha v\beta 5$ integrins regulate cell adhesion, and inhibitors of these integrins suppress tumor growth in certain pre-clinical models. Several integrin-targeted drugs are in clinical trials as potential compounds for the treatment of cancer. Among them, cilengitide is a novel integrin antagonist for the treatment of glioblastoma. The multimodal anti-glioma effects are based on its cytotoxic, anti-angiogenic, anti-invasive, and synergetic effects. Preclinical studies showed a promising synergy between cilengitide and radiochemotherapy in order to normalize tumor vasculature and attenuate tumor invasion. Cilengitide is currently being assessed in phase III trials for patients with glioblastoma multiforme and in phase II trials for other types of cancers, demonstrating promising therapeutic outcomes to date. The results of these and other clinical studies are expected with great hope and interest. A more clear understanding of the benefits and pitfalls of each approach can then lead to the design of strategies to derive maximal benefit from these therapies.

Key words: malignant glioma, integrin, cilengitide, angiogenesis, invasion

Introduction

Malignant gliomas are the most common type of primary brain tumor. Their treatment has involved a combination of surgery, radiation, and chemotherapy, yet these modalities rarely extend the life of the patient to more than one year from diagnosis. Several modalities have been and continue to be tested for the treatment of these tumors. Malignant gliomas remain a challenging tumor to treat, and a variety of experimental therapies have failed to show effectiveness in clinical trials.[44] The pathophysiological processes of angiogenesis and tumor cell invasion play pivotal roles in glioma development and growth from the earliest stages.[5] The formation of abnormal tumor vasculature and glioma cell invasion along white matter tracts are believed to be the major reasons for the resistance of these tumors to treatment. This angiogenesis or invasion causes the production of many different angiogenic or invasive factors, respectively, such as vascular endothelial growth factor receptor (VEGFR) platelet-derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR), hepatocytes growth factor, integrins, etc. Among these factors, the overexpression of integrins is well documented.[39,60] Emerging evidence indicates that integrins promote the adhesion, migration, and angiogenesis of glioblastoma.[61]

Received February 27, 2012; Accepted April 9, 2012

Integrins

Integrins are a superfamily of cell adhesion receptors that bind to extracellular matrix (ECM) ligands, cell-surface ligands, and soluble ligands. Integrins are heterodimeric transmembrane cell surface receptors that play a key role in the crosstalk between the cell and its surrounding stroma.[55] Twenty-four different integrin heterodimers are currently recognized, and are formed by the combination of at least 18 α-subunits and 8 β-subunits. Upon ligation to extracellular ligands such as fibronectin, vitronectin, collagen, and fibrinogen, the integrin dimers activate downstream signaling pathways in concert with growth factor receptors, including PDGFR, EGFR, and VEGFR. The physical interaction of integrins with ECM proteins promotes signal transduction, gene expression, proliferation, apoptosis regulation, angiogenesis, invasion, and metastasis.[18]

$\alpha v\beta 3$ and $\alpha v\beta 5$ integrins are usually expressed at low levels in most adult epithelia but can be highly upregulated in some tumors. Human gliomas also overexpress $\alpha v\beta 3$ and $\alpha v\beta 5$ integrins.[4,50] These integrins are expressed on certain tumor cells during their growth and on activated endothelial cells during tumor angiogenesis and invasion of the surrounding tissue.[59,60]

Cilengitide

A vast number of integrin antagonists have been reported such as monoclonal antibodies, peptide and peptidomimetic antagonists, and small molecules.[17,34,54] $\alpha v\beta 3$ and $\alpha v\beta 5$ integrins regulate cell adhesion,[27] and inhibitors of these integrins suppress tumor growth in certain pre-clinical models.[30,54] Currently, several compounds targeting integrins are in clinical trials as potential drugs for the treatment of numerous diseases including cancer.[3] Among them, cilengitide is the first integrin antagonist in clinical phase III for the treatment of glioblastoma and in phase II trials for several other

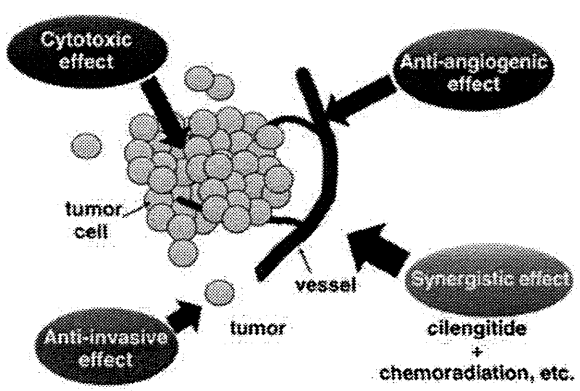

Fig. 1 Anti-glioma effects of cilengitide. The multimodal anti-glioma mechanisms for cilengitide are based on its cytotoxic, anti-angiogenic, anti-invasive, and synergistic effects.

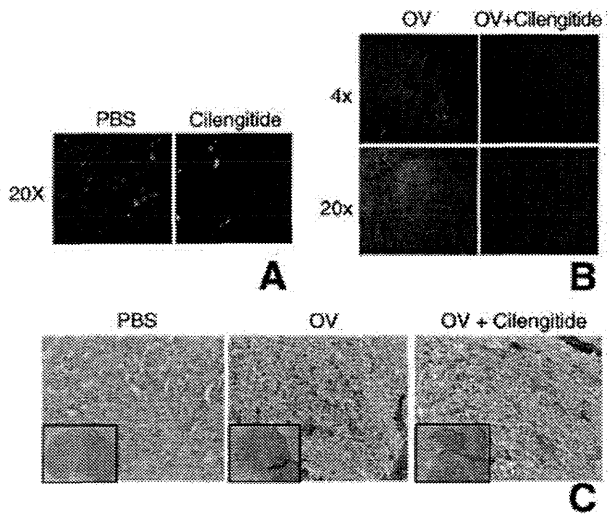

Fig. 2 Effect of cilengitide treatment on angiogenesis in rat glioma and increased oncolytic virus (OV) present in tumor tissue of rats with normalized vasculature. Rats bearing brain tumors were treated with 30 μg of cilengitide or with phosphate-buffered saline (PBS) 3 days after intracerebral glioma cell implantation. Rats were injected with fluorescein isothiocyanate (FITC)-conjugated dextran via the tail vein 5 minutes before they were killed on day 10 after glioma cell injection. Brains were harvested and sectioned for analysis. A: Fluorescence microscopy images of FITC-dextran–perfused tumor blood vessels derived from rats treated with PBS (*left*) or cilengitide (*right*). Original magnification, ×20. B: Fluorescence microscopy images of rat brain sections from OV-infected rats pretreated with PBS (*left column*) or cilengitide (*right column*). Original magnification, *upper row*: ×4, *lower row*: ×20. C: Immunohistochemistry staining of herpes simplex virus (HSV) particles in rat brain tumors treated with PBS (*left*), and infected with OV with (*right*) or without cilengitide pretreatment (*center*). Note the increased presence of OV indicated by increased staining of HSV in tissue samples treated with cilengitide prior to OV insult. Original magnification, ×20. *Insets* show representative low magnification images of the indicated tumor section stained for HSV. Original magnification, ×4. Reproduced with permission from Figs. 5A, 5C, and 7C of Kurozumi et al.: Effect of tumor microenvironment modulation on the efficacy of oncolytic virus therapy. *J Natl Cancer Inst* 99: 1768–1781, 2007,[25] ©2007, Oxford University Press.

tumors. This drug is the only anti-angiogenic small molecule showing subnanomolar antagonistic activity for $\alpha v\beta 3$ and affinities in the low nanomolar range for $\alpha v\beta 5$ and $\alpha 5\beta 1$. Cilengitide was shown to influence cellular adhesion to $\alpha v\beta 3$ ligands, to induce increased apoptosis after the detachment of $\alpha v\beta 3$- and $\alpha v\beta 5$-expressing cells in vitro,[55] and to block the growth of human xenografts in nude mice.[36] Moreover, cilengitide demonstrated anti-angiogenic and anti-tumor activity in different animal models.[9,31] Cilengitide has multimodal anti-glioma effects such as cytotoxic, anti-angiogenic, anti-invasive, and synergetic effects (Fig. 1).

I. Cytotoxic effects of cilengitide

Recent studies reported that cilengitide exerts direct cytotoxic effects on glioma cells via an as yet unknown mechanism.[33,35,41] Several studies have shown that various cells are dependent on integrin-mediated adhesion to specific ECM proteins for their growth and survival and that detachment induces apoptotic cell death.[2,6,11,20,41] Cilengitide reportedly induces apoptosis in αv-integrin-expressing tumor cell lines by detaching tumor cells from vitronectin and tenascin, which are matrix proteins that are essential for tumor growth and invasion.[55] Apoptosis in response to lack of adhesion or inappropriate adhesion has been termed anoikis.[49] Cilengitide induces anoikis in brain tumor cells by inhibiting the phosphorylation of focal adhesion kinase (FAK), Src, and Akt.[2,41] In addition to apoptosis, detachment has also been associated with the induction of autophagy.[14] Autophagy may contribute to the cytotoxic effects of cilengitide.[29] We showed that cilengitide treatment of $\alpha v\beta 3$-expressing glioma cells induced changes in cell morphology, cell detachment, and decreased cell viability in vitro.[43] Anoikis of glioma cells was induced by intraperitoneal cilengitide injection.

