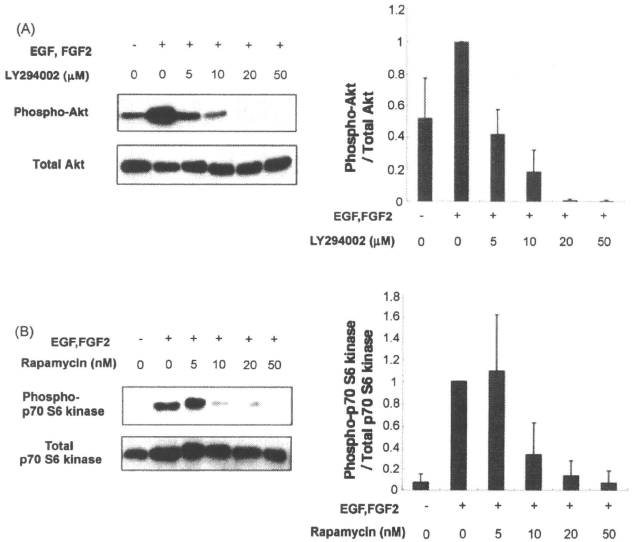


Fig. 1. Inhibition of growth factor-mediated activation of PI3K and mTOR by LY294002 and rapamycin, respectively, in neural stem/progenitor cells. Neural stem/progenitor cells derived from E14.5 mouse embryos were cultured in the absence of EGF and FGF2 for 24 h and then stimulated with the growth factors in the absence or presence of the indicated concentrations of LY294002 (A) or rapamycin (B). Cells were harvested 30 min after the growth factor stimulation and subjected to immunoblot analysis for phospho(Ser473)- and total Akt (A) and for phospho(Thr389)- and total p70 S6 kinase (B). Representative immunoblot images (Left) and results of quantitative densitometric analysis (Right) of 3 independent experiments are shown. The graphs show the means + SD of relative expression levels of phospho-Akt (A) and phospho-p70 S6 kinase (B) compared to their levels in the presence of EGF and FGF2 but in the absence of the inhibitors, which were arbitrarily set to 1.

and FGF2 (20 ng/ml), to enrich neural stem/progenitor cell population. EGF and FGF2 were added to the culture medium every day unless indicated otherwise. Under this culture condition, cell aggregates known as neurospheres were formed within 2 or 3 days after seeding, and neurospheres were mechanically dissociated and reseeded at 3–4-day intervals. After 4 or 5 rounds of this passaging procedure, the cells comprising the neurospheres (i.e., passage 4 or 5 neurospheres; Supplementary Fig. S1A, a) expressed Sox2 but no or barely detectable level of glial fibrillary acidic protein (GFAP) and βIII-tubulin (Fig. 3, lane 1; Supplementary Fig. S1B). They were also capable of generating neurons, astrocytes, and oligodendrocytes under the differentiation culture condition (Supplementary Fig. S1A, b–d), indicating that neurospheres at passages 4 and 5 are highly enriched in multipotent neural stem/progenitor cells. Neural stem/progenitor cells were maintained by culturing in the neural stem/progenitor cell culture medium unless otherwise indicated. To induce differentiation, dissociated cells from passage 4 or 5 neurospheres were cultured in the differentiation culture condition (DMEM/F12 containing 10% fetal bovine serum and PS) for defined periods.

Neurosphere formation assays were carried out according to Yoshimatsu et al. [20], with some modifications. For the primary neurosphere formation assay, passage 4 or 5 neurospheres prepared from E14.5 mouse embryos as above were completely dissociated, and the dissociated cells were suspended in the neural stem/progenitor cell culture medium containing EGF/FGF2 at a density of 5000 cells/ml. Aliquots (200 μl) were transferred to each well of 96-well plates. The numbers of primary neurospheres were counted after 3-day culture.

For the secondary neurosphere formation assay, cells cultured in the neural stem/progenitor cell culture medium containing EGF/FGF2 at a density of 2.5×10^5 cells/ml in the presence or absence of inhibitors were collected and, being washed once with the culture medium, mechanically dissociated and resuspended in the neural stem/progenitor cell culture medium containing EGF/FGF2 at a density of 5000 cells/ml. Aliquots (200 μl) of this cell suspension were then transferred to each well of 96-well plates, and the numbers of secondary neurospheres were counted after 3-day culture.

For cell growth and viability assays, cells were plated in the neural stem/progenitor cell culture medium containing EGF/FGF2 at a density of 2×10^5 cells/ml and cultured for 3 days. The cell number was then determined using a hemocytometer, and cellular viability was examined by the dye exclusion method (0.1% trypan blue).

For immunoblot analysis, cells were lysed in the lysis buffer (10 mM Tris–HCl [pH 7.4], 0.1% SDS, 1% sodium deoxycholate, 0.15 M NaCl, 1 mM EDTA, 1% protease inhibitor cocktail set III [Calbiochem]). For the analysis of phosphorylated proteins, cells were lysed in the lysis buffer supplemented with phosphatase inhibitors (20 mM β-glycerophosphate, 10 mM NaF, 0.1 mM Na_3VO_4). After determination of protein concentrations using the BCA protein assay kit (Pierce), cell lysates containing equal amounts of protein were separated by SDS-PAGE and transferred to a polyvinylidene difluoride membrane. The membrane was probed with a primary antibody and then with an appropriate HRP-conjugated secondary antibody according to the protocol recommended by the manufacturer of each antibody. Blots were visualized on X-ray films using Immobilon Western Chemiluminescent HRP Substrate

(Millipore). For densitometric analysis, developed films were scanned and band densitometry was done using Image J (National Institutes of Health). Densitometry results were normalized to total Akt for phospho-Akt, to total p70 S6 kinase for phospho-p70 S6 kinase, and to β-actin for Sox2 and GFAP.

Statistical test for difference of means between two independent groups were done using two-sided, unpaired Student's *t*-test. *P* values less than 0.05 were considered statistically significant.

Previous studies indicated that PTEN negatively regulates proliferation and self-renewal of neural stem/progenitor cells [8,9]. Since PTEN counteracts the function of PI3K, we expected that PI3K pathway activation by EGF and FGF2 may play a role in the maintenance of neural stem/progenitor cells. To test this idea, we used neural stem/progenitor cells derived from the forebrain neuroepithelium of E14.5 mouse embryo. When neural stem/progenitor cells were stimulated with EGF/FGF2 after 24 h starvation, the PI3K pathway was activated as indicated by the increased phosphorylation of Akt (which signals downstream of PI3K) (Fig. 1A). We then examined the effect of inhibiting this EGF/FGF2-mediated PI3K activation on neural stem/progenitor cells using a PI3K inhibitor LY294002. In neural stem/progenitor cells, LY294002 inhibited PI3K in a concentration-dependent manner: it inhibited EGF/FGF2-mediated PI3K activation down to the basal (unstimulated) level at 5 μM and almost completely at 20 μM (Fig. 1A). We next tested its effect on the growth and survival of neural stem/progenitor cells. Significant growth inhibition and marginal reduction of viability were observed when cells were treated with 5 μM LY294002 (Supplementary Fig. S2), suggesting that intact PI3K activity may be required for full proliferation and survival of neural stem/progenitor cells. We also asked whether the activation of PI3K by EGF/FGF2 is required for maintaining the stem cell state of neural stem/progenitor cells *per se*, i.e., the self-renewal capacity. For this purpose, we conducted neurosphere formation assay to monitor the self-renewal capacity of neural stem/progenitor cells [7,13] and also checked the expression levels of the stem cell marker Sox2 as well as the differentiation markers such as GFAP (astrocyte marker) and βIII-tubulin (neuronal marker) [1,4,10,20]. Contrary to our expectation, LY294002 neither inhibited formation of primary and secondary neurospheres by neural stem/progenitor cells (Fig. 2) nor altered the expression levels of Sox2, GFAP, and βIII-tubulin (Fig. 3, lane 2). These results suggested that neural stem/progenitor cells maintain their capacity to self-renew even under a PI3K-inhibited condition. Of note, these experiments were done at a concentration of LY294002 (5 μM), which had minimal negative effect on cell survival, since reduced viability could cause artificial reduction of neurospheres in the absence of reduced self-renewal. Nevertheless, the failure of LY294002 at 5 μM to inhibit self-renewal was unlikely to be attributed to its incomplete inhibition of the PI3K activity, since LY294002 failed to reduce the expression of Sox2 even at higher concentrations (Supplementary Fig. S3A) where it completely blocked the activity of PI3K (Fig. 1A). Thus, PI3K activation by EGF/FGF2 appeared to be required for the growth of neural stem/progenitor cells but may not be essential for the maintenance of their stem cell state.

