ACTC1 as an invasion and prognosis marker in glioma

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OBJECTIVE Glioma is a major class of brain tumors, and glioblastoma (GBM) is the most aggressive and malignant type. The nature of tumor invasion makes surgical removal difficult, which results in remote recurrence. The present study focused on glioma invasion and investigated the expression of actin, alpha cardiac muscle 1 (ACTC1), which is 1 of 6 actin families implicated in cell motility.

METHODS mRNA expression of ACTC1 expression was analyzed using quantitative real-time polymerase chain reaction (qRT-PCR) in 47 formalin-fixed, paraffin-embedded glioma tissues that were graded according to WHO criteria: Grade I (n = 4); Grade II (n = 12); Grade III (n = 6); and Grade IV (n = 25). Survival was analyzed using the Kaplan-Meier method. The relationships between ACTC1 expression and clinical features such as radiological findings at the time of diagnosis and recurrence, patient age, Karnofsky Performance Scale status (KPS), and the MIB-1 index were evaluated.

RESULTS The incidence of ACTC1 expression as a qualitative assessment gradually increased according to WHO grade. The hazard ratio for the median overall survival (mOS) of the patients with ACTC1-positive high-grade gliomas as compared with the ACTC1-negative group was 2.96 (95% CI, 1.03–8.56). The mOS was 6.28 years in the ACTC1-negative group and 1.26 years in the positive group (p = 0.037). In GBM patients, the hazard ratio for mOS in the ACTC1-positive GBMs as compared with the ACTC1-negative group was 2.86 (95% CI 0.97–8.45). mOS was 3.20 years for patients with ACTC1-negative GBMs and 1.08 years for patients with ACTC1-positive GBMs (p = 0.048). By the radiological findings, 42.9% of ACTC1-positive GBM patients demonstrated invasion toward the contralateral cerebral hemisphere at the time of diagnosis, although no invasion was observed in ACTC1-negative GBM patients (p = 0.013). The recurrence rate of GBM was 87.5% in the ACTC1-positive group; in contrast, none of the ACTC1-negative patients demonstrated distant recurrence (0.007). No remarkable relationship was demonstrated among ACTC1 expression and patient age, KPS, and the MIB-1 index.

CONCLUSIONS ACTC1 may serve as a novel independent prognostic and invasion marker in GBM.

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KEY WORDS ACTC1; astrocytoma; cytoskeletal filament; glioblastoma; high-grade glioma; invasion; prognosis; oncology

GLIOMASTOMA (GBM) displays an aggressive proliferation of tumor cells, and patients demonstrate very short survival despite treatments including surgical removal, irradiation, and chemotherapy.³³ The 5-year survival rate of GBM patients is 4.7% to

13.8%.^{9,24,32} GBM patients frequently develop recurrence, not only locally but also in remote areas of brain. The infiltration of glioma cells, which constitutes a limiting factor of complete tumor removal, results in poor prognosis. The progression-free survival (PFS) and overall survival (OS)

ABBREVIATIONS ACTC1 = actin, alpha cardiac muscle 1; CE-T1 = contrast-enhanced T1-weighted; CI = confidence interval; FFPE = formalin-fixed, paraffin-embedded; FLAIR = fluid-attenuated inversion recovery; GAPDH = glyceraldehyde 3-phosphate dehydrogenase; GBM = glioblastoma; HGG = high-grade glioma; IDH = isocitrate dehydrogenase; KPS = Karnofsky Performance Scale; LGG = low-grade glioma; MGMT = O⁶-methylguanine-DNA methyltransferase; mOS = median overall survival; mPFS = median progression-free survival; OS = overall survival; PFS = progression-free survival; qRT-PCR = quantitative real-time polymerase chain reaction.

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in patients with GBM can be quite variable with poor prediction of survival.