II. Anti-angiogenic effects of cilengitide

Angiogenesis is the formation of new blood vessels by the rerouting or remodeling of existing vessels, and is believed to be the primary method of vessel formation in gliomas. Cilengitide was highly effective in suppressing blood vessel growth, thereby inhibiting the orthotopic growth of human glioblastoma cells in animals.[63] Reduction in the size of tumors increased survival in mice with orthotopic brain tumors treated with cilengitide compared to mice treated with an inactive peptide.[31] Therefore, brain tumors, which are highly angiogenic, may be more susceptible to growth inhibition by integrin antagonists. Angiogenesis requires three distinct steps: 1) blood vessel breakdown; 2) degradation of the vessel basement membrane and surrounding ECM; and 3) migration of endothelial cells and the formation of new blood vessels.[57] During the third step, endothelial cells proliferate and begin to migrate toward tumor cells expressing pro-angiogenic compounds. Endothelial cell activation upregulates the expression of cell surface adhesion/migration molecules.[22] Specifically, $\alpha v\beta 3$ integrin is upregulated in endothelial cells during angiogenesis, enhancing endothelial cell adhesion and migration.[7,8] Cilengitide might prevent the third step of angiogenesis and reduce the size of tumor vessels.

III. Anti-invasive effects of cilengitide

Glioma cell invasion requires four distinct steps: 1) detachment of the invading cells from the primary tumor mass; 2) adhesion to the ECM; 3) degradation of the ECM; and 4) cell motility and contractility.[42] During the second step, the molecules allowing glioma cells to adhere to the ECM are integrins, $\alpha v\beta 3$ integrin in particular, which binds to fibronectin, vitronectin, and tenascin-C in the ECM.[57] Integrin $\alpha v\beta 3$ plays a central role in glioma invasion.[27] Inhibition of integrin $\alpha v\beta 3$ decreased glioma cell motility in vitro.[45] Cilengitide might inhibit the second step, thereby suppressing the invasion of glioma. Although most of the animal models with glioma xenografts have tumors with borders that are easily distinguished and dissected from normal brain tissue, we recently established two novel invasive animal glioma models (J3T-1 and J3T-2) that reflect the invasive phenotype of human malignant gliomas.[21] These models were particularly beneficial to investigate the anti-invasive effects of cilengitide. Cilengitide suppressed the invasiveness of these animal glioma models. The borders of cilengitide-treated J3T-2 tumors (angiogenesis-independent invasive tumors) were more easily distinguished than the borders of control J3T-2 tumors.[43]

IV. Other anti-tumor effects of cilengitide

Treatment of glioblastoma cells with cilengitide led to a significant and dose-dependent decrease in the intracellular levels of hypoxia-inducible factor 1 (HIF-1) under hypoxic conditions.[51] Hypoxia stimulates the $\alpha v\beta 3$ and $\alpha v\beta 5$ integrin pathways through FAK and that hypoxia activates FAK in glioblastoma cell lines.[51] This study suggests that $\alpha v\beta 3$ and $\alpha v\beta 5$ are activated by hypoxia and are key regulators of the response of glioma to hypoxic conditions by controlling HIF-1 degradation. Cysteine-rich protein 61 (CYR61), a member of the CCN (CYR61/CTGF/NOV) family of matricellular proteins that regulate cell growth, differentiation, survival, angiogenesis, and migration in development, tissue remodeling, and

repair,[26] and $\alpha v\beta 5$ integrin (a receptor for CYR61) are expressed by tumor cells as critical molecules that cooperate to promote local invasion and distant metastases.[38] Importantly, a function-blocking anti-αv monoclonal antibody (17E6) and cilengitide inhibits CYR61-mediated angiogenesis, invasion, and metastasis.[37,38]

V. Synergistic effects of combination with radiation, chemotherapy, or other new therapeutic strategies

An increased understanding of the molecular mechanisms in the tumorigenesis of glioblastomas has led to the evaluation of targeted agents as potential radiosensitizers.[16,28] Preclinical studies showed a promising synergy between cilengitide and radiochemotherapy (RCT) in order to normalize tumor vasculature and attenuate tumor invasion. The combination of an integrin antagonist and radiotherapy (RT) showed a significant delay of tumor growth in glioblastoma xenografts compared with either treatment individually.[1] Irradiation of tumors reduces the local tumor growth, but at the same time upregulates $\alpha v\beta 3$ expression[1] and enhances local invasion.[54] Hence, cilengitide conceivably may normalize the tumor vasculature, lower tumor interstitial fluid pressure, and improve vascular function and tumor oxygenation.[1,10,46,47,58] Such activity of cilengitide may promote enhanced responsiveness to RT.[62] Most recently, dramatically enhanced antitumor activity of RT was induced by cilengitide in 2-week-old intracranial U251 gliomas in nude rats, but only when cilengitide was given at 4–8 hours before radiation.[35]

Cilengitide acts primarily to block survival pathways, and its enhanced antitumor activity may occur in combination with conventional cytotoxic or pro-apoptotic therapies.[58] As a new therapeutic approach, we demonstrated that anti-angiogenic cilengitide treatment before oncolytic virus (OV) treatment enhanced the antitumor efficacy of the virus (Fig. 2).[13,25] This study showed that pretreatment of gliomas with cilengitide reduced inflammation, vascular hyperpermeability, and leukocyte infiltration of tumor tissue upon treatment with the OV. The reduction of host immune responses by cilengitide treatment enhanced the anticancer efficacy of OV treatment by increasing the propagation of this virus in tumors. We also reported that oncolytic herpes simplex virus 1 infection of tumors induced angiogenesis and upregulated the expression of CYR61.[24] In order to test the role of CYR61-mediated integrin activation in OV-induced angiogenesis, we showed the impact of cilengitide on OV treatment-induced angiogenesis.[23,26]

Clinical Trials

I. Completed clinical trials

The first clinical trial using cilengitide was reported in a phase I trial in patients with recurrent glioblastoma.[32,40] This multi-institutional phase I trial was designed to determine the maximum-tolerated dose of cilengitide (EMD 121974) and to evaluate the use of perfusion magnetic resonance imaging in patients with glioblastoma. In this study, cilengitide demonstrated an unexpected single agent activity for these tumors with limited toxicity for doses up to 2,400 mg/m^2. A multicenter, open-label, phase II study was conducted to evaluate the activity and safety of cilengitide in glioblastoma patients at their first recurrence.[47] As previous clinical studies showed responses at both the lower and higher dose levels, reported follow-up (>4 years) data recently showed that the long-term survival rates were consistently greater with 2000 mg (10.0% after 54 months) versus 500 mg (2.4% after 54 months).[12] Another phase II trial to evaluate the efficacy and tumor delivery of cilengitide in patients with recurrent glioblastoma detected in all tumor specimens with higher levels in the group receiving 2000 mg dosing while corresponding plasma concentrations were low.[15] This study provides evidence that with established dosing, cilengitide is adequately delivered to the tumor.

Preclinical studies revealed that cilengitide in combination with RT and chemotherapy could have enhanced anti-tumor activity.[1,35] A phase II pilot trial added cilengitide (500 mg) to standard chemoradiotherapy with temozolomide (TMZ).[52] In a multicenter pilot study, the progression-free survival (PFS) rates at 6 months in primary endpoint were greater than historical controls (69% versus 54%). Median survival was 16.1 months, with a 2-year survival rate of 35%. These results suggested cilengitide acted as a chemosensitizer but not as a toxic substance. Interestingly, the authors of this study also showed that this treatment was more effective in patients whose tumors had O^6-methylguanine-deoxyribonucleic acid methyltransferase (MGMT) promoter methylation, exhibiting longer PFS and overall survival (OS).[48] On the basis of these results, international, randomized, controlled phase III (CENTRIC) and phase II (CORE) trials were launched in 2008.

II. Clinical trials of cilengitide currently in progress

CENTRIC: The CENTRIC study was designed to test the efficacy and tolerability of cilengitide in patients with newly diagnosed glioblastoma with a

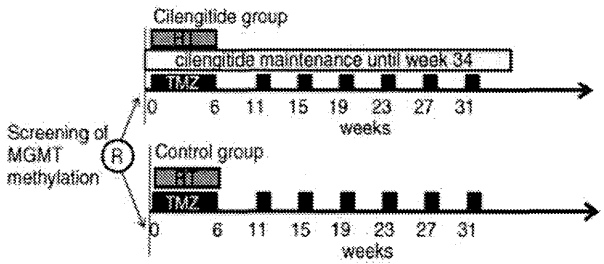

Fig. 3 Design of the CENTRIC study. An estimated 504 patients (approximately 250 patients in each treatment arm) from ~200 centers worldwide will be randomized. *Shaded columns*: focal radiotherapy (RT) 5×/week for 6 weeks (30×2 Gy, total dose 60 Gy); *open column*: cilengitide 2000 mg intravenously 2×/week until week 34, for 18 months (until week 77) optional thereafter; *closed columns*: temozolomide (TMZ) 75 mg/m² per oral daily for 6 weeks (during RT), followed by 150–200 mg/m² per oral on days 1–5 every 28 days for 6 cycles. MGMT: O⁶-methylguanine-deoxyribonucleic acid methyltransferase gene promoter, R: randomization. Modified from Stupp et al.: Cilengitide in newly diagnosed glioblastoma with MGMT promoter methylation: protocol of a multicenter, randomized, openlabel, controlled phase III trial (CENTRIC) [meeting abstract]. *J Clin Oncol* 28: 15s, 2010 (suppl; abstr TPS152).[53]