Recent studies demonstrated that combinatorial inhibition of PI3K and mTOR inhibits proliferation and survival of glioma cells more effectively than inhibition of either alone [5,16], suggesting the possibility that PI3K and mTOR could function independently and complement each other with respect to the expression of certain phenotypes. We therefore surmised that a similar relationship between PI3K and mTOR might be operative in the maintenance of the stem cell state of neural stem/progenitor cells and examined the effect of inhibiting mTOR alone or in combination with PI3K inhibition on neural stem/progenitor cells. As shown in Fig. 1B, the specific mTOR inhibitor rapamycin efficiently inhibited the activation of mTOR by EGF/FGF2 at 10 nM and higher concentrations, which was monitored by the phosphorylation status of Thr389 of p70 S6 kinase (downstream marker of mTOR signaling). We therefore tested the effect of rapamycin at concentrations between 10 nM and 50 nM in the subsequent assays. At 20 nM, rapamycin significantly retarded growth and marginally reduced cell viability, suggesting that mTOR is also required for efficient proliferation and survival of neural stem/progenitor cells (Supplementary Fig. S2). We then tested the effect of rapamycin on the capacity of neural stem/progenitor cells to self-renew. Similar to LY294002, rapamycin had no apparent effect on neurosphere formation (Fig. 2) and on the expression levels of Sox2, GFAP, and βIII-tubulin (Fig. 3 and Supplementary Fig. S3B). Thus, the results suggest that mTOR inhibition alone, though sufficient to inhibit the growth of neural stem/progenitor cells, does not affect the stem cell state of neural stem/progenitor cells.

Given that inhibition of PI3K or mTOR alone had no effect on the stem cell state of neural stem/progenitor cells, we next examined the effect of blocking PI3K and mTOR at the same time. Concomitant use of LY294002 and rapamycin inhibited, though modestly, the formation of primary neurospheres (Fig. 2B). Because the combination of these inhibitors also caused small but significant reduction in the viability of neural stem/progenitor cells (Supplementary Fig. S2B), it was formally possible that the inhibition of primary neurosphere formation was secondary to the reduced viability. However, strikingly, inhibition of neurosphere formation became even more pronounced when the cells treated with the combination of the two inhibitors for 3 days were subjected to secondary neurosphere formation assay in their "absence" (Fig. 2C). Thus, the results strongly suggested that the 3-day treatment with the inhibitors irreversibly compromised the capacity of neural stem/progenitor cells to self-renew. Consistent with this idea, Sox2 expression apparently decreased after 3-day combinatorial treatment with LY294002 and rapamycin, accompanied by parallel increase of GFAP but not βIII-tubulin (Fig. 3). Furthermore, whereas treatment with either LY294002 or rapamycin alone only reduced the size of neurospheres (but not the formation/number of neurospheres) in line with their negative effect on neural stem/progenitor cell proliferation, concomitant treatment with these two inhibitors caused neural stem/progenitor cells to adhere to the culture dish and extend cellular processes, which is a morphological alteration characteristic of differentiating cells (Supplementary Fig. S4). Altogether, these lines of evidence suggest that neural stem/progenitor cells exit the stem cell state and become committed to differentiation into the astrocytic lineage once both PI3K and mTOR are inhibited but not when either is active.

In this study, we investigated the mechanism by which neural stem/progenitor cells are maintained by EGF/FGF2. Using neural stem/progenitor cells derived from mouse embryo forebrain, we demonstrated that both PI3K and mTOR are activated by EGF/FGF2 and that concurrent, but not individual, inhibition of PI3K and mTOR effectively reduces the ability of neural stem/progenitor cells to self-renew, making them committed to differentiation into the astrocytic lineage. Notably, we also observed that the activities of PI3K and mTOR drop sharply when neural stem/progenitor cells were induced to undergo differentiation in the differentiation culture condition in the absence of EGF/FGF2 (Supplementary Fig. S5). Together, these results suggest that activation of two independent intracellular signals by EGF/FGF2 – one through PI3K and the other through mTOR – may play a crucial role in maintaining the undifferentiated, stem cell state and that the maintenance may be achieved in a complementary manner by the two independent, parallel signaling pathways.

It remains to be shown at the molecular level how PI3K and mTOR complement each other in maintaining the stem cell state of neural stem/progenitor cells. However, intriguingly, we found

in the course of this study that the phosphorylation status of 4E-BP1, another well-characterized substrate for mTOR in addition to p70 S6 kinase [14], is regulated in a similar manner to the stem cell state in neural stem/progenitor cells. In sharp contrast to p70 S6 kinase, whose phosphorylation at Thr389 was clearly inhibited by rapamycin alone at 10 nM (Fig. 1B), phosphorylation of 4E-BP1 at Thr37/46 was only partially inhibited by rapamycin even at 50 nM (Supplementary Fig. S6). However, when neural stem/progenitor cells were treated with rapamycin concomitantly with LY294002, which alone similarly inhibited phosphorylation of 4E-BP1 only partially, 4E-BP1 phosphorylation was dramatically reduced (Supplementary Fig. S6). This result suggests that, at least in the neural stem/progenitor cells used in this study, inhibition of mTOR alone may not be sufficient to efficiently reduce phosphorylation of 4E-BP1 and that PI3K can maintain 4E-BP1 phosphorylation in mTOR-independent manner. This is apparently at variance with a previous work demonstrating that either rapamycin or LY294002 alone was sufficient to inhibit phosphorylation of 4E-BP1 in HEK293 cells, which was consistent with a model of linear signaling pathway leading from growth factor receptor through PI3K to mTOR [6]. Thus, the mode of regulation of 4E-BP1 phosphorylation by PI3K and mTOR may depend on cell type, and the results of this study suggest that a parallel rather than a linear signaling pathway model may be operative in neural stem/progenitor cells in which PI3K and mTOR function in a complementary manner to phosphorylate 4E-BP1 (Supplementary Fig.

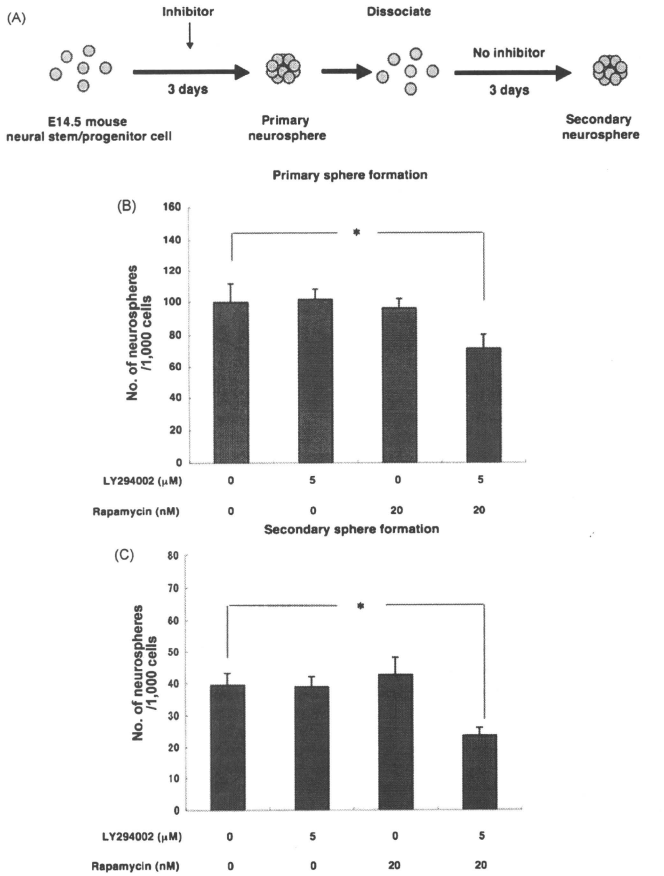
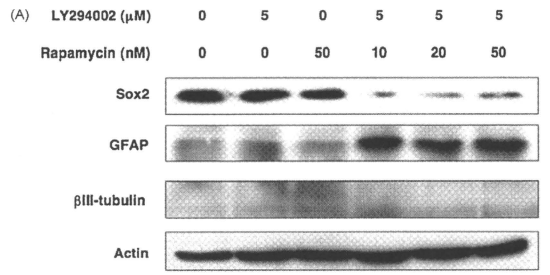


Fig. 2. Effect of PI3K and mTOR inhibition on self-renewal of neural stem/progenitor cells. (A) Schematic presentation of the experimental procedure. (B and C) Effect of PI3K and mTOR inhibition on primary (B) and secondary (C) neurosphere formation. Neural stem/progenitor cells from E14.5 mouse embryos were subjected to neurosphere formation assay in the absence or presence of the indicated inhibitors. Note that, whereas primary neurospheres were formed in the presence of the indicated inhibitors, secondary neurospheres were formed in their absence. The data in (B) and (C) are expressed as means + SD of 3 independent experiments. *$P < 0.05$.