Glioma has 2 major characteristics: proliferation and infiltration. The former may be associated with local recurrence after incomplete tumor removal, and the latter is related to remote recurrence that is separate from the original tumor location. Patients with recurrence without infiltration resulting in local regrowth can be good candidates for surgical reexploration; however, patients with recurrence caused by the infiltration of tumor cells, which leads to remote recurrence especially at deep-seated locations or multiple areas, are not. The difficulty of tumor removal due to tumor infiltration has been strongly related to poor prognosis in glioma.^{17,27,39} Although the process of infiltration has been analyzed by molecular signatures such as matrix metalloproteinase, CD44, neural cell adhesion molecule, cadherin, integrin, and hepatocyte growth factor, the clinical relevance of these markers has not yet been fully studied.^{20,22,35} Elucidating the regulatory mechanisms of GBM infiltration holds promise for improving prognosis.

One of the causes of remote recurrence by glioma infiltration is considered enhancement of cell motility provided by the cytoskeletal filaments that consists of 3 major components: actin filaments, microtubules, and intermediate filaments. Among these cytoskeleton components, actin polymerization provides a transfer of intracellular materials and cell motility, including chemotaxis, mitogenesis, and path finding by axons.^{5,26,29} Actin dynamics-mediated cellular protrusion, which is often the first step in cell locomotion, is essential for cell invasion.³⁴ In this study, we focused on the glioma cell expression of actin, alpha cardiac muscle 1 (ACTC1) in various WHO-defined glioma grades in relation to prognosis and radiological studies in order to determine if ACTC1 may serve as a novel independent prognostic and invasion marker.

Methods

Patients and Tissue

Newly diagnosed consecutive patients between 2 and 80 years of age with diagnosed and histologically confirmed WHO Grade I to IV gliomas by several independent pathologists at our university hospital were eligible for this study. All tumors were maximally resected to preserve neurological function and followed with radiochemotherapy, as reported by Stupp et al. for high-grade gliomas (HGGs; i.e., Grade III and IV).³³ The glioma tissues obtained in the operating room between November 2001 and October 2014 were fixed in liquid buffered formalin. A total of 47 formalin-fixed, paraffin-embedded (FFPE) tissues, including 4 WHO Grade I samples, 12 WHO Grade II samples, 6 WHO Grade III samples, and 25 WHO Grade IV samples, were analyzed. Written informed consent was obtained from each patient in accordance with the local ethics guidelines. This study was approved by the Institutional Review Board of Sapporo Medical University.

Surveillance and Follow-Up

OS and PFS were analyzed using the Kaplan-Meier survival analysis.

Tumor progression is defined by increasing tumor size, new areas of tumor, or unequivocal neurological deterioration.¹⁹ Since the oldest surgery of those cases was performed in 2001, this patient was followed up for 12.4 years.

RNA Extraction, cDNA Synthesis, and Real-Time qRT-PCR

FFPE samples were sectioned using a microtome and collected in 2-ml microtubes. RNA extraction was performed using deparaffinization solution (Qiagen) and the RNeasy FFPE Kit (Qiagen) according to the manufacturer's protocol. The total RNA concentration and A260/A280 ratio were measured using a NanoDrop Lite spectrophotometer (Thermo Fisher Scientific Inc.). Samples in which the A260/A280 ratio was less than 1.8 were excluded. Samples with an RNA concentration less than 40 ng/μl were prepared using a centrifugal concentrator. The QuantiTect Reverse Transcription Kit (Qiagen) was used to reverse transcribe 500 ng of total RNA. PCR reactions, which were 10 μl in volume, were prepared with 2 μl cDNA diluted to 1:5. TaqMan Universal Master Mix II with UNG and TaqMan Gene Expression assays for glyceraldehyde 3-phosphate dehydrogenase (GAPDH; Hs02758991_g1) and ACTC1 (Hs00606316_m1) were purchased from Thermo Fisher Scientific Inc. Quantitative real-time polymerase chain reaction (qRT-PCR) was performed in quintuplicate using PRISM7500 (Thermo Fisher Scientific Inc.), and the entire experiment was repeated twice. The presence of ACTC1 on qRT-PCR was the criterion for defining a sample as ACTC1 positive, and the absence of ACTC1 classified a sample as negative. The thermal cycling parameters were 50°C for 2 minutes and 95°C for 10 minutes, followed by 60 cycles of 95°C for 15 seconds and 60°C for 1 minutes. The cycle threshold value of each gene of interest was calculated with reference to the GAPDH value. After obtaining written informed consent, the fold change was determined using the $2^{-\Delta\Delta C_t}$ method relative to the calibrator, which was obtained during surgery with internal and external decompression because of trauma.³