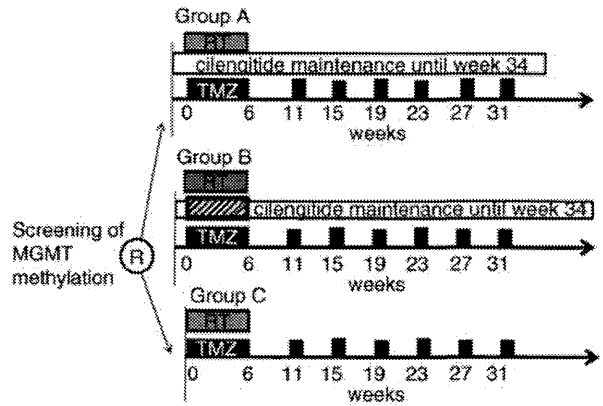

Fig. 4 Design of the randomized part of the CORE study. An estimated 252 patients from up to 85 centers in the US, Canada, Europe, and Asia will be randomized in the second part of the study. *Shaded columns*: focal radiotherapy (RT) 5×/week for 6 weeks (30×2 Gy, total dose 60 Gy); *open columns*: cilengitide 2000 mg intravenously 2×/week until week 34; *hatched column*: cilengitide 2000 mg intravenously 5×/week during weeks 1–6; *closed columns*: temozolomide (TMZ) 75 mg/m² per oral daily for 6 weeks (during RT), followed by 150–200 mg/m² per oral on days 1–5 every 28 days for 6 cycles. MGMT: O⁶-methylguanine-deoxyribonucleic acid methyltransferase gene promoter, R: randomization. Modified from Nabors et al.: Cilengitide in patients with newly diagnosed glioblastoma multiforme and unmethylated MGMT gene promoter: Protocol of a multicenter, randomized, open-label, controlled phase II study [meeting abstract]. *J Clin Oncol* 28: 15s, 2010 (suppl; abstr TPS151).[39]

methylated MGMT gene promoter.[53] In the investigational arm, patients receive cilengitide at a dose of 2000 mg intravenously twice weekly in combination with standard RCT (concomitant RT/TMZ for 6 weeks, followed by 6 cycles of TMZ maintenance therapy). Patients in the control arm receive only standard RCT. The treatment duration is 18 months for patients in the cilengitide group and 8 months for those in the control group. Patients in the cilengitide group are allowed to continue with cilengitide after completion of the 18 months of the study treatment until the occurrence of progression disease or unacceptable toxicity, or withdrawal for any other reason. The study design is shown in Fig. 3.

CORE: The CORE study is investigating the efficacy and safety of 2 regimens of cilengitide in glioblastoma patients with an unmethylated MGMT promoter.[39] CORE is a multicenter, open-label, phase II study. Cilengitide (2000 mg intravenously over 60 min) is administered at 4 hours before RT and TMZ is given orally for 7 days a week after the completion of cilengitide infusion at least 1 hour before RT. The primary objective of this study is to investigate the OS time in subjects receiving 2 different regimens of 2000 mg of cilengitide in combination with RT and TMZ standard therapy. Secondary objectives of this study are 1) to evaluate PFS time, 2) to evaluate the safety and tolerability of the combination of cilengitide with standard RT and TMZ therapy, and 3) to evaluate the pharmacokinetic profile of cilengitide (Fig. 4).

III. Other on-going trials (Table 1)

Several preclinical studies have shown an enhanced antitumor effect of cilengitide when administered in combinatorial therapeutic regimens. RCT with cilengitide or cetuximab is being investigated in a randomized, non-comparative trial in patients with newly diagnosed MGMT-promoter unmethylated glioblastoma (CeCil).[19] Chemoresistance was examined in the MGMT unmethylated population, building on preclinical data, prior experience with cilengitide, and the combination of low dose TMZ and procarbazine (ExCentric).[19] Cediranib maleate and cilengitide may stop the growth of tumor cells by blocking blood flow to the tumor; therefore, the co-administration of cediranib maleate and cilengitide may kill more tumor cells. This phase I trial is studying the side effects and best dose

Table 1 Clinical trials of cilengitide currently in progress*

Trial	Estimated no. of patients	Disease setting	Purpose/Treatment	Start date
Phase II ExCentric	48	newly diagnosed GBM (unmethylated gene promoter status)	evaluate safety and efficacy/ cilengitide + RT + TMZ + PCB	November 2009
Phase II Cecil	108	newly diagnosed GBM (unmethylated gene promoter status)	evaluate safety and efficacy/ cilengitide or cetuximab + RT + TMZ	September 2009
Phase I	28	newly diagnosed GBM	evaluate safety and dosage/ cilengitide + sunitinib maleate	January 2010
Phase I	52	progressive/recurrent GBM	evaluate safety and dosage/ cilengitide + cediranib maleate	March 2010

*Data are available on the Web at http://www.clinicaltrials.gov.[19] GBM: glioblastoma multiforme, PCB: procarbazine, RT: radiotherapy, TMZ: temozolomide.

of cediranib maleate when given together with cilengitide in patients with progressive or recurrent glioblastoma.[19] Sunitinib is an oral, small-molecule, multi-targeted receptor tyrosine kinase inhibitor for the treatment of renal cell carcinoma and imatinib-resistant gastrointestinal stromal tumor. Cilengitide in combination with sunitinib is being investigated in patients with advanced solid tumors or glioblastoma.[19]

Conclusions

The management of glioblastoma remains a challenging area in oncology. Angiogenesis and invasion are undoubtedly critical in the development and survival of glioblastoma. Cilengitide is the first integrin antagonist for the treatment of glioblastoma. Integrins are expressed in tumor cells and tumor endothelial cells, and are important in angiogenesis and invasion in glioma. Cilengitide is currently being assessed in phase III trials for glioblastoma patients and phase II trials for other types of cancers, with promising therapeutic outcomes to date. The CENTRIC controlled phase III study was launched in 2008, with primary outcome measures due in September 2012. The results of this and other clinical studies are expected with great hope and interest. A more clear understanding of the benefits and pitfalls of each approach can then lead to the design of strategies to derive maximal benefit from these therapies.

Funding

Cilengitide was generously provided by Merck KGaA (Darmstadt, Germany) and Cancer Therapy Evaluation Program, the National Cancer Institute at the National Institutes of Health (Bethesda, Maryland, USA).

Conflict of Interest

No author has any conflict of interest to declare.

References

1) Abdollahi A, Griggs DW, Zieher H, Roth A, Lipson KE, Saffrich R, Grone HJ, Hallahan DE, Reisfeld RA, Debus J, Niethammer AG, Huber PE: Inhibition of alpha(v)beta3 integrin survival signaling enhances antiangiogenic and antitumor effects of radiotherapy. *Clin Cancer Res* 11: 6270–6279, 2005
2) Alghisi GC, Ponsonnet L, Ruegg C: The integrin antagonist cilengitide activates alphaVbeta3, disrupts VE-cadherin localization at cell junctions and enhances permeability in endothelial cells. *PLoS One* 4: e4449, 2009
3) Avraamides CJ, Garmy-Susini B, Varner JA: Integrins in angiogenesis and lymphangiogenesis. *Nat Rev Cancer* 8: 604–617, 2008
4) Bello L, Francolini M, Marthyn P, Zhang J, Carroll RS, Nikas DC, Strasser JF, Villani R, Cheresh DA, Black PM: Alpha(v)beta3 and alpha(v)beta5 integrin expression in glioma periphery. *Neurosurgery* 49: 380–390, 2001
5) Bello L, Giussani C, Carrabba G, Pluderi M, Costa F, Bikfalvi A: Angiogenesis and invasion in gliomas. *Cancer Treat Res* 117: 263–284, 2004
6) Bouchard V, Demers MJ, Thibodeau S, Laquerre V, Fujita N, Tsuruo T, Beaulieu JF, Gauthier R, Vezina A, Villeneuve L, Vachon PH: Fak/Src signaling in human intestinal epithelial cell survival and anoikis: differentiation state-specific uncoupling with the PI3-K/Akt-1 and MEK/Erk pathways. *J Cell Physiol* 212: 717–728, 2007
7) Brooks PC, Clark RA, Cheresh DA: Requirement of vascular integrin alpha v beta 3 for angiogenesis. *Science* 264: 569–571, 1994
8) Brooks PC, Montgomery AM, Rosenfeld M, Reisfeld RA, Hu T, Klier G, Cheresh DA: Integrin alpha v beta 3 antagonists promote tumor regression by inducing