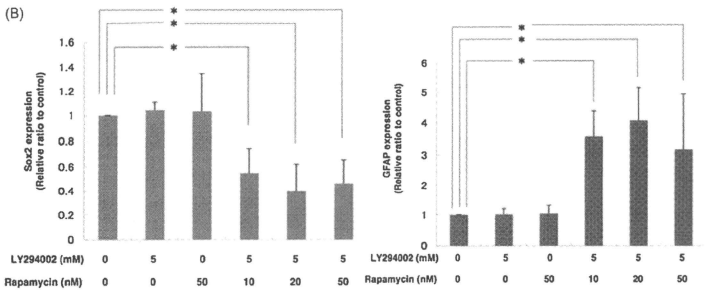


Fig. 3. Effect of PI3K and mTOR inhibition on Sox2, GFAP, and βIII-tubulin expression in neural stem/progenitor cells. Neural stem/progenitor cells derived from E14.5 mouse embryos cultured in the absence or presence of the indicated inhibitors for 3 days were subjected to immunoblot analysis for Sox2, GFAP, and βIII-tubulin. The actin blot is for loading control and normalization in densitometric analysis. Representative immunoblot images (Upper) and results of quantitative densitometric analysis (Lower) of 3 independent experiments are shown. The graphs show the means + SD of relative expression levels of Sox2 (Lower left) and GFAP (Lower right) compared to their levels in the absence of both inhibitors, which were arbitrarily set to 1. $^*P < 0.05$.

S7). Given this similarity of regulation – parallel and complementary regulation by PI3K and mTOR – of stem cell state and 4E-BP1 phosphorylation in neural stem/progenitor cells, it is speculated that some molecule(s) regulated similarly to 4E-BP1 (or 4E-BP1 itself) is likely involved in the regulation of the stem cell state of neural stem/progenitor cells.

Previous studies using *Pten* conditional knockout mice clearly demonstrated increased self-renewal capacity of PTEN-deficient neural stem/progenitor cells [8,9], suggesting the possibility that supra-physiological activation of the PI3K pathway may have contributed to the increase of self-renewal capacity. Nevertheless, to date, whether and how PI3K is involved in the regulation of the stem cell state of neural stem/progenitor cells under physiological conditions (e.g., in genetically unmodified neural stem/progenitor cells) has not been demonstrated and therefore remains largely elusive. One reason for this apparent lack of demonstration may be the redundancy of regulation we have disclosed in this study—parallel activation of mTOR could mask the phenotype change that would otherwise become manifest after PI3K inhibition, thus making it difficult to delineate the potential involvement of PI3K. Indeed, in one study [20], treatment of neural stem/progenitor cells with LY294002 failed, just as in our study, to inhibit secondary neurosphere formation, in line with the idea that transient inhibition of PI3K alone is not sufficient to inhibit self-renewal of neural stem/progenitor cells. On the other hand, another study demonstrated inhibition of neurosphere formation by rapamycin [15]. Although the effect of rapamycin on the expression of neural stem cell markers remains to be shown in that study, the results are essentially consistent with ours and together support the idea that mTOR plays a role in the maintenance of the stem cell state of neural stem/progenitor cells. A major and important discrepancy between the study and ours is that rapamycin alone was sufficient to inhibit neurosphere formation in that study but not in ours. Presumably, the most simple and plausible explanation reconciling this discrepancy may be that PI3K was not sufficiently activated in the neural stem cell culture condition of that study, rendering the stem cell state largely dependent on mTOR. In support of this idea, EGF and FGF2 were used at relatively lower concentrations in that study than conventionally used [7,13].

In conclusion, we have revealed in this study not only that PI3K and mTOR activated by EGF and FGF2 contribute to the maintenance of the stem cell state of neural stem/progenitor cells but that they do so independently of each other and in a complementary manner. Our findings thus shed a new light on the novel regulatory mechanism of neural stem/progenitor cell maintenance and could help understand how the fate of neural stem/progenitor cells is controlled during development of the nervous system.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neulet.2009.12.067.

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ORIGINAL ARTICLE

Association of stem cell marker CD133 expression with dissemination of glioblastomas

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Abstract Dissemination of glioblastoma was once considered rare but is now increasingly encountered with longer survival of glioblastoma patients. Despite the potential negative impact of dissemination on clinical outcome, however, molecular markers useful for prediction of dissemination risk still remains ill defined. We tested in this study for an association between the expression of stem cell marker CD133 and the risk of dissemination in 26 cases of glioblastoma (16 with dissemination and 10 without dissemination). The protein expression of CD133 was examined by western blot analysis of tumor specimens, and the CD133 expression levels were quantified by densitometry and normalized to β-actin. The results indicated that CD133 expression levels are significantly higher in glioblastomas with dissemination (mean 10.3, range 0.20-27.8) than in those without (mean 1.18, range 0.07-3.58). The results suggest that CD133 could be a molecular predictor of glioblastoma dissemination, and also give rise to an intriguing idea that CD133-positive cancer stem cells may be implicated in the initiation of disseminated lesions.

Keywords Glioblastoma · Dissemination · Cancer stem cell · CD133

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Introduction

Malignant gliomas are the most common and intractable primary neoplasms of the central nervous system. Despite aggressive treatments, recurrence is inevitable and fatal in most cases of malignant glioma. Recurrent tumors usually arise locally close to the primary tumor site, but they occasionally appear as disseminated lesions at sites distant from the primary tumor [1, 3, 9, 12, 19, 23, 24, 35, 37, 44]. The incidence of disseminating malignant gliomas, which was once considered rare [44], ranges from 8% to 27% [1, 9, 23, 24] and reaches as high as 44% in one recent report, which could be ascribed to longer survival of the patients

analyzed [17]. Given the apparent negative impact of dissemination on survival of patients with malignant gliomas [1, 24], control of dissemination will have increasingly greater clinical significance as progress is made in local control of the tumors. Understanding the biology that underlies dissemination would help improve diagnosis and treatment of disseminated tumors, yet information on genetic and/or molecular aspects of dissemination is still very limited [2, 15, 17, 18, 21].

The cancer stem cell hypothesis holds that tumors are composed of a rare subpopulation of cancer stem cells having the ability to self-renew indefinitely and initiate tumor formation and of the other majority of tumor cells having limited ability to divide and therefore incapable of initiating tumor formation [25]. Recent studies have documented existence of such cancer stem cells in several tumor types including gliomas [10, 27, 34, 38], and the cell surface antigen CD133 has been established as a useful marker molecule for identifying cancer stem cells of glioma (glioma stem cells) [31]. CD133 is expressed preferentially in glioma stem cells, and the $CD133^+$ but not the $CD133^-$ population of glioma has been shown to retain the ability to self-renew and, upon orthotopic transplantation into immunodeficient mice, initiate formation of tumor that recapitulates the characteristics of the original tumor from which it is derived [4, 28–30, 43]. According to this cancer stem cell hypothesis, the cells that give rise to disseminated lesions should be "cancer stem cells" capable of initiating tumors, since those cells are presumed to be individual (single) tumor cells migrating away from the primary tumor to the distant sites of dissemination. If this is actually the case, then, it is expected that the proportion of the cancer stem cell population within primary tumors would be a critical factor determining the chance of developing dissemination.

In this study, in an attempt to identify molecular predictors of dissemination, we investigated whether the expression level of CD133, which is expected to reflect the proportion of glioma stem cells within a tumor, is associated with development of dissemination in glioblastoma cases.