Immunohistochemical Analysis

The FFPE blocks were sectioned into slides with a thickness of 3 μm and then deparaffinized. Sections were immunostained using BGX-Ki 67 (BioGenex) according to the manufacturer's instructions with the anti-Ki 67 monoclonal antibody. The MIB-1 index was calculated as the percentage of positively stained tumor cell nuclei in areas with the greatest degree of immunostaining.²⁸

Radiological Evaluation

Radiological analysis was performed by 2 neuroradiologists who were independent of this study. A tumor that extended beyond the corpus callosum toward the direction of the contralateral hemisphere was judged as invasive, especially at the time of diagnosis. A tumor that occurred at another location without direct connection with the cavity after tumor removal was judged as recurrence.

Statistical Analysis

Data were statistically analyzed, and they are presented

as the mean values $\pm$ the standard error of the mean. Differences among groups were assessed using the Kruskal-Wallis test, and the Mann-Whitney U-test and Spearman test were used to identify group differences. All statistical analyses were performed using SPSS (version 22) (IBM Corp.). Differences were deemed statistically significant if $p < 0.05$. Comparison of OS and PFS according to ACTC1 expression was performed with the log-rank test.

Results

Expression Of ACTC1 in Low-Grade Gliomas and HGGs

Qualitative assessment of the positivity rate of ACTC1 using real-time qRT-PCR indicated an increase in HGGs as compared with low-grade gliomas (LGGs: Grades I and II). The incidence was 31.3% for LGGs and 58.1% for HGGs ($p = 0.081$) (Fig. 1A). The expression of ACTC1 mRNA as a quantitative assessment was significantly elevated in HGGs ($p = 0.024$) (Fig. 1B).

Survival and Progression

The OS and PFS for patients with Grade II to IV gliomas and only Grade IV gliomas are shown in Fig. 2 and Table 1. For the HGG group, the median survival benefit in the ACTC1-negative group was 5.02 years; the median OS (mOS) was 6.28 years in ACTC1-negative HGG patients and 1.26 years in ACTC1-positive HGG patients ($p = 0.037$) (Table 1 and Fig. 2A). The hazard ratio, which was adjusted by fitting the Cox proportional-hazard models for mOS in the ACTC1-positive HGG patients as compared with the ACTC1-negative patients, was 2.96 (95% CI 1.03–8.56). These data indicate 5 times longer OS in ACTC1-negative HGG patients compared with ACTC1-positive patients. The median PFS (mPFS) benefit in the ACTC1-negative group was 1.63 years; the mPFS was 2.08 years in ACTC1-negative HGG patients and 0.50 years in ACTC1-positive HGG patients ($p = 0.032$). The hazard ratio

for mPFS was 2.54 (95% CI 1.10–5.83) (Table 1 and Fig. 2B).

The mOS for the Grade IV GBM patients was 3.20 years in ACTC1-negative GBM patients and 1.08 years in ACTC1-positive GBM patients ($p = 0.048$), indicating a median survival benefit in the ACTC1-negative group of 2.12 years (Table 1 and Fig. 2C). The hazard ratio for mOS in the ACTC1-positive GBM patients as compared with the ACTC1-negative patients was 2.86 (95% CI 0.97–8.45). These data indicate about 3-times longer overall survival in ACTC1-negative GBM patients compared with ACTC1-positive patients. The mPFS benefit in the ACTC1-negative group was 0.69 years; mPFS was 1.13 years in ACTC1-negative GBM patients and 0.44 years in ACTC1-positive GBM patients ($p = 0.020$). The hazard ratio for mPFS was 2.82 (95% CI 1.14–6.98) (Table 1 and Fig. 2D). Thus, both OS and PFS were prolonged in the ACTC1-negative groups in patients with Grade II to IV, Grade III and IV, and Grade IV only gliomas.