apoptosis of angiogenic blood vessels. *Cell* 79: 1157–1164, 1994
9) Buerkle MA, Pahernik SA, Sutter A, Jonczyk A, Messmer K, Dellian M: Inhibition of the alpha-nu integrins with a cyclic RGD peptide impairs angiogenesis, growth and metastasis of solid tumours in vivo. *Br J Cancer* 86: 788–795, 2002
10) Burke PA, DeNardo SJ, Miers LA, Lamborn KR, Matzku S, DeNardo GL: Cilengitide targeting of alpha(v)beta(3) integrin receptor synergizes with radioimmunotherapy to increase efficacy and apoptosis in breast cancer xenografts. *Cancer Res* 62: 4263–4272, 2002
11) Chi A, Norden AD, Wen PY: Inhibition of angiogenesis and invasion in malignant gliomas. *Expert Rev Anticancer Ther* 7: 1537–1560, 2007
12) Fink K, Mikkelsen T, Nabors LB, Ravin P, Plotkin SR, Schiff D, Hicking C, Picard M, Reardon DA: Long-term effects of cilengitide, a novel integrin inhibitor, in recurrent glioblastoma: a randomized phase IIa study [meeting abstract]. *J Clin Oncol* 28: 15s, 2010 (suppl; abstr 2010)
13) Fujii K, Kurozumi K, Ichikawa T, Yoshida K, Onishi M, Michiue H, Chiocca EA, Kaur B, Date I: The integrin antagonist cilengitide enhances anti-tumor effect of vasculostatin-expressing oncolytic virus. Poster presented at: Congress of Neurological Surgeons 2011 Annual Meeting; Oct 1–6, 2011; Washington, DC, USA. Available from: http://2011.cns.org/posterbrowser.aspx. Accessed Jun 26, 2012
14) Fung C, Lock R, Gao S, Salas E, Debnath J: Induction of autophagy during extracellular matrix detachment promotes cell survival. *Mol Biol Cell* 19: 797–806, 2008
15) Gilbert MR, Kuhn J, Lamborn KR, Lieberman F, Wen PY, Mehta M, Cloughesy T, Lassman AB, Deangelis LM, Chang S, Prados M: Cilengitide in patients with recurrent glioblastoma: the results of NABTC 03-02, a phase II trial with measures of treatment delivery. *J Neurooncol* 106: 147–153, 2012
16) Gorski DH, Beckett MA, Jaskowiak NT, Calvin DP, Mauceri HJ, Salloum RM, Seetharam S, Koons A, Hari DM, Kufe DW, Weichselbaum RR: Blockage of the vascular endothelial growth factor stress response increases the antitumor effects of ionizing radiation. *Cancer Res* 59: 3374–3378, 1999
17) Heckmann D, Kessler H: Design and chemical synthesis of integrin ligands. *Methods Enzymol* 426: 463–503, 2007
18) Hood JD, Cheresh DA: Role of integrins in cell invasion and migration. *Nat Rev Cancer* 2: 91–100, 2002
19) ClinicalTrials.gov: registry and results database of federally and privately supported clinical trials. Available from: http://clinicaltrials.gov/ct2/home. Accessed Jun 26, 2012
20) Hynes RO: Integrins: bidirectional, allosteric signaling machines. *Cell* 110: 673–687, 2002
21) Inoue S, Ichikawa T, Kurozumi K, Maruo T, Onishi M, Yoshida K, Fujii K, Kambara H, Chiocca EA, Date I: Novel animal glioma models that separately exhibit two different invasive and angiogenic phenotypes of human glioblastomas. *World Neurosurg* Epub 2011 Nov 7
22) Jouanneau E: Angiogenesis and gliomas: current issues and development of surrogate markers. *Neurosurgery* 62: 31–52, 2008
23) Kireeva ML, Lam SC, Lau LF: Adhesion of human umbilical vein endothelial cells to the immediate-early gene product Cyr61 is mediated through integrin alphavbeta3. *J Biol Chem* 273: 3090–3096, 1998
24) Kurozumi K, Hardcastle J, Thakur R, Shroll J, Nowicki M, Otsuki A, Chiocca EA, Kaur B: Oncolytic HSV-1 infection of tumors induces angiogenesis and upregulates CYR61. *Mol Ther* 16: 1382–1391, 2008
25) Kurozumi K, Hardcastle J, Thakur R, Yang M, Christoforidis G, Fulci G, Hochberg FH, Weissleder R, Carson W, Chiocca EA, Kaur B: Effect of tumor microenvironment modulation on the efficacy of oncolytic virus therapy. *J Natl Cancer Inst* 99: 1768–1781, 2007
26) Leask A, Abraham DJ: All in the CCN family: essential matricellular signaling modulators emerge from the bunker. *J Cell Sci* 119: 4803–4810, 2006
27) Leavesley DI, Ferguson GD, Wayner EA, Cheresh DA: Requirement of the integrin beta 3 subunit for carcinoma cell spreading or migration on vitronectin and fibrinogen. *J Cell Biol* 117: 1101–1107, 1992
28) Lee CG, Heijn M, di Tomaso E, Griffon-Etienne G, Ancukiewicz M, Koike C, Park KR, Ferrara N, Jain RK, Suit HD, Boucher Y: Anti-vascular endothelial growth factor treatment augments tumor radiation response under normoxic or hypoxic conditions. *Cancer Res* 60: 5565–5570, 2000
29) Lomonaco SL, Finniss S, Xiang C, Lee HK, Jiang W, Lemke N, Rempel SA, Mikkelsen T, Brodie C: Cilengitide induces autophagy-mediated cell death in glioma cells. *Neuro Oncol* 13: 857–865, 2011
30) MacDonald TJ, Stewart CF, Kocak M, Goldman S, Ellenbogen RG, Phillips P, Lafond D, Poussaint TY, Kieran MW, Boyett JM, Kun LE: Phase I clinical trial of cilengitide in children with refractory brain tumors: Pediatric Brain Tumor Consortium Study PBTC-012. *J Clin Oncol* 26: 919–924, 2008
31) MacDonald TJ, Taga T, Shimada H, Tabrizi P, Zlokovic BV, Cheresh DA, Laug WE: Preferential susceptibility of brain tumors to the antiangiogenic effects of an alpha(v) integrin antagonist. *Neurosurgery* 48: 151–157, 2001
32) Mas-Moruno C, Rechenmacher F, Kessler H: Cilengitide: the first anti-angiogenic small molecule drug candidate design, synthesis and clinical evaluation. *Anticancer Agents Med Chem* 10: 753–768, 2010
33) Maurer GD, Tritschler I, Adams B, Tabatabai G, Wick W, Stupp R, Weller M: Cilengitide modulates attachment and viability of human glioma cells, but not sensitivity to irradiation or temozolomide in vitro. *Neuro Oncol* 11: 747–756, 2009
34) Meyer A, Auernheimer J, Modlinger A, Kessler H:

Targeting RGD recognizing integrins: drug development, biomaterial research, tumor imaging and targeting. *Curr Pharm Des* 12: 2723–2747, 2006
35) Mikkelsen T, Brodie C, Finniss S, Berens ME, Rennert JL, Nelson K, Lemke N, Brown SL, Hahn D, Neuteboom B, Goodman SL: Radiation sensitization of glioblastoma by cilengitide has unanticipated schedule-dependency. *Int J Cancer* 124: 2719–2727, 2009
36) Mitjans F, Meyer T, Fittschen C, Goodman S, Jonczyk A, Marshall JF, Reyes G, Piulats J: In vivo therapy of malignant melanoma by means of antagonists of alphav integrins. *Int J Cancer* 87: 716–723, 2000
37) Mitjans F, Sander D, Adan J, Sutter A, Martinez JM, Jaggle CS, Moyano JM, Kreysch HG, Piulats J, Goodman SL: An anti-alpha v-integrin antibody that blocks integrin function inhibits the development of a human melanoma in nude mice. *J Cell Sci* 108(Pt 8): 2825–2838, 1995
38) Monnier Y, Farmer P, Bieler G, Imaizumi N, Sengstag T, Alghisi GC, Stehle JC, Ciarloni L, Andrejevic-Blant S, Moeckli R, Mirimanoff RO, Goodman SL, Delorenzi M, Ruegg C: CYR61 and alphaVbeta5 integrin cooperate to promote invasion and metastasis of tumors growing in preirradiated stroma. *Cancer Res* 68: 7323–7331, 2008
39) Nabors LB, Fink K, Reardon DA, Lesser GJ, Trusheim J, Raval SN, Hicking C, Picard M, Mikkelsen T: Cilengitide in patients with newly diagnosed glioblastoma multiforme and unmethylated MGMT gene promoter: Protocol of a multicenter, randomized, open-label, controlled phase II study [meeting abstract]. *J Clin Oncol* 28: 15s, 2010 (suppl; abstr TPS151)
40) Nabors LB, Mikkelsen T, Rosenfeld SS, Hochberg F, Akella NS, Fisher JD, Cloud GA, Zhang Y, Carson K, Wittemer SM, Colevas AD, Grossman SA: Phase I and correlative biology study of cilengitide in patients with recurrent malignant glioma. *J Clin Oncol* 25: 1651–1657, 2007
41) Oliveira-Ferrer L, Hauschild J, Fiedler W, Bokemeyer C, Nippgen J, Celik I, Schuch G: Cilengitide induces cellular detachment and apoptosis in endothelial and glioma cells mediated by inhibition of FAK/src/AKT pathway. *J Exp Clin Cancer Res* 27: 86, 2008
42) Onishi M, Ichikawa T, Kurozumi K, Date I: Angiogenesis and invasion in glioma. *Brain Tumor Pathol* 28: 13–24, 2011
43) Onishi M, Ichikawa T, Kurozumi K, Fujii K, Yoshida K, Inoue S, Michiue H, Chiocca EA, Kaur B, Date I: Anti-angiogenic and anti-invasive effect of cilengitide on experimental glioma. Poster presented at: Congress of Neurological Surgeons 2011 Annual Meeting; Oct 1–6, 2011; Washington, DC, USA. Available from: http://2011.cns.org/posterbrowser.aspx. Accessed Jun 26, 2012
44) Penas-Prado M, Gilbert MR: Molecularly targeted therapies for malignant gliomas: advances and challenges. *Expert Rev Anticancer Ther* 7: 641–661, 2007
45) Platten M, Wick W, Wild-Bode C, Aulwurm S, Dichgans J, Weller M: Transforming growth factors beta(1) (TGF-beta(1)) and TGF-beta(2) promote glioma cell migration via Up-regulation of alpha(V)beta(3) integrin expression. *Biochem Biophys Res Commun* 268: 607–611, 2000
46) Reardon DA, Fink KL, Mikkelsen T, Cloughesy TF, O'Neill A, Plotkin S, Glantz M, Ravin P, Raizer JJ, Rich KM, Schiff D, Shapiro WR, Burdette-Radoux S, Dropcho EJ, Wittemer SM, Nippgen J, Picard M, Nabors LB: Randomized phase II study of cilengitide, an integrin-targeting arginine-glycine-aspartic acid peptide, in recurrent glioblastoma multiforme. *J Clin Oncol* 26: 5610–5617, 2008
47) Reardon DA, Nabors LB, Stupp R, Mikkelsen T: Cilengitide: an integrin-targeting arginine-glycine-aspartic acid peptide with promising activity for glioblastoma multiforme. *Expert Opin Investig Drugs* 17: 1225–1235, 2008
48) Reardon DA, Neyns B, Weller M, Tonn JC, Nabors LB, Stupp R: Cilengitide: an RGD pentapeptide alphanubeta3 and alphanubeta5 integrin inhibitor in development for glioblastoma and other malignancies. *Future Oncol* 7: 339–354, 2011
49) Schaller MD: Biochemical signals and biological responses elicited by the focal adhesion kinase. *Biochim Biophys Acta* 1540: 1–21, 2001
50) Schnell O, Krebs B, Wagner E, Romagna A, Beer AJ, Grau SJ, Thon N, Goetz C, Kretzschmar HA, Tonn JC, Goldbrunner RH: Expression of integrin alphavbeta3 in gliomas correlates with tumor grade and is not restricted to tumor vasculature. *Brain Pathol* 18: 378–386, 2008
51) Skuli N, Monferran S, Delmas C, Favre G, Bonnet J, Toulas C, Cohen-Jonathan Moyal E: Alphavbeta3/alphavbeta5 integrins-FAK-RhoB: a novel pathway for hypoxia regulation in glioblastoma. *Cancer Res* 69: 3308–3316, 2009
52) Stupp R, Hegi ME, Neyns B, Goldbrunner R, Schlegel U, Clement PM, Grabenbauer GG, Ochsenbein AF, Simon M, Dietrich PY, Pietsch T, Hicking C, Tonn JC, Diserens AC, Pica A, Hermisson M, Krueger S, Picard M, Weller M: Phase I/IIa study of cilengitide and temozolomide with concomitant radiotherapy followed by cilengitide and temozolomide maintenance therapy in patients with newly diagnosed glioblastoma. *J Clin Oncol* 28: 2712–2718, 2010
53) Stupp R, Van Den Bent MJ, Erridge SC, Reardon DA, Hong Y, Wheeler H, Hegi M, Perry JR, Picard M, Weller M: Cilengitide in newly diagnosed glioblastoma with MGMT promoter methylation: protocol of a multicenter, randomized, openlabel, controlled phase III trial (CENTRIC) [meeting abstract]. *J Clin Oncol* 28: 15s, 2010 (suppl; abstr TPS152)
54) Tabatabai G, Weller M, Nabors B, Picard M, Reardon D, Mikkelsen T, Ruegg C, Stupp R: Targeting integrins in malignant glioma. *Target Oncol* 5: 175–181, 2010