Materials and methods

Patient population

The subjects of this study were patients with newly diagnosed glioblastoma multiforme (GBM) who were treated at the neurosurgical departments of the participating institutions and for whom snap-frozen samples of the primary tumor available for the following expression analysis have been obtained upon informed consent. The subject patients consisted of two groups: "patients with dissemination" and "patients without dissemination". "Patients with dissemination" were those who had evidence of dissemination documented on magnetic resonance (MR) imaging during the clinical course. "Patients without dissemination" were those who survived 12 months or longer without any evidence of dissemination on MR images taken during the course. Dissemination was defined as the appearance of an enhanced nodule(s) and/or diffuse enhancement of the leptomeningeal space at sites distant from (i.e., not contiguous to) the primary tumor location on T_1-weighted MR images with contrast enhancement. Patients were treated according to each institution's protocol, and outpatient follow-up was done at 1-2 month intervals. MR examinations were conducted at least every 2-3 months.

Clinical data acquisition

Clinical records including the MR images were reviewed for each patient. We recorded patient age at diagnosis, sex, pathological diagnosis, location of the primary tumor, extent of surgical resection, and history of radiation therapy and chemotherapy. The extent of resection was described as total (100% resected), subtotal (95% $\leq$, <100% resected), partial (5% $\leq$, <95% resected), and biopsy. The date of primary diagnosis, the date dissemination was detected, final outcome, the date of death (the date of the latest clinical follow-up for living patients) were recorded to calculate time interval between primary diagnosis and dissemination (= timing of dissemination) and overall survival.

Western blot analysis of CD133 expression

Samples from the main body of the primary tumors, which were snap frozen in liquid nitrogen at the time of tumor resection and stored at −80°C until use, were lysed in the lysis buffer (62.5 mM Tris-HCl pH 6.8, 2% sodium dodecyl sulfate (SDS), 10% glycerol) and sonicated. After determination of protein concentration using the BCA protein assay kit (Pierce), cell lysates were separated by SDS-polyacrylamide gel electrophoresis (PAGE) and transferred to a polyvinylidene difluoride membrane. The membrane was, after being blocked in Tris-buffered saline with 5% nonfat dry milk, probed with anti-CD133 primary antibody (W6B3C1, Milteny Biotechnology, 1:3,000) and then with an anti-mouse horseradish peroxidase (HRP)-conjugated secondary antibody (Santa Cruz Biotechnology, 1:2,000) using Can Get Signal (Toyobo). The membrane was also probed with anti-β-actin (Sigma, 1:6,000 in phosphate-buffered saline-0.1% Tween 20 [PBS-T] with 5% nonfat dry milk) and then with an anti-mouse HRP-conjugated secondary antibody (Santa Cruz Biotechnology, 1:3,000 in PBS-T with 5% nonfat milk). Blots were visualized on X-ray films using Immobilon Western Chemiluminescent HRP Substrate (Millipore).

Developed films were scanned and band densitometry was done using Image J (National Institutes of Health).

CD133 densitometry results were normalized to β-actin. A positive control sample (patient ID: D4) was always run on each gel and used as a standard for comparison of results from different gels.

Statistical analysis

Mann-Whitney *U* test was used to test for statistically significant difference of CD133 expression and age between two independent groups. Chi-square test and Fisher's exact probability test were used to examine the difference of sex, tumor location, and initial therapies (the extent of surgical resection, with or without radiation and chemotherapy). Correlation of CD133 expression with timing of dissemination and overall survival was assessed using the nonparametric Spearman rank correlation assay (correlation coefficient: r_s). Two-sided values of $P<0.05$ were considered statistically significant.

Results

Patient characteristics

A total of 26 GBM patients were available for analysis in this study, and their baseline characteristics are summarized in Table 1. Of the 26 patients analyzed, 16 were patients with dissemination, and 10 were without dissemination. Of the 16 patients, with dissemination, 14 were adult (>17 years old) and two were pediatric patients, with the median age of 58.5 years (range 8–74 years). The location of the primary tumor was spinal in one patient and supratentorial in the other 15. Of the 10 patients without dissemination, all were adult, with the median age of 54 years (range 44-74 years). The location of the primary tumor was supratentorial in all patients without dissemination. In principle, both patients with and without dissemination received initial therapies consisting of surgical resection, radiation therapy, and chemotherapy. There were no statistically significant differences in age, sex, tumor location, and the initial therapies (the extent of surgical resection, whether or not accompanied by radiation and chemotherapy) between the two groups. The median overall survival was 14 months (range 5-129 months) for patients with dissemination and 29.5 months (range 12-88 months) for those without.

CD133 expression in GBMs with and without dissemination

To investigate CD133 expression, we subjected samples of the primary tumors from patients with and without dissemination to western blot analysis. Varying levels of CD133 expression was observed in tumors from patients with dissemination, ranging from very high levels to nearly undetectable. In contrast, the expression levels of CD133 in patients without dissemination were uniformly low: CD133 was almost undetectable in the majority of patients, and was at most barely detectable even in patients with highest expression (Fig. 1). We then quantified CD133 expression by densitometry. Since inevitable inclusion of red blood cells within the tumor samples made determination of protein concentration somewhat inaccurate, CD133 expression level was normalized to β-actin level, an internal control for protein loading. The levels of CD133 expression after normalization to β-actin (CD133/β-actin ratio) were remarkably higher in patients with dissemination (mean 10.3, range 0.20-27.8, $n=16$) than in those without dissemination (mean 1.18, range 0.07-3.58, $n=10$). This difference of CD133/β-actin levels was statistically significant ($P<0.05$, Fig. 2a), and the difference remained significant even when the analysis was limited to adult GBM patients with (mean 9.48, range 0.20–22.7, $n=14$) and without (mean 1.18, range 0.07–3.58, $n=10$) dissemination ($P<0.05$, Fig. 2b).

CD133 expression and timing of dissemination

The median time interval between primary diagnosis and dissemination (timing of dissemination) for 16 patients with dissemination was 8 months (range 0–107 months) (Table 1). Dissemination occurred within 1 year after primary diagnosis in 10 patients (62.5%) and later than 1 year in six (37.5%). We examined whether there is any correlation between timing of dissemination and CD133 expression in primary tumors, and found positive correlation between the two parameters ($r_s=0.67$, $n=16$), which was statistically significant ($P<0.05$, Fig. 3).

CD133 expression and overall survival in adult GBMs with dissemination

To test whether CD133 expression in the primary tumor is associated with overall survival of patients independent of dissemination, we examined if any correlation exists between the two parameters among patients with dissemination. To exclude confounding factors and conduct analysis on a uniform group of patients, analysis was limited to adult GBMs, and patients who were alive at the time of latest follow-up were censored. The result indicated that there is no significant correlation between the level of CD133/β-actin ratio and overall survival in patients with dissemination ($r_s=0.55$, $n=10$; Fig. 4a). When the patients with dissemination were grouped into those who survived 12 months or longer and those who survived less than 12 months, the mean CD133/β-actin ratio was higher in the former group than in