Radiological Findings at the Time of Diagnosis in GBMs

As for invasion, 42.9% of tumors demonstrated invasion toward the contralateral cerebral hemisphere in ACTC1-positive GBM patients, although none of the instances of invasion were observed in ACTC1-negative GBM patients ($p = 0.013$). The finding of deep-seated invasion to the contralateral cerebral hemisphere was observed in ACTC1-positive GBMs at the time of diagnosis. The typical invasion pattern was progression through the corpus callosum. Frontal tumors demonstrated progression through the genu and the anterior part of the body of the corpus callosum (Fig. 3A and B), and occipital tumors spread through the splenium of the corpus callosum (Fig. 3C). In contrast, the ACTC1-negative GBMs indicated topographical unity near the surface of the cortex. Even though the maximum diameter of the ACTC1-negative tumors was 62 mm, no invasion was observed (Fig. 3D). Two other patients with ACTC1-negative tumors revealed similar radiological findings (Fig. 3E and F, respectively).

FIG. 1. Positivity rate and expression of ACTC1. mRNA expression for ACTC1 was analyzed in 47 FFPE glioma samples with WHO-defined grades: Grade I+II is LGG ($n = 16$), and Grade III+IV is HGG ($n = 31$). The results are expressed as the relative mRNA expression with respect to the WHO grade. **A:** The positivity rate of ACTC1 in HGGs was higher than in LGGs ($p = 0.081$). **B:** The expression of ACTC1 mRNA as a quantitative assessment was significantly elevated in HGGs as compared with LGGs ($p = 0.024$). The error bars indicate the standard error of the mean. FC = fold change.

TABLE 1. OS and PFS of ACTC1-negative and -positive gliomas*

Variable	ACTC1		p Value	Hazard Ratio	95% CI	Benefit (yrs)
	Negative (yrs)	Positive (yrs)				
Median OS						
Grade III+IV	6.28	1.26	0.037	2.96	1.03–8.56	5.02
Grade IV	3.20	1.08	0.048	2.86	0.97–8.45	2.12
Median PFS						
Grade III+IV	2.08	0.50	0.032	2.54	1.10–5.83	1.63
Grade IV	1.13	0.44	0.020	2.82	1.14–6.98	0.69

* A total of 18 patients with ACTC1-positive HGG (Grade III and IV) and 13 patients with ACTC1-negative gliomas were compared. Among these, 14 patients with ACTC1-positive GBMs and 11 patients with ACTC1-negative GBMs were further investigated.

Radiological Findings at the Time of Recurrence in GBMs

Among recurrent GBM cases, 87.5% of ACTC1-positive tumors demonstrated remote recurrence, but no remote recurrence was observed in ACTC1-negative patients ($p = 0.007$). A representative case of ACTC1-positive GBM originating from the left putamen revealed heterogeneous ring enhancement without apparent perifocal edema (Fig. 4A and B). Recurrence was observed in the right temporal lobe at 20.3 months after the initial surgery followed by chemoradiotherapy (Fig. 4C and D). The recurrent lesion was in the contralateral side with presumed tumor spread through the commissural tracts. A case of an ACTC1-negative GBM tumor, which was initially a single lesion, occurred at the temporal lobe with perifocal edema (Fig.

4E and F). Because the tumor was confined to a superficial location, gross-total removal of the enhanced tumor was accomplished. At 13.3 months after initial treatment, only local recurrence without any invasion toward multiple lobules or remote recurrence was observed (Fig. 4G and H).

Analysis of the HGGs indicated a 90.9% rate of remote recurrence in the ACTC1-positive group. In contrast, none of the ACTC1-negative patients demonstrated distant recurrence ($p = 0.002$; Pearson's chi-square test). Among the observed recurrent cases, 21.4% were of Grade III gliomas, and all ACTC1-positive tumors demonstrated the remote recurrence of Grade III tumors. In contrast, all ACTC1-negative Grade III tumors remained localized to the initial tumor site.