55) Taga T, Suzuki A, Gonzalez-Gomez I, Gilles FH, Stins M, Shimada H, Barsky L, Weinberg KI, Laug WE: alpha v-Integrin antagonist EMD 121974 induces apoptosis in brain tumor cells growing on vitronectin and tenascin. *Int J Cancer* 98: 690–697, 2002
56) Takada Y, Ye X, Simon S: The integrins. *Genome Biol* 8: 215, 2007
57) Tate MC, Aghi MK: Biology of angiogenesis and invasion in glioma. *Neurotherapeutics* 6: 447–457, 2009
58) Tentori L, Dorio AS, Muzi A, Lacal PM, Ruffini F, Navarra P, Graziani G: The integrin antagonist cilengitide increases the antitumor activity of temozolomide against malignant melanoma. *Oncol Rep* 19: 1039–1043, 2008
59) Varner JA, Cheresh DA: Integrins and cancer. *Curr Opin Cell Biol* 8: 724–730, 1996
60) Varner JA, Cheresh DA: Tumor angiogenesis and the role of vascular cell integrin alphavbeta3. *Important Adv Oncol* 69–87, 1996
61) Wild-Bode C, Weller M, Wick W: Molecular determinants of glioma cell migration and invasion. *J Neurosurg* 94: 978–984, 2001
62) Winkler F, Kozin SV, Tong RT, Chae SS, Booth MF, Garkavtsev I, Xu L, Hicklin DJ, Fukumura D, di Tomaso E, Munn LL, Jain RK: Kinetics of vascular normalization by VEGFR2 blockade governs brain tumor response to radiation: role of oxygenation, angiopoietin-1, and matrix metalloproteinases. *Cancer Cell* 6: 553–563, 2004
63) Yamada S, Bu XY, Khankaldyyan V, Gonzales-Gomez I, McComb JG, Laug WE: Effect of the angiogenesis inhibitor Cilengitide (EMD 121974) on glioblastoma growth in nude mice. *Neurosurgery* 59: 1304–1312, 2006

Address reprint requests to: Kazuhiko Kurozumi, MD, PhD, Department of Neurological Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan.
e-mail: kkuro@md.okayama-u.ac.jp

Brain Tumor Pathol (2012) 29:17–24
DOI 10.1007/s10014-011-0058-9

ORIGINAL ARTICLE

Atypical and ischemic features of embolized meningiomas

Ken Matsuda · Hiroaki Takeuchi · Yoshikazu Arai · Ryuhei Kitai · Tetsuya Hosoda · Kenzo Tsunetoshi · Hidetaka Arishima · Kazufumi Sato · Ken-ichiro Kikuta

Received: 14 April 2011 / Accepted: 4 July 2011 / Published online: 26 July 2011

Abstract Preoperative embolization (POE) of meningiomas is widely used to facilitate surgical removal and to reduce intraoperative blood loss. The resulting necrosis and enhanced proliferation have been reported to affect subsequent histologic grading. However, there was little concern about ischemic features, for example small cells resembling atypical meningiomas, cytoplasmic vacuoles resembling clear cell meningioma, intercellular discohesion resembling rhabdoid meningioma, and perivascular cuffs resembling papillary meningioma. Therefore, the extent of these ischemic features was scored and Ki-67 staining indices were investigated in a POE group composed of 29 specimens of meningiomas treated with POE and compared with equivalent results for a non-POE group composed of 29 meningiomas that were not treated with POE. Small cells with high N/C ratios, cytoplasmic vacuoles, intercellular discohesion, and perivascular cuffs were significantly increased in the POE group (versus the non-POE group, $p < 0.05$). There were no significant differences of the Ki-67 index between the POE group (2.2%) and the non-POE group (1.9%) ($p = 0.49$). Our results suggest that small cell change resulting in necrosis may be followed by POE, and that clear cell-like, rhabdoid cell-like, or pseudopapillary pattern identified in meningiomas may also be induced by POE. Therefore, histological findings and determination of grading should be evaluated cautiously in cases of embolized meningiomas.

K. Matsuda · H. Takeuchi (✉) · Y. Arai · R. Kitai · T. Hosoda · K. Tsunetoshi · H. Arishima · K. Sato · K. Kikuta
Department of Neurosurgery, Faculty of Medical Sciences, University of Fukui, 23-3 Matsuoka-Shimoaizuki, Eiheiji, Fukui 910-1193, Japan
e-mail: takeu@u-fukui.ac.jp

Keywords Meningioma · Embolization · Atypical · Ischemia · Ki-67

Introduction

Preoperative embolization (POE) of meningiomas is commonly performed to minimize intraoperative bleeding and softening of the tumor, thereby facilitating surgery and reducing the need for transfusions [1–6]. The procedure itself can induce necrosis with associated regenerative or reparative atypia and compensatory proliferative activity [7–10]. Perry et al. [11] reported that embolized meningiomas had higher frequencies of necrosis, nuclear atypia, macronucleoli, sheeting, high mitotic index, and brain invasion in comparison with nonembolized counterparts. Therefore, histologically overgrading may occur in meningiomas treated with POE, resulting in their classification as atypical [7–11].

In our experience, small cells resembling atypical meningioma, tumor cells with cleared vacuoles within the cytoplasm resembling clear cell meningiomas, intercellular discohesion with intracytoplasmic glassy homogenous appearance resembling rhabdoid meningiomas, and the formation of perivascular papillary structure caused by dehiscence during processing or disappearance of tumor cells far from the vessels resembling papillary meningiomas have been identified in some cases with meningiomas receiving POE. These morphological findings are suggested to be ischemic changes associated with POE. However, there have been no reports about these histological findings in meningiomas treated by POE. Therefore, we investigated atypical and ischemic features of embolized meningioma in patients with meningioma receiving surgical resection after POE.

Materials and methods

The study was approved by the institutional review board of the University of Fukui Hospital. After approval, the discharge databases of the neurosurgical department were reviewed to identify all patients who had undergone surgical resection receiving POE from January 1, 1998 to December 31, 2010. The available hospital chart and clinical records were reviewed retrospectively to extract relevant data. The POE group comprised 29 patients with meningiomas, and the 29 specimens that were obtained from their first operations after POE. The POE used large poly(vinyl alcohol) (PVA) particles (150–250 μm), and were performed in the Neurosurgical Department at the University of Fukui Hospital between 1998 and 2010. All meningiomas with POE had been embolized for 3 to 355 h (mean ± SD 81.9 ± 70.1 h) before surgical extirpation. The non-POE group comprised 29 patients with meningiomas, and the 29 specimens that were obtained from their first operations without POE, which were identified from the files of the Neurosurgical Department at the University of Fukui Hospital. The full course of follow-up and the occurrence of complications were also evaluated in each patient after discharge from the POE or operation. Follow-up periods were measured from the date of the first surgery for the meningioma in both the POE and non-POE groups.

Embolization technique

Embolization was performed via the middle meningeal or occipital artery only. The ophthalmic artery, the meningohypophyseal trunk, and pial feeders were not used as access for embolization. Embolization was performed through a standard microcatheter (TurboTracker 18 or Excel 14; Target Therapeutics/Boston Scientific, Fremont, CA, USA). Poly(vinyl alcohol) particles of 150–250 μm (Contour Emboli; Target Therapeutics) were used as an embolic agent. Particles were mixed with a nonionic contrast medium, and the mixture was diluted to approximately 50% with saline. Under fluoroscopic control, the mixture was slowly injected until stagnation of the contrast agent in the feeding artery was accomplished.

Histopathologic assessment

Meningiomas were classified in accordance with the World Health Organization, 2007 [12]. In a large series of embolized meningiomas, Perry et al. suggested that application of normal grading criteria (except for geographic necrosis) remained highly predictive of increased recurrence of meningiomas qualifying as atypical. Therefore, atypical meningioma (Grade II) was diagnosed by criteria proposed by Perry in embolized meningiomas [11]. Designation as atypical meningioma in the POE group required high mitotic index (4 or more mitoses per any 10 consecutive high-power fields (HPFs)), brain invasion, or the presence of at least 3 of the following 4 characteristics: sheeting (patternless) architecture, macronucleoli, hypercellularity, small cells with high nuclear/cytoplasmic ratio. Designation as atypical meningioma in the non-POE group required high mitotic index (4 or more mitoses per any 10 consecutive HPFs), brain invasion, or the presence of at least 3 of the following 5 characteristics: sheeting (patternless) architecture, macronucleoli, hypercellularity, small cells with high nuclear/cytoplasmic ratio, geographic necrosis. Brain invasion was defined by tumor penetration through the pial surface, rather than simple extension along Virchow–Robin spaces. Diagnosis of anaplastic meningioma (Grade III) required either an excessive mitotic index (≥20/10 HPF) or a loss of meningothelial differentiation at the light-microscopic level (i.e., sarcoma, carcinoma, or melanoma-like morphology) in the POE and non-POE groups.