Table 1 Baseline clinical characteristics of study subject

Patient ID	Age	Sex	Histopathology	Location	Initial therapies			Dissemination	TOD (months)	Outcome	OS (months)
					Surgery	Radiation (Gy)	Chemotherapy				
D1	13	F	GBM	Lt. F	Total	Local 60	CDDP, VP-16	+	47	Alive	56
D2	69	F	GBM	Rt. P-O	Total	Local 60	ACNU iv	+	18	Dead	25
D3	59	M	GBM	Lt. O	Total	Local 60	ACNU iv	+	8	Dead	12
D4	67	F	GBM	Rt. T	Total	Local 60	ACNU iv	+	14	Dead	16
D5	64	M	GBM	Lt. T	Total	Local 60	CDDP, VP-16, INF-β	+	8	Dead	10
D6	45	F	GBM	Rt. F	Total	Local 60	ACNU iv	+	8	Dead	10
D7	71	M	GBM	Rt.P-O	Partial	Local 60	–	+	4	Dead	6
D8	58	F	GBM	Lt. T	Total	Local 60	–	+	107	Dead	129
D9	17	M	GBM	Lt. O	Total	Local 60	ACNU iv	+	18	Alive	46
D10	55	F	GBM	Rt. F-T	Partial	Local 60	ACNU iv	+	12	Alive	29
D11	8	F	GBM	Spinal	Partial	Local 44	CDDP,VP-16,IFM	+	0	Dead	5
D12	69	F	GBM	Lt. T	Partial	Local 60	ACNU iv	+	0	Alive	26
D13	74	M	GBM	Blt. F	Partial	Local 60	ACNU iv	+	8	Alive	9
D14	61	M	GBM	Rt. T	Total	Local 60	MTX it	+	6	Dead	10
D15	57	F	GBM	Lt. F	Subtotal	Local 60	ACNU iv	+	5	Dead	7
D16	49	M	GBM	Rt. T	Total	Local 60+24	ACNU ia, MTX it	+	5	Dead	17
ND1	52	F	GBM	Rt. P	Total	Local 60	ACNU iv	–	–	Dead	15
ND2	52	M	GBM	Lt. F	Total	Local 60	ACNU iv	–	–	Dead	12
ND3	74	M	GBM	Rt. F	Total	Local 60	ACNU iv	–	–	Dead	21
ND4	62	F	GBM	Lt. F	Total	–	–	–	–	Dead	40
ND5	56	F	GBM	Lt. T	Subtotal	Local 60	ACNU iv,INF-β	–	–	Dead	45
ND6	72	F	GBM	Rt. F	Partial	Local 60	ACNU iv	–	–	Dead	19
ND7	50	M	GBM	Lt. insular	Partial	Local 60	ACNU iv	–	–	Alive	24
ND8	51	M	GBM	Rt. F	Total	Local 60	ACNU iv	–	–	Alive	88
ND9	44	M	GBM	Rt. insulo-operculum	Subtotal	Whole 30+Local 30	ACNU iv	–	–	Alive	70
ND10	64	M	GBM	Rt. hippocampus	Total	Local 60	–	–	–	Alive	35

GBM glioblastoma, *TOD* timing of dissemination, *OS* overall survival, *F* denotes frontal, *P-O* parieto-occipital, *O* occipital, *T* temporal, *F-T* fronto-temporal, *T-O* temporo-occipital, *P* parietal, *Lt* left, *Rt* right, *Blt* bilateral, *iv* intravenous injection, *ia* intraarterial injection, *it* intrathecal injection

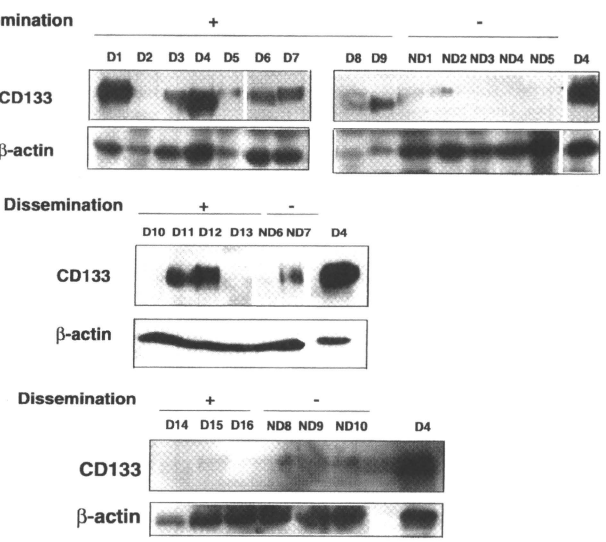

Fig. 1 Western blot analysis of CD133 expression in primary tumor samples from glioblastoma patients with and without dissemination. Primary tumor samples from patients with (D1–D16) and without (ND1–ND10) dissemination was analyzed by western blotting for CD133 expression. Actin blots show the relative amount of protein loaded onto each lane

the latter (13.9 for those who survived ≥12 months and 7.1 for those who survived <12 months), although the difference was not statistically significant (Fig. 4b).

Discussion

In this study, to explore the possibility that CD133, a glioma stem cell marker, could be a molecular predictor of dissemination, we investigated whether there is significant association between CD133 expression in the primary tumors from GBM patients and development of dissemination. The results clearly indicated that CD133 expression levels assessed by western blot analysis are significantly higher in patients with dissemination than in those without, suggesting that CD133 protein expression may be a useful indicator of dissemination risk in GBM cases. The findings also prompt us to put forward a novel hypothesis that CD133-positive glioma stem cells could be a potential source/seed of dissemination.

We analyzed in this study, for patients without dissemination, only those who survived 12 months or longer without the evidence of dissemination. This inclusion criterion was added for the purpose of preventing "potentially disseminating cases (cases in which dissemination would have occurred if the patient had survived longer)" from being included in the group of patients without dissemination. The median or mean time interval between primary diagnosis and dissemination falls within 6-12 months in the majority of previous studies [17, 24]; summarized in Ref. [39], which was also the case in this study. We therefore expect that, with this inclusion criterion, we could have substantially reduced, albeit not totally eliminated, the risk of including potentially disseminating cases in the group of patients without dissemination. As one potential drawback of this inclusion criterion, it is possible that CD133 expression levels are low in patients without dissemination in this study just because we have selected patients with favorable prognosis, which may be associated with low CD133 expression. However, this is unlikely to be the case for two reasons. First, no significant correlation was observed between CD133 expression level and overall survival when the analysis was conducted on patients (adult GBMs) with dissemination, suggesting that CD133 expression may not be a prognostic factor of overall survival independent of dissemination (Fig. 4a). Of note, when we divided the patients with dissemination into two groups—those surviving 12 months or longer and those surviving less than 12 months—the CD133 expression levels tended to be even higher in the former group than in the latter (Fig. 4b). Thus, it seems unlikely that we have artificially selected patients with low CD133 expression by limiting the analysis of patients without dissemination to those who survived 12 months or longer. Second, in line with our finding, a recent study failed to show significant association between CD133 expression and overall survival

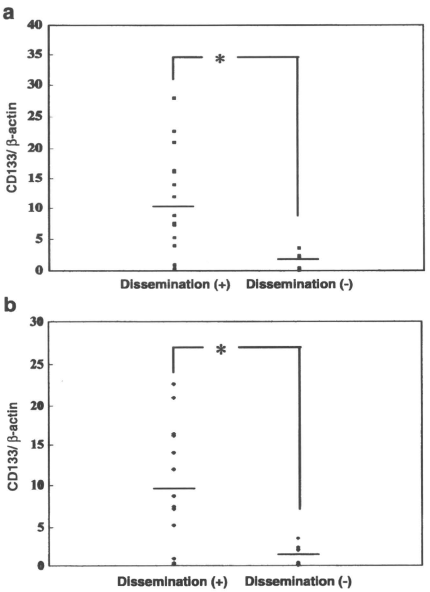

Fig. 2 Association between dissemination and CD133 expression in glioblastoma patients. CD133/β-actin ratio of primary tumors from patients with (dissemination +) and without (dissemination −) dissemination. *Bars* indicate mean values. **a** All subjects (16 patients with dissemination and 10 without dissemination) were included in the analysis. **b** Analysis was limited to adult patients (14 with dissemination and 10 without dissemination); $^{*}P<0.05$

of 72 adult GBM patients [7]. Although another report, in which a series of grade 2-4 astrocytoma patients were analyzed, suggested that CD133 expression may be associated with overall survival of astrocytoma patients, it remains to be shown in that study whether it holds true if the analysis is limited to grade 4 (i.e., GBM) patients [45]. Together, these observations are in support of the idea that CD133 expression may be associated with dissemination rather than with overall survival of GBM patients. It may, however, deserve emphasizing here that association of CD133 expression with dissemination does not necessarily preclude its association with overall survival. Given that CD133 is a marker of glioma stem cells that represent only a small fraction within the entire tumor, the association between CD133 expression and overall survival would become more apparent as the remaining majority of tumor cells, i.e., the non-stem cell population, becomes better controlled in the future.

Whereas higher CD133 expression is thus associated with increased risk of dissemination, the results of this study also suggested that it is not associated with "shorter" interval between primary diagnosis and dissemination. This could be naturally understood if we assume that the timing of dissemination mainly depends on the time required for formation of tumor mass, for which the intrinsic growth properties of tumor cells, irrespective of whether they are stem cells or not, are considered to be key determinants. In line with this idea, higher MIB-1 labeling index has been associated with shorter time interval before development of dissemination in patients with disseminating GBM [17].