FIG. 2. Kaplan-Meier estimates of OS and PFS in the patients who had gliomas with the presence or absence of ACTC1 expression. **A:** The median survival of the HGG group (Grade III+IV) was 6.28 years for the 13 patients with ACTC1-negative HGG compared with 1.26 years for the 18 patients with ACTC1-positive HGG ($p = 0.037$). **B:** mPFS was 2.08 years for ACTC1-negative HGG patients and 0.50 years for ACTC1-positive HGG patients ($p = 0.032$). The median survival was 1.08 years for the 14 patients with ACTC1-positive GBMs compared with 3.20 years for the 11 patients with ACTC1-negative GBMs. **C:** The survival benefit in the ACTC1-negative group was 2.12 years ($p = 0.048$). **D:** The mPFS was 1.13 years for ACTC1-negative GBM patients and 0.44 years for ACTC1-positive GBM patients ($p = 0.020$). The benefit of mPFS in the ACTC1-negative group was 0.69 years. Survival distributions were compared with the use of the log-rank test.

FIG. 3. MRI findings at the time of diagnosis. **A and B:** Findings obtained in patients with ACTC1-positive GBMs. A 60-year-old woman presented with symptoms of headache and nausea (A) and a 53-year-old man presented with symptoms of seizure (B). Both patients demonstrated tumor invasion toward the contralateral cerebral hemisphere through the genu and anterior third of the corpus callosum. **C:** Image obtained in a 71-year-old woman with an ACTC1-positive GBM who presented with headache, showing invasion of the GBM through the splenium of the corpus callosum. **D–F:** In contrast, ACTC1-negative GBMs developed at localized areas near the surface of the cortex and were associated with focal neurological deficits. A 19-year-old woman presented with symptoms of progressive sensory aphasia (D), a 63-year-old woman presented with sensory aphasia (E), and a 61-year-old woman presented with left hemiparesis.

FIG. 4. MRI findings at the time of recurrence. **A–D:** A 57-year-old man with an ACTC1-positive GBM presented with left sensory disturbance at the time of diagnosis. FLAIR (A) and contrast-enhanced T1-weighted MR (B) images showed that the tumor originated from the left putamen with heterogeneous ring enhancement. Recurrence was observed in the right temporal lobe on FLAIR (C) and contrast-enhanced T1-weighted (D) images at 20.3 months after initial surgery followed by chemoradiotherapy. **E–H:** A 19-year-old woman with a ACTC1-negative GBM presented with progressive sensory aphasia. FLAIR (E) and contrast-enhanced T1-weighted (F) images indicated that the tumor was localized at the posterior temporal lobe with peritumoral edema. At 13.3 months after initial treatment, only local recurrence without any invasion toward multiple lobules or remote recurrence was observed on FLAIR (G) and contrast-enhanced T1-weighted images.

FIG. 5. Scatter plot analysis of ACTC1 mRNA expression with respect to patient age, KPS, and the MIB-1 index. No relationship was determined among ACTC1 mRNA expression and patient age (A), KPS (B), or the MIB-1 index (C).

Relationship Among ACTC1 Expression and MIB-1 Index, Patient Age, and Karnofsky Performance Scale Status

No relationship was observed between ACTC1 expression and patient age (Fig. 5A), Karnofsky Performance Scale status (KPS) (Fig. 5B), and MIB-1 index (Fig. 5C). Because both KPS and patient age are considered to have a relationship with the prognosis of GBM, ACTC1 expression may be an independent marker of glioma prognosis, apart from the KPS score and patient age. Since the MIB-1 index is commonly used as a marker of proliferation, the lack of a relationship between ACTC1 expression and the MIB-1 index suggests that ACTC1 expression differs from proliferation markers.