Atypical histopathologic characteristics, including increased cellularity, prominent nucleoli, small cells with high nuclear/cytoplasmic (N/C) ratios except for small cells with hyperchromatic and concentrated nuclei associated with ischemic change and infiltrating inflammatory cells, sheet-like growth, and geographic necrosis, were investigated. Evaluation of these was scored as follows: 1, partial; 2, island; 3, global. Ischemic histopathologic characteristics, including cellular swelling with cleared vacuoles within the cytoplasm resembling clear cell meningiomas (cytoplasmic vacuoles), intercellular discohesion with intracytoplasmic glassy homogenous appearance resembling rhabdoid meningioma (intercellular discohesion), and the formation of perivascular papillary structure caused by dehiscence during processing or disappearance of tumor cells far from the vessels resembling papillary meningiomas (perivascular cuffs), were also evaluated by use of the scores mentioned above. Additional 5–6-μm sections were cut from archival paraffin-embedded blocks for immunohistochemical study. Immunostaining for Ki-67 (Dako, Glostrup, Denmark; 1:200 dilution) was performed with microwave antigen retrieval in 0.01 M citrate buffer, pH 6.0, for 7 min. The Ki-67 staining index was expressed as the percentage of positive nuclei derived by manually counting 1000 nuclei in the region of maximum staining in nonnecrotic regions for each tumor [13].

Statistical methods

Statistical analysis of the differences between the early and late groups was performed by use of the Student's *t* test. A *p* value of below 0.05 was considered significant.

Results

Table 1 shows individual data for clinicopathologic features and scores in the POE group. Table 2 shows individual data for clinicopathologic features and scores in the non-POE group.

Clinical findings

In the POE group, there were 16 women and 13 men who ranged in age from 25 to 87 years (mean 63 years) at the time of their operations. The tumors were located in the cerebral convexity in 5 patients, in the skull base in 14, in the parasagittal in 9, and in the falx in 1. No complications were encountered in the 29 patients treated with POE. The patients' follow-up periods ranged from 8 to 150 months (mean 64 months) and tumor recurrence or regrowth was identified in 8 patients. In the non-POE group, there were 16 women and 13 men who ranged in age from 36 to 85 years (mean 61 years). The tumors were located in the cerebral convexity in 6, in the skull base in 14, in the parasagittal in 8, and in the falx in 1. The patients' follow-up periods ranged from 6 to 277 months (mean 121 months) and recurrence or regrowth was identified in 7 patients. There were no statistical differences between those data.

Histopathologic findings

Histological subtypes and mean estimated scores of each atypical and ischemic feature are listed in Table 3.

Atypical and ischemic features in embolized meningiomas

High mitotic activity (4 or more mitoses per 10 HPF) was identified in 4 patients (14%) in the POE group and in the non-POE group. Brain invasion was identified in 7 patients (24%) in the POE group and 4 patients (13%) in the non-POE group. Increased cellularity was identified in 13 patients (45%) in the POE group and in 10 patients (34%) in the non-POE group. Prominent nucleoli were identified in 5 patients (17%) in the POE group and in 3 patients (10%) in the non-POE group. Small cells with high nuclear/cytoplasmic (N/C) ratios were identified in 15 patients (52%) in the POE group and in 5 patients (17%) in the non-POE group. Sheet-like growth was identified in 19 patients (66%) in the POE group and in 17 patients (59%) in the non-POE group. Geographic necrosis was identified in 11 patients (38%) in the POE group and in 9 patients (31%) in the non-POE group. 11 patients (38%) in the POE group and 7 patients (24%) in the non-POE group were designated atypical meningioma (Grade II). There were no patients with anaplastic meningioma (Grade III) in this study (Table 3).

Cytoplasmic vacuoles (clear cell-like) were identified in 21 patients (72%) in the POE group and in 12 patients (41%) in the non-POE group. Intercellular discohesion (rhabdoid cell-like) was identified in 12 patients (41%) in the POE group and in 1 patient (3%) in the non-POE group. Intercellular discohesion identified in embolized meningiomas resembled rhabdoid meningiomas. However, there were few discrete cytoplasmic inclusions. Perivascular cuffs (pseudopapillary-like) were identified in 14 patients (48%) in the POE group and in 3 patients (10%) in the non-POE group. Although perivascular cuffs identified in embolized meningiomas resembled papillary meningiomas, there were few findings of a perivascular orientation of tumor cells.

Mean scores in the POE group (versus the non-POE group) were significantly increased in small cells with high N/C ratios (0.59 vs. 0.17, $p = 0.004$), cytoplasmic vacuoles (1.0 vs. 0.52, $p = 0.02$), intercellular discohesion (0.41 vs. 0.03, $p = 0.0005$), and perivascular cuffs (0.48 vs. 0.1, $p = 0.001$). Mean scores in the POE group were not significantly elevated in geographic necrosis (0.83 vs. 0.38, $p = 0.06$), increased cellularity (0.45 vs. 0.34, $p = 0.43$), prominent nucleoli (0.17 vs. 0.10, $p = 0.46$), or sheet-like growth (0.69 vs. 0.72, $p = 0.84$) (Table 3).

The Ki-67 staining index in embolized meningiomas

The Ki-67 index in nonnecrotic regions ranged from 0.1 to 9.1% (mean 2.2%) in the POE group, and from 0.1 to 8.6% (mean 1.9%) in the non-POE group. There were no significant differences in the Ki-67 index between the POE group and the non-POE group ($p = 0.49$). High Ki-67 indices (>3%) were encountered in 8 cases (28%) in the POE group and in 7 cases in the non-POE group (24%). In the POE group, 6 (75%) of the 8 patients with high Ki-67 indices had recurrent tumors. In the non-POE group, 6 (86%) of the 7 patients with high Ki-67 indices had recurrent tumors.

Representative case (Case No. 29 in the POE group)

A vascular-rich atypical meningioma was incidentally identified in the right frontal convexity of a 66-year-old man who underwent tumor resection 7 days after POE with large PVA particles (Fig. 1a, b, c). Classical whorl formation and geographic necroses were identified in the tumor. Mitotic figures were inconspicuous (Fig. 1d, e). Cytoplasmic vacuoles, intercellular discohesion, and perivascular cuffs were identified in the same tumor specimen (Fig. 1f, g, h). Ki-67 immunoreactive cells were not apparent and Ki-67 index was 2.1% (Fig. 1i).

Table 1 Clinicopathologic features and scores of the 29 patients with meningioma in the POE group

No.	Age	Gender	Site	Interval (POE to Op) (days)	Pathology	Atypical features							Ischemic features			Ki67 index (%)	Recurrence	Follow up (months)
						Mitosis (per 10 HPF)	Brain invasion[a]	Increased cellularity[a]	Prominent nucleoli[a]	Small cell with high N/C ratios[a]	Sheet-like growth[a]	Geographic necrosis[a]	Cytoplasmic vacuoles[a]	Intercellular discohesion[a]	Perivascular cuffs[a]			
1	56	M	PS	0	Me	0	0	0	0	0	0	0	1	1	1	0.8		145
2	82	F	SR	2	Papillary	4	1	1	0	0	1	3	1	1	1	6.7	+	63(D)
3	83	F	Con	2	Trans	0	0	0	0	0	0	0	0	0	0	0.3		100
4	75	M	SR	2	Angio	0	0	0	0	0	1	0	1	0	0	0.2		86
5	76	M	SR	2	Me	1	0	0	0	1	1	0	1	0	1	2.5	+	83
6	54	F	PS	2	Me	0	0	0	0	1	0	0	0	0	0	0.1		79
7	25	M	SR	2	Atypical	3	1	1	0	2	1	0	2	0	0	1.9	+	64
8	57	F	Tent	1	Psa	0	0	0	0	1	0	0	1	0	1	0.1		61
9	44	F	PS	2	Microcystic	0	0	0	0	0	1	0	2	0	0	1.7		60
10	59	M	PS	2	Angio	0	0	0	0	1	0	0	3	0	0	0.9		54
11	57	F	SR	2	Fib	0	0	0	0	1	0	2	2	1	0	0.2		52
12	58	M	PS	2	Atypical	5	1	1	0	1	1	2	1	0	1	9.1	+	50
13	70	M	Tem. fossa	2	Atypical	4	1	1	0	1	1	2	0	1	1	6.9	+	58(D)
14	77	M	SR	2	Atypical	0	0	1	0	1	1	0	0	0	0	3.7	+	42
15	87	F	Con	2	Fib	0	0	0	0	1	0	0	1	0	0	0.8		17
16	59	F	Con	2	Atypical	0	0	1	0	1	1	0	0	1	0	0.6		14
17	75	F	PS	1	Atypical	1	1	1	1	0	1	0	1	0	0	4.3		13
18	67	M	PS	2	Atypical	0	0	1	1	0	2	0	1	0	1	1.4		8
19	49	F	SR	15	Me	2	0	0	1	0	1	2	1	0	0	4.2		150
20	68	F	SR	6	Me	1	0	0	0	0	1	2	2	1	1	2.3		121
21	60	M	PS	6	Me	0	0	0	0	0	1	2	0	1	1	0.4		98
22	63	F	SR	5	Atypical	4	1	1	0	0	1	3	1	1	1	3.8	+	94
23	43	F	PS	5	Atypical	2	1	1	1	0	1	0	1	1	0	3.5	+	83
24	53	F	SR	4	Me	0	0	0	0	0	0	0	0	1	1	0.6		81
25	70	F	Falx	5	Me	0	0	0	0	1	0	2	2	0	0	0.1		59
26	57	F	Con	7	Fib	0	0	1	0	1	0	2	1	1	0	1.2		55
27	80	M	SR	5	Me	1	0	0	0	2	1	2	2	0	1	2		43
28	61	M	Tent	7	Atypical	2	0	1	0	1	1	0	0	0	1	1.7		14
29	66	M	Con	7	Atypical	1	0	1	1	0	1	0	1	1	1	2.1		11