Recently, we have reported that nestin expression may be associated with dissemination of central nervous system (CNS) germ cell tumors [26]. Although it remains to be shown whether cancer stem cells exist in CNS germ cell tumors and whether nestin expression, presumed to be a marker for multi-lineage progenitor cells [41], could also be a marker for such cancer stem cells of CNS germ cell tumor, the association of nestin expression and dissemination suggests the possibility that cancer stem cells are the potential source of dissemination in CNS germ cell tumors. Thus, the results of the present study, in conjunction with this earlier report of ours [26], support the emerging hypothesis that brain tumor stem cells may play an important role in brain tumor dissemination within the CNS. Although, in the present study, the exact identity of the cells expressing CD133 remains to be determined, demonstrating the expression of stem cell markers including CD133 by tumor cells in future immunohistochemical studies would help further validate this hypothesis. In this respect, nestin has been classically regarded as a marker for glioma stem cell, and nestin immunohistochemistry has

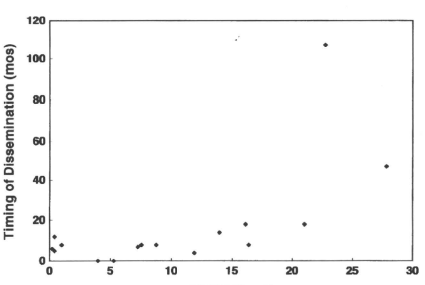

Fig. 3 Relationship between CD133 expression level and timing of dissemination in glioblastoma patients. A scatter plot of time interval between primary diagnosis and dissemination (timing of dissemination) in relation to CD133/β-actin ratio in 16 glioblastoma patients with dissemination. Correlation coefficient, $r_s=0.67$, $n=16$, $P<0.05$

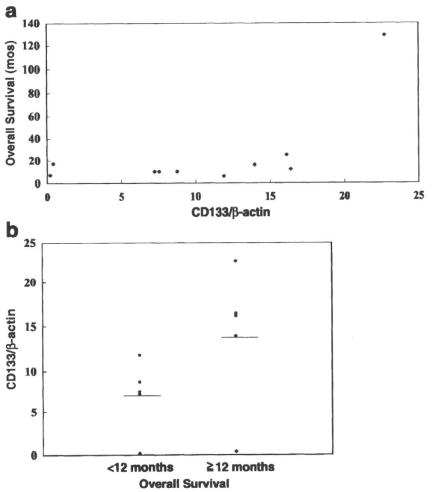

Fig. 4 Relationship between CD133 expression level and overall survival of adult glioblastoma patients with dissemination. **a** A scatter plot of overall survival in relation to CD133/β-actin ratio in adult glioblastoma patients with dissemination. Correlation coefficient, r_s= 0.55, n=10, P>0.05. **b** CD133/β-actin ratio in adult glioblastoma patients who survived 12 months or longer (≥12 months) and less than 12 months (<12 months). *Bars* indicate mean values. In **a** and **b**, live patients at the time of latest follow-up were censored from the analysis. Consequently, a total of 10 patients were analyzed

been well established [8]. We therefore conducted a pilot immunohistochemical analysis of our GBM samples for nestin expression and found that the level of nestin expression in tumor cells is indeed higher in primary tumors from patients with dissemination than in those from patients without dissemination (unpublished data, the Tohoku Brain Tumor Study Group). However, the difference of nestin expression was much less pronounced compared with that of CD133 demonstrated in this study, with substantial overlap of nestin expression levels in the two groups. This could be explained by the idea that nestin is a less specific marker for stem cells than CD133 is, given the recent reports suggesting that nestin expression may not be restricted to stem cells [6, 20].

The results of the present study suggest that stem cell marker CD133 may be a novel molecular marker for dissemination of GBM. Our data clearly indicated that there is little overlap of CD133 expression between the two groups of patients with and without dissemination (Fig. 2). Importantly, whereas the expression levels of CD133 in patients with dissemination somewhat varied, those in patients without dissemination were uniformly low. This finding implies that, although low CD133 expression may not exclude the possibility of dissemination, high levels of CD133 expression may be associated with a high risk of dissemination. Thus, CD133 expression could become a useful predictor for selective identification of patients at high risk of dissemination. On the other hand, some tumors in this study disseminated despite low CD133 expression. This could be explained by the recent observations that there may exist a distinct class of glioma stem cells that do not express CD133 [5, 16, 22, 40]. To date, several clinical parameters such as young age, male sex, incomplete tumor removal, multiple resections, ventricular entry, and proximity of the tumor to the ventricular system, have been suggested as possible risk factors of dissemination [1, 3, 12, 19], but their significance as predictors of dissemination still remains to be shown. As for genetic and molecular markers of dissemination, gain at the *1p36* chromosomal region [18], *PTEN* mutation [15, 17], and tissue inhibitor of metalloproteinase 2 (TIMP-2) expression [21] have been associated with dissemination. Although the significance of these genetic/molecular markers as risk factors of dissemination also remains to be established, they could become a useful predictor, because the incidence of these genetic abnormalities (*1p36* gain, *PTEN* mutation) and the expression level of TIMP-2 in patients with dissemination were markedly higher than in control patients. Both increased TIMP-2 expression and inactivation of PTEN by mutation are presumed to contribute to dissemination via promotion of glioma cell migration/invasion away from the primary tumor, an essential step in the process of dissemination [17, 21]. However, it would be intriguing to speculate that *PTEN* mutation also contributes to dissemination by increasing the population of glioma stem cells within the primary tumors, given the observations that PTEN negatively regulates the population size of neural stem cells which are considered to share characteristic stem cell properties with glioma stem cells [13, 14]. Aside from whether and how these genetic and molecular markers are involved in dissemination, these markers are expected to help predict the risk of dissemination and their significance as predictors of dissemination will be tested and verified in future studies.

Currently, radiation therapy and intrathecal chemotherapy are the major treatment options for disseminated lesions in malignant glioma cases, yet their effects against established tumors are nonetheless limited [32, 33, 36, 42]. Given the principle idea that therapy resistance of tumor cells develop in a time-dependent manner [11], those treatments would be more effective if delivered prophylactically before disseminated lesions form discrete masses. Thus, patients would benefit from prophylactic radiation therapy and/or intrathecal chemotherapy against dissemina-

tion if we knew in advance that disseminated tumors would eventually develop during the course. In this respect, although the results of this present study need to be confirmed in large-scale studies in the future, our results suggest that CD133 could become a useful molecular predictor to prospectively identify such patients at high risk of dissemination, alone or in combination with other dissemination markers reported to date.

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Comments

Karl Frei, Zurich, Switzerland

In this study, the authors tested for an association between the expression of the stem cell marker CD133 and the risk of dissemination in 26 cases of glioblastomas (10 without and 16 with dissemination). The tumor specimens were examined by western blot analysis and the CD133 expression levels were quantified by densitometry and normalized to β-actin.

The authors suggest from their data that CD133 might be a molecular predictor of glioblastoma dissemination and that CD133-positive cancer stem cells may be implicated in the initiation of disseminated lesions.

This intriguing idea has to be taken with caution due to the following reasons:

1. The study is only based on western blot analysis and no immunohistochemistry has been performed to confirm the biochemical data.

2. The western blot analysis was not done in a quantitative manner.

The number of 26 cases is low and the observed variability (five negative cases in each group) high.

Oliver Heese, Hamburg, Germany

In this study, the authors investigated the association between the expression of CD133 and the risk of tumor dissemination in 26 cases of glioblastoma multiforme in order to identify new potential predictors of an unvaivorable outcome of this tumor. The CD133 expression was measured on protein level by western blot. Dissemination was defined as appearance of enhanced nodules and/or diffuse enhancement of the leptomeningeal space at distance sites of the primary tumor location on contrast enhanced MRI.

The results indicated that significant higher CD133 level were found in GMB patients with a disseminated tumor progression. These findings imply that high levels of CD133 expression may be associated with a high risk of dissemination whereas low CD133 expression may not exclude the possibility of dissemination.

Despite the recent surge of interest in CD133+ brain tumor stem cells, the clinical significance of this cell population remains unclear and with this study an interesting hypothesis is made. Striking evidence suggests a dynamic process of expression of CD133 positive cells.

Since the amount of CD133 positive cells may vary in a process of tumor progression the measured western blot data of the initial tumor specimen may not represent the amount of CD33 positive cells during tumor dissemination or during local tumor progression. In addition for future analysis, in order to correlate the relationship between CD133 expression and clinical prognosis not only the quantity of CD133 positive cells should be taken into account but also the quality of CD133+ cells in in vitro and in vivo model systems have to be evaluated.