HPF high-power field, *N/C* nuclear/cytoplasmic, *M* male, *F* female, *Con* convexity, *PS* parasagittal, *SR* sphenoid ridge, *Tent* tentoria, *Angio* angiomatous, *Fib* fibrous, *Me* meningothelial, *Psa* psammomatous, *Trans* transitional, *D* dead

[a] 1, partial; 2, island; 3, global

Table 2 Clinicopathological features and scores of the 29 patients with meningioma in the non-POE group

No.	Age	Gender	Site	Pathology	Atypical features							Ischemic features			Ki67 index (%)	Recurrence	Follow up (months)
					Mitosis (per 10 HPF)	Brain invasion[a]	Increased cellularity[a]	Prominent nucleoli[a]	Small cell with high N/C ratios[a]	Sheet-like growth[a]	Geographic necrosis[a]	Cytoplasmic vacuoles[a]	Intercellular discohesion[a]	Perivascular cuffs[a]			
1	52	M	PS	Atypical	4	1	1	0	0	2	1	0	0	1	4.2	+	95(D)
2	77	F	SR	Fib	0	0	1	1	0	0	1	0	0	0	2.8	–	114
3	84	F	Con	Atypical	1	0	1	0	1	1	1	1	0	0	3.3	–	112
4	79	F	PS	Fib	0	0	0	0	0	0	0	0	0	0	0.7	–	155
5	67	M	SR	Angio	0	0	0	0	0	0	0	2	0	0	3.7	+	202(D)
6	48	F	PS	Me	0	0	0	0	0	1	0	0	0	0	0.2	–	83
7	36	F	SR	Me	0	0	1	0	0	2	0	0	0	0	2.6	–	13
8	53	F	Tent	Atypical	0	0	1	0	0	1	1	0	0	0	0.2	–	140
9	58	F	Con	Microcystic	0	0	0	0	0	0	0	2	0	0	0.5	–	127
10	48	M	SR	Me	0	0	0	0	0	0	0	0	0	0	2.1	–	153
11	65	M	SR	Fib	0	0	0	0	0	1	0	1	0	0	0.4	–	104
12	65	M	PS	Atypical	1	1	1	0	1	3	1	0	0	1	3.5	+	144
13	51	F	SR	Me	0	0	0	0	0	1	0	1	0	0	1.3	–	149
14	78	M	SR	Me	0	0	0	0	1	1	0	0	0	0	0.9	–	130
15	85	M	Con	Angio	0	0	0	0	0	0	0	1	0	0	0.1	–	6
16	58	F	Con	Fib	0	0	0	0	0	0	0	0	0	0	0.1	–	7
17	48	M	PS	Atypical	5	1	1	0	0	1	1	1	0	0	8.6	+	139(D)
18	64	M	Con	Me	0	0	0	1	0	1	0	1	0	0	2.1	–	60
19	50	M	SR	Me	0	0	0	0	0	0	0	0	0	0	1.7	–	88
20	82	F	SR	Me	0	0	0	0	0	0	0	0	0	0	0.6	–	103
21	46	M	PS	Fib	0	0	0	0	0	1	0	1	0	0	0.7	–	116
22	64	F	SR	Atypical	4	1	1	1	1	1	2	1	0	0	3.6	+	213(D)
23	44	F	PS	Trans	0	0	0	0	0	0	0	0	0	0	0.3	–	277
24	64	F	SR	Me	0	0	0	0	0	1	0	0	1	0	1.4	–	219
25	72	F	Falx	Fib	0	0	0	0	0	0	0	0	0	0	0.2	–	246
26	59	F	Con	Fib	1	0	0	0	0	0	1	0	0	0	2.8	+	30
27	70	F	SR	Fib	0	0	0	0	0	1	0	0	0	0	0.1	–	91
28	72	M	Tent	Me	0	0	1	0	0	1	0	1	0	0	1.1	–	105
29	38	M	PS	Atypical	5	0	1	0	1	1	2	2	0	1	4	+	101

HPF high-power field, *N/C* nuclear/cytoplasmic, *M* male, *F* female, *Con* convexity, *PS* parasagittal, *SR* sphenoid ridge, *Tent* tentoria, *Angio* angiomatous, *Fib* fibrous, *Me* meningothelial, *Trans* transitional, *D* dead

[a] 1, partial; 2, island; 3, global

Table 3 Summary of histologic subtypes, and scores of atypical and ischemic features in the POE group and in the non-POE group

	POE group (29 cases)	Non-POE group (29 cases)	*p* value
Histologic subtype(s)			
Meningothelial	9 (31%)	10 (34%)	
Transitional	1 (3%)	1 (3%)	
Angiomatous	2 (7%)	2 (7%)	
Fibrous	3 (10%)	8 (28%)	
Microcystic	1 (3%)	1 (3%)	
Psammomatous	1 (3%)	0	
Atypical	11 (38%)	7 (24%)	
Papillary	1 (3%)	0	
Mean score of atypical features			
Increased cellularity	0.45	0.34	0.43
Prominent nucleoli	0.17	0.10	0.46
Small cells with high N/C ratios	0.59	0.17	0.004*
Sheet-like growth	0.69	0.72	0.84
Geographic necrosis	0.83	0.38	0.06
Mean score of ischemic features			
Cytoplasmic vacuoles	1.0	0.52	0.02*
Intercellular discohesion	0.41	0.03	0.0005*
Perivascular cuffs	0.48	0.1	0.001*
Ki-67 index [mean ± SD (%)]	2.2 ± 2.3	1.9 ± 1.9	0.49

N/C nuclear/cytoplasmic

* Statistically significant difference ($p < 0.05$)

Discussion

Microscopic necrosis has been identified in 40–89% of previously reported specimens [3, 6–8, 11]. However, in our study, geographic necroses were observed in only 38% of embolized meningiomas with small cells, intercellular discohesion and perivascular cuffs being identified instead, and most meningiomas with geographic necrosis were island or global pattern. In previous clinicopathologic reports of embolized meningiomas, the embolization methods and embolic agents used were varied with regard to devices, embolic agent development, and treatment strategy. Embolic agents which have been used are platinum microcoils, particles such as PVA, collagen, and gelfoam, and liquids such as dehydrated alcohol [8–11]. In general, despite good embolization results in most cases, marked necrosis was uncommon and complete necrosis was never observed, despite complete elimination of the angiographic blush [11]. PVA particles are widely used in the POE of meningiomas. Small particles (45–150 μm) induce better devascularization of the meningioma with a positive effect on blood loss during surgery compared with larger particles [6, 14]. However, it has been suggested that small particles carry a higher risk of hemorrhagic and ischemic complications in the POE of meningiomas [15, 16]. In embolized meningioma treated with small particles, embolization material was visible together with necrosis, such that a comparable stage of degenerative changes in the tumor should lead to cautious grading [17]. It has been postulated that necrosis as a result of deep penetration of the particles causes the tissue to be more vulnerable to bleeding. It is possible that penetration of the particles into the draining veins of the tumor may block the outflow, increasing the risk of hemorrhage [6, 14, 15, 18]. Therefore, we used large PVA particles (150–250 μm) in the POE of meningiomas, and there were no complications concerning POE. In most of our cases with POE, embolization material was invisible in the tumors, and the large particles were believed to remain in the feeding vessels. Small cells, clear cells, rhabdoid-like cells, and pseudopapillary pattern may be associated with embolization using large PVA particles instead of development of necrosis.

POE to induce necrosis with atypical features of meningiomas, for example sheeting architecture, macronucleoli, hypercellularity, and small cells with high nuclear/cytoplasmic ratio, has discussed in relation to the potential for overgrading benign meningiomas as atypical (Grade II), possibly resulting in prognostic inaccuracies and overly aggressive therapy. Perry et al. suggested that embolized meningiomas fulfilling criteria excluding necrosis for atypical meningioma are associated with high recurrence and therefore should be treated as having an aggressive biologic potential. Furthermore, the high frequency of atypical meningiomas (41%) in embolized meningioma series is most likely to reflect patient selection