Since various regimens target local tumor control of glioblastomas such as radiosurgery or local chemotherapy, disseminated tumor growth is difficult to treat and predictors are important in order to indentify this subpopulation of patients and CD133 expression may be one molecular parameter for prediction of the clinical course of a glioma patient group.

Michel Mittelbronn, Frankfurt, Germany

In their current manuscript, Sato et al. present CD133 as a molecular predictor for glioblastoma dissemination and suggest that CD133-positive stem cells may be implicated in the initiation of disseminated lesions. This very interesting finding might be—if constantly reproducible—a useful tool for the prediction of glioma growth and could impinge on treatment strategies. However, what is not proven in the present study and probably unprovable to date, is the open question if CD133 upregulation in glioblastoma is the cause of dissemination or rather a bystander effect or consequence of other conditions within gliomas. The authors strongly favor the hypothesis that the source of CD133 expression might be related to tumor stem cells (although not proven by means of immunohistochemistry or FACS analysis). Subsequently, I would like to provide some additional interpretation of the western blot data. CD133 is frequently considered as a marker for neural, hematopoietic, and brain tumor initiating stem or progenitor cells, however, the distribution of CD133 expression in brain tumors has remained controversial. CD133-positive cells not closely related to tumor vessels have been reported to reside in pseudopalisading areas of necrosis (1). These areas are mainly subjected to low oxygen concentrations. From cell experiments, it is known that cultured glioma cells are capable to express CD133 when kept under hypoxic conditions without immediately being considered as stem cells (2, 3). Furthermore, emerging studies point out that anti-angiogenic approaches in high grade gliomas either lead to (1) reactivating angiogenesis through upregulation of other proangiogenic factors, (1) invading normal CNS tissue via upregulation of matrix metalloproteinases-2, 9, 12, and sparc (secreted protein,

acidic, cysteine-rich) or even drive expression of critical genes associated with aggressiveness, invasiveness and poor survival in glioma patients (4, 5). Taking into account the fact that glioma cells upregulate CD133 under hypoxic conditions and that hypoxia strongly leads to a more migratory phenotype, one could assume that the findings of Sato et al. could more likely reflect a secondary CD133 upregulation in more migratory or disseminating glioblastomas.

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14. 後頭蓋窩正中部に発生した巨大腫瘍

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臨床経過

症　例：16歳，女性

主　訴：頭痛，複視

既往歴・家族歴：特記事項なし

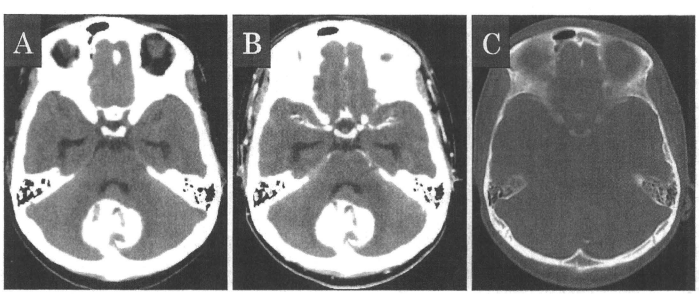
現病歴：2006年1月より起床時の頭痛を自覚．後頭部優位の持続性の頭痛であった．2007年1月初旬より右方視時の複視が出現し，近医眼科を受診．右外転神経麻痺を指摘され，当科紹介となった．

初診時神経学的所見：後頭部を中心とした強い持続性の頭痛および右外転神経麻痺を認めた．四肢麻痺や失調は認められなかった．

身体外表所見：項部には紅斑，皮膚小孔などの外表面の異常は認められなかった．

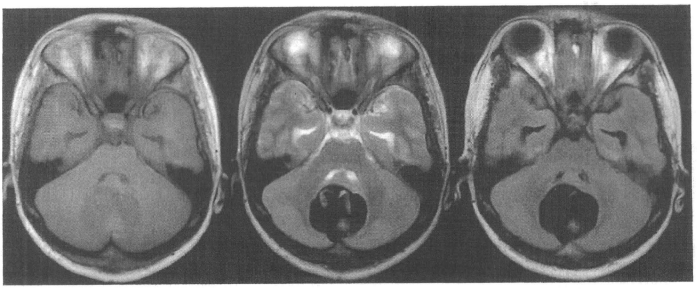
画像所見：入院時CTでは後頭蓋窩正中の両側小脳半球間にhigh densityのmass lesionを認め，第1頸椎下端まで伸展していた．内部には索状のiso density areaを認めた．Bone windowでは，病変の主体は骨よりもCT値が低く，一部に石灰化と考えられるhigh densityの部分を認めた（**Fig. 1**）．

MRIでは，T1強調画像でiso intensityからややlow intensity，T2強調画像，FLAIRでlow intensity，内部にiso intensityを示す索状物を認めた．周囲小脳実質を圧排しているが，明らかな浮腫やMRI信号強度の変化は認められなかった．拡散強調画像では，病変は全体的にlow intensityであった．病変の圧排による非交通性の水頭症を認めた．ガドリニウム造影MRIでは腫瘍後端に一部造影される部分を認めたが，その他の大部分には明らかな造影効果は認めなかった（**Fig. 2**）．脳血管撮影では病変による血管の圧排を認めたが，明らかな腫瘍濃染やfeederは認められなかった．

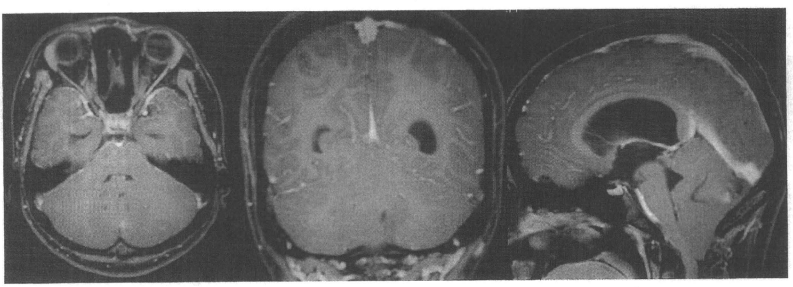


Plane　　CE（+）　　Bone window

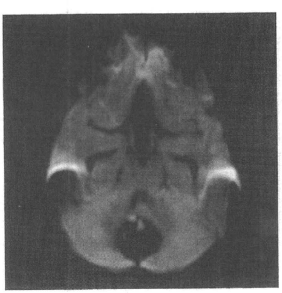
Fig. 1



T1　　T2　　FLAIR



Gd-MRI



DWI

Fig. 2

Q1 画像所見よりどのような腫瘍を考えるか？

A1 画像からの鑑別診断

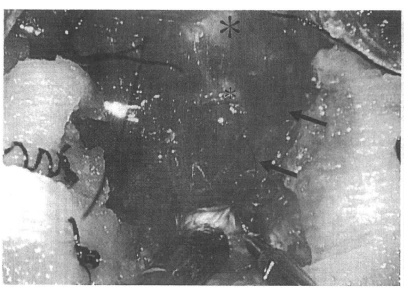
後頭蓋窩の正中部に発生した腫瘍である．硬膜に付着部をもち，CT でも high density を示すことから髄膜腫が鑑別に挙げられるが，MRI T2 強調画像では low intensity を呈し，また明らかな腫瘍濃染は認められないことから非典型的と考えられた．拡散強調画像は low intensity を呈しており，細胞密度が低い腫瘍と考えられ，腫瘍内部に一部石灰化と思われる部や柵状の構造物の存在が示唆されることから，奇形腫（teratoma），類皮嚢胞（dermoid cyst）などが鑑別疾患に挙げられた．

手術所見

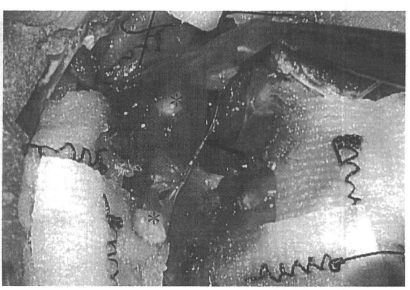
手術は腫瘍が foramen magnum を超え第 1 頚椎まで進展していることから，midline skin incision，suboccipital craniotomy＋第 1 頚椎 laminectomy でアプローチした．腫瘍内容は黄褐色で粘稠度がきわめて高い液体であった．内溶液中に石灰化成分と毛髪を認め，類皮嚢胞が強く疑われた（Fig. 3）．類皮嚢胞の本体と思われる比較的白色の部分には，PICA から細い feeder の流入がみられた．Feeder を焼灼処理し，全周性に丁寧に剝離を進め，肉眼的に全摘出した．

病理組織学的所見

病理組織としては，重層扁平上皮からなる上皮組織と，皮下組織として線維性結合組織，血管，毛嚢・毛髪，および石灰化が認められた．明らかな異型細胞は認められなかった（Fig. 4）．

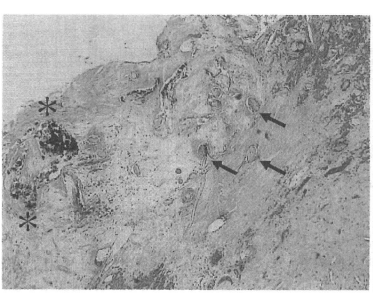


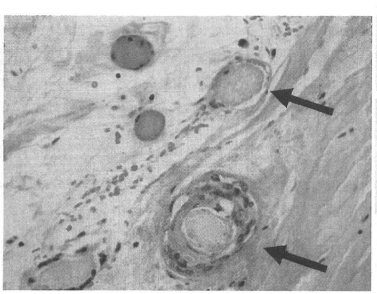
＊：石灰化，→：毛髪



＊：石灰化，→：毛髪

Fig. 3





＊：石灰化，→：毛嚢，毛髪

Fig. 4

Q2 病理所見からの診断は？

Table 1

Authors	Journal	Year	No. of cases	Location	CT density
Sanchez-M, et al.	*J Neurosurg*	2006	1	CM	High
Layadi, et al.	*Pediatr Neurosurg*	2005	2	CM	Low
van Calenbergh, et al.	*Surg Neurol*	2005	1	4th V	Low
Caldarelli, et al.	*J Neurosurg*	2004	8	CM：4 cases	Low
Ciurea, et al.	*Surg Neurol*	2004	1	4th V	Low
Neugraschl, et al.	*Eur Radiol*	2002	1	CM	High
Our case				CM	High

CM：cisterna magna, 4th V：forth ventricle

Table 2

Authors	Case	MRI		Pathological findings
		T1WI	T2WI	
Sanchez-M, et al.	A 16-year-old male	High	Low	Microbleeding
Neugraschl, et al.	A 73-year-old male	Mixed	Low	Intratumoral hemorrhage
Our case	A 16-year-old female	Low-iso	Low	Calcification
Usual dermoid		High	Low	

A2 病理診断：類皮嚢胞 dermoid cyst

一般に類皮嚢胞の嚢胞壁は厚く，嚢胞内容は毛髪や脂肪性分泌物，汗を混じ酒粕様である．毛嚢や汗腺，皮脂腺などの皮膚の付属器官があれば類皮嚢胞と診断できる．対して類表皮嚢胞（epidermoid cyst）は，異所性の扁平上皮に囲まれた嚢胞で，皮膚の付属器を欠く．嚢胞壁は薄く内部に膜状の keratin 物質を入れていて，嚢胞内は肉眼的に白く光沢があり，真珠腫（pearly tumor）ともいわれている．奇形腫（teratoma）は三胚葉組織の異常増殖からなる腫瘍であり，皮膜を有し皮膚付属器や軟骨，脂肪組織，神経などの組織の無秩序な増殖を呈する．それぞれの組織が分化している場合と未分化な場合があり，成熟奇形腫（mature teratoma）と未熟奇形腫（immature teratoma）といわれる．未熟な場合には脳組織が多く，神経管構造が存在すれば未熟の目安になる．類皮嚢胞には軟骨や骨の小片を混じることがあるので，これのみでは奇形腫との鑑別はできない．逆に類皮嚢胞が，成熟奇形腫の一部としてみられることがあり，病理診断に難渋することがある．特に松果体の成熟奇形腫では，類皮嚢胞と類表皮嚢胞が奇形腫の大部分を占めることがしばしばである．

まとめ

類皮嚢胞は，全頭蓋内腫瘍の中でも 0.1～0.7％と頻度としてはまれであり，正中部に多く発生し，小児から若年者にみられるという特徴がある[3)6)]．発生部位は第四脳室と小脳虫部が多い．対して類表皮嚢胞は，小脳橋角部や傍鞍部，頭蓋骨板間層，脊髄などに好発し，中年以降に発症する．類皮嚢胞は胎生期の外胚葉組織の迷入であると考えられ，時に皮膚と連続する先天性皮膚洞（congenital dermal sinus）を有し感染を合併することが知られているが[4)]，本症例では明らかな外表異常や感染症状，既往は認められなかった．後頭蓋窩に発生した類皮嚢胞の報告は，近年の文献を渉猟しえた範囲でも少数であり[1)2)4)5)7)8)]，中でも本症例のように CT 上 high density であった報告は，本症例を含めわずか 3 例であった（**Table 1**）．

一般的に類皮嚢胞は，CT では low density，MRI では T1 強調画像で high intensity，T2 強調画像では low intensity を示すといわれている．CT で high density を示した 3 例の画像所見，病理所見を比較すると（**Table 2**），他の 2 症例では病理組織学的に腫瘍内出血を呈しており[2)3)]，これにより CT 上 high density を呈したものと考えられたが，今回の症例では，腫瘍内出血は認めず石灰化成分を多く含んだ高粘稠度の内容物であり，これが CT で high density として非典型的な画像所見を示した原因と考えられた．

治療法としては，全摘出術にて根治可能と考えられ，放射線治療，化学療法は効果がないと考えられている．類表皮嚢胞と同様に，内容物の漏出で髄膜炎症状 chemical meningitis を呈することがある．髄膜炎症状が強い場合にはステイロイド投与が有効とされている．本症例では，全摘出後に髄膜炎もきたさず，術前より存在していた水頭症，頭痛，複視も消失した．

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術中MRIシステムを用いた脳腫瘍手術

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A New Intraoperative MRI System in Brain Tumor Surgery

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Summary: *Background of Study*: Extensive surgical removal of brain tumors lengthens life expectancy. As dynamic changes, such as brain shift, can occur during surgical procedures, intraoperative MRI is an important tool for safe and maximum resection of tumors. In July 2008, new operating room equipped with an intraoperative high-field MRI (1.5T) system, neuronavigation, and fluorescence diagnosis system opened at the author's institution, Yamagata University Hospital.

Methods: Preoperatively, 3.0T MR studies, including morphological study, multivoxel MR spectroscopy, tractography and functional MRI, were performed. If the tumor was located in or near an eloquent area, we performed MEP (motor evoked potential)/SEP (sensory evoked potential) monitoring and/or awake surgery using cortical and subcortical stimulation. Intraoperative MRI was performed after total resection or to obtain updated information on brain positioning during the removal of deep-seated tumors.

Results: Using this new suite, we have safely treated various brain tumors, including gliomas, metastatic brain tumors, meningiomas, and pituitary adenomas. Gross total removal was achieved in over 70% of malignant tumors. Intraoperative MR images contributed to improving the tumor resection rate and overall results.

Key Words: Intraoperative MRI system, Brain surgery
使用機種: Achieva 3.0T (Philips) Signa 1.5T (GE), Neuronavigation (Brain Lab)

はじめに

fMRIやDiffusion Tensor Imagingなどの新しい画像診断法の進歩により脳腫瘍手術の計画をより綿密に立てることが可能となった．運動誘発電位や覚醒下手術などのモニタリング・マッピングを用いて最大限の安全な摘出を図ることが浸潤性に発育し病理学的には全摘出が不可能と考えられる悪性神経膠腫にとってもoptimalな治療であると認識されるようになってきている．現在ナビゲーションシステムや超音波診断装置の併用による摘出率の向上が試みられているが，brain shiftや超音波診断装置の解像度の問題もあり，術中に正確に残存腫瘍，摘出率を把握することは困難である．2008年7月，当科で1.5テスラ術中MRIシステム（1.5T MRI (GE)，neuronavigation (Brain LAB)，術中蛍光診断・術中血管撮影機能FL400，FL800（KARL-STORZ）搭載手術顕微鏡（Leica），MRI・CT対応operation table (MAQUET)）を導入したので，新インテリジェント手術システムとその使用経験につき報告する．

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