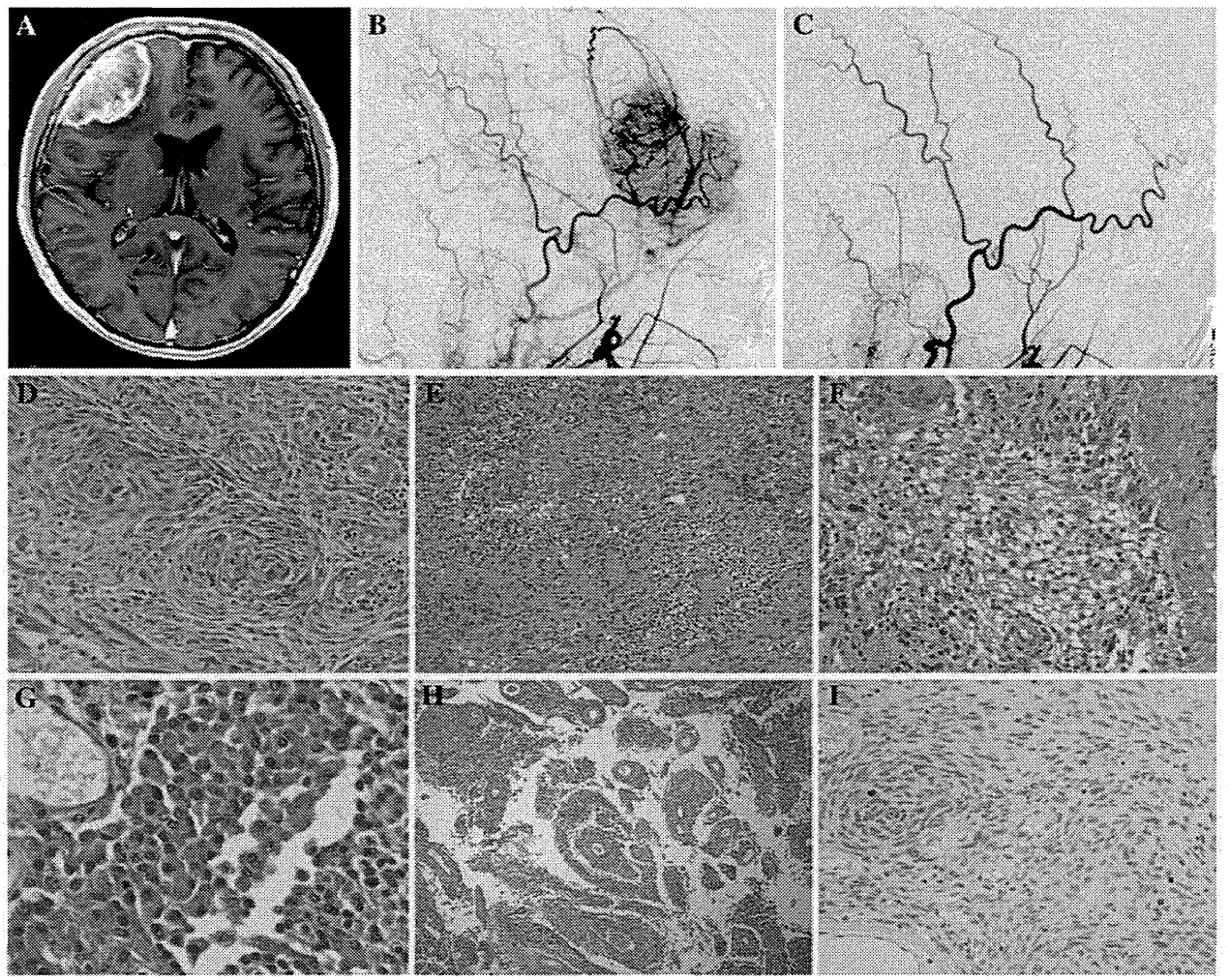

Fig. 1 Representative meningioma showing devascularization by preoperative embolization (POE). The axial contrast-enhanced T1-weighted magnetic resonance image shows an enhanced dural-based mass on the right frontal convexity (**a**). The lateral view of the preembolization external carotid angiogram shows marked staining typical of an angiographically hypervascular meningioma (**b**). The lateral view of the external carotid angiogram after embolization with PVA particles shows no residual tumor staining (**c**). Classical histologic features of meningiomas including whorl formation were identified in the tumor (×200, original magnification) (**d**). Geographic necroses were identified in the same tumor specimen. However, mitotic figures were not apparent (×200, original magnification) (**e**). Tumor cells with cleared vacuoles within the cytoplasm resemble clear cell meningioma (×400, original magnification) (**f**). Intercellular discohesion with intracytoplasmic glassy homogenous appearance resembles rhabdoid meningioma. However, there are few discrete cytoplasmic inclusions (×400, original magnification) (**g**). Tumor cells were located around the feeding vessels and disappeared far from the vessels, resembling papillary meningiomas. However, there were few findings of a perivascular orientation of tumor cells (**h**). Ki-67 immunoreactive cells were inconspicuous (Ki-67 index 2.1%) (**i**)

biases, rather than embolization artifacts [11]. Perry et al. [19] reported that the frequency of atypical meningioma was approximately 20% in nonembolized meningiomas. In our study, recurrence in patients diagnosed as atypical meningioma was reduced in the POE group (55%, and 86% in the non-POE group). In addition, high frequency of atypical subtype (38 vs. 24%) and significant increase in small cells with high N/C ratios, clear cell-like, rhabdoid cell-like, or pseudopapillary pattern in the POE group suggest that embolization effects may have the potential for overgrading benign as atypical or anaplastic in embolized meningiomas.

Small cells were frequently identified in embolized meningiomas. However, there has been little attention to small cells in previous literature. Small cells identified in embolized meningiomas may include ischemic cell change with hyperchromatic and concentrated nuclei and infiltrating inflammatory cells. In addition, it is difficult to discriminate these from each other. In our study, small cells excluding hyperchromatic small cells and inflammatory

cells also increased in embolized meningiomas. Our study suggests that small cell change resulting in necrosis may be followed by preoperative embolization in meningiomas.

Elevated Ki-67 proliferative indices have been identified in benign-appearing embolized meningiomas and relatively limited to perinecrotic foci. Therefore, proliferative activity in embolized meningiomas does not always reflect genuine tumor proliferation and should not be used to assess malignancy [9, 10]. However, in our study, Ki-67 indices of embolized meningiomas measured in nonnecrotic regions were considered to be similar to those of nonembolized meningiomas. The finding of significantly elevated proliferative indices (e.g., $\geq$4 mitoses per 10 HPF) in embolized meningiomas, particularly in nonnecrotic regions may be interpreted as evidence of a truly aggressive biologic potential rather than postembolization artifact, as suggested by Perry [11]. In our study, 6 (75%) of the 8 patients with embolized meningiomas and high Ki-67 indices had recurrent tumors. Although numbers of patients were small in this study, elevated Ki-67 index estimated in nonnecrotic regions may be also useful for predicting recurrence of embolized meningiomas.

Conclusions

Ischemic histologic features including small cells with high N/C ratios (atypical cells), cytoplasmic vacuoles (clear cell-like), intercellular discohesion (rhabdoid cell-like), and perivascular cuffs (pseudopapillary pattern) were identified in embolized meningiomas. Furthermore, when large PVA particles were used in POE, intravascular embolization material was inconspicuous. Therefore, histological findings and determination of grading should be evaluated cautiously in cases of embolized meningiomas.

References

1. Dean BL, Flom RA, Wallace RC et al (1994) Efficacy of endovascular treatment of meningiomas: evaluation with matched samples. Am J Neuroradiol 15:1675–1680
2. Grand C, Bank WO, Balériaux D et al (1993) Gadolinium-enhanced MR in the evaluation of preoperative meningioma embolization. Am J Neuroradiol 14:563–569
3. Manelfe C, Lasjaunias P, Ruscalleda J (1986) Preoperative embolization of intracranial meningiomas. Am J Neuroradiol 7:963–972
4. Nelson PK, Setton A, Choi IS, Ransohoff J, Berenstein A (1994) Current status of interventional neuroradiology in the management of meningiomas. Neurosurg Clin N Am 5:235–259
5. Richter HP, Schachenmayr W (1983) Preoperative embolization of intracranial meningiomas. Neurosurgery 13:261–268
6. Wakhloo AK, Juengling FD, Van Velthoven V, Schumacher M, Hennig J, Schwechheimer K (1993) Extended preoperative poly(vinyl alcohol) microembolization of intracranial meningiomas: assessment of two embolization techniques. Am J Neuroradiol 14:571–582
7. Nakasu S, Nakajima M, Nakazawa T, Nakasu Y, Handa J (1997) P53 accumulation and apoptosis in embolized meningiomas. Acta Neuropathol 93:599–605
8. Ng H-K, Poon W-S, Goh K, Chan MSY (1996) Histopathology of post-embolized meningiomas. Am J Surg Pathol 20:1224–1230
9. Patsouris E, Laas R, Hagel C, Stavrou D (1998) Increased proliferative activity due to necroses induced by pre-operative embolization in benign meningiomas. J Neurooncol 40:257–264
10. Paulus W, Meixensberger J, Hofmann E, Roggendorf W (1993) Effect of embolization of meningioma on Ki-67 proliferation index. J Clin Pathol 46:876–877
11. Perry A, Chicoine MR, Filiput E, Miller JP (2001) Clinicopathologic assessment and grading of embolized meningiomas: a correlative study of 64 patients. Cancer 92:701–711
12. Perry A, Louis DN, Scheithauer BW, Budka H, Von Deimling A (2007) Meningiomas. In: Louis DN, Ohgaki H, Wiestler OD, Cavenee WK (eds) WHO classification of tumours of the central nervous system. IARC press, Lyon, pp 164–172
13. Takeuchi H, Kubota T, Kabuto M, Kitai R, Nozaki J, Yamashita J (1997) Prediction of recurrence in histologically benign meningiomas, proliferating cell nuclear antigen and Ki-67 immunohistochemical study. Surg Neurol 48:501–506
14. Bendszus M, Rao G, Burger R et al (2000) Is there a benefit of preoperative meningioma embolization? Neurosurgery 47:1306–1312
15. Carli DF, Sluzewski M, Beute GN, van Rooij WJ (2010) Complications of particle embolization of meningiomas: frequency, risk factors, and outcome. Am J Neuroradiol 31:152–154
16. Kallmes DF, Evans AJ, Kaptain GJ et al (1997) Hemorrhagic complications in embolization of a meningioma: case report and review of the literature. Neuroradiology 39:877–880
17. Mawrin C, Perry A (2010) Pathological classification and molecular genetics of meningiomas. J Neurooncol 99:379–391
18. Latchaw RE (1993) Preoperative intracranial meningioma embolization: technical considerations affecting the risk-to-benefit ratio. Am J Neuroradiol 14:583–586
19. Perry A, Stafford SL, Scheithauer BW, Suman VJ, Lohse CM (1997) Meningioma grading: an analysis of histologic parameters. Am J Surg Pathol 21:1455–1465