Discussion

We discovered that ACTC1 is expressed in a subset of glioma cases with a progressive increase from low to high grade (WHO Grade I–IV). In addition, the OS and PFS of patients who had ACTC1-positive tumors were remarkably poor when compared with ACTC1-negative tumors. The mOS of our GBM sample (both ACTC1-positive and -negative tumors) is 18.36 months, which is consistent with previous studies.^{1,18,19} The mOS of GBM patients treated by surgical removal and radiotherapy was 12.1 months.^{1,18,19} Survival was extended to 14.6 months by adding temozolo-

mid.³³ With a survival benefit of 2.5 months, temozolomide treatment became widely used as a standard chemotherapy agent for GBM.³³ mOS was extended to 16.7 months in a Phase III study (AVAglio).⁸ Among our GBM patients, the mOS of ACTC1-positive GBMs was 13.0 months, whereas that of ACTC1-negative patients was 38.4 months. Thus, ACTC1-positive GBM patients indicated much poorer survival than ACTC1-negative GBM patients. The survival benefit of 25.4 months in ACTC1-negative GBM patients was much longer than the benefit obtained by chemotherapy. Since survival was statistically different in the absence or presence of ACTC1 expression, ACTC1 may be considered a prognostic factor. In clinical studies that explore new therapeutic approaches for GBM, analysis of ACTC1-positive and -negative tumors may be informative.

Many genetic factors, such as phosphatase and tensin homolog deletion on chromosome 10, epidermal growth factor receptor, and tumor protein p53, were reported as prognostic factors. Among those, isocitrate dehydrogenase (IDH) mutation and methylation of the promotor of O⁶-methylguanine-DNA methyltransferase (MGMT) have been given heavy prognostic weight. However, the methylation of MGMT promotor identified in 45% to 75% of GBM tumors is strictly considered as a predictive factor for the treatment by temozolomide, not a prognostic fac-

tor.^{2,13,15,23} Mutation of IDH is the most commonly used factor for the prognosis of HGGs. IDH mutation is considered to be a favorable factor in HGGs.^{12,25,37,38} For patients with GBM, the mOS was 31 months for mutated IDH as compared with 15 months for patients with wild-type IDH. The survival benefit in the presence of IDH mutation was 16 months.³⁸ The survival benefit of 25.4 months by ACTC1-negative GBMs was much longer than the benefit obtained by the mutation of IDH.

In our study, the radiological findings of the ACTC1-positive GBM patients revealed tumor invasion toward the contralateral hemisphere at the time of initial diagnosis and remote recurrence at the time of recurrence. The invasion and remote recurrence of ACTC1-positive GBM cells in HGGs occurred through the commissural, association, or other projection fibers.¹ Actin relates to cell motility in normal cells and invasion or metastasis in cancer cells.²⁶ Actin is present in the cytoplasm just inside of the cell membrane. In the normal state, actin regulates cell motility and shape by repeating the expansion of the filaments by polymerization and contraction of the filaments by depolymerization.^{11,21,31} Thus, the expression of ACTC1 in GBM cells may be mechanistically related to their greater invasiveness than ACTC1-negative cells.

No clear relationship was determined between ACTC1 expression and KPS score or patient age. Prognostic factors have been associated with age, performance status, degree of surgical removal of the tumor, and postsurgical treatment. Favorable factors were young age, good KPS prior to surgery, and maximum tumor removal,^{4,7,14,16,30,36} which were associated with a good response to chemotherapy and irradiation. Since ACTC1 expression has no relationship with the previously mentioned prognostic factors, ACTC1 may be considered an independent prognostic factor.

No relationship was observed between ACTC1 expression and the MIB-1 index, which detects the Ki-67 antigen. Ki-67, which was initially detected as an autoantibody in the blood of leukemia patients in 1992, is expressed during the G₁, S, G₂, and M stages of the cell cycle.⁶ Since Ki-67 was detected inside the nucleus of active cells, the MIB-1 antibody, which detects Ki-67, is considered a marker of proliferation. The lack of an association between ACTC1 and the Ki-67 index suggests that the poor prognosis of ACTC1-positive GBM patients is due to higher tumor cell invasion along the nerve tracts, not an increase in cell proliferation. Thus, ACTC1 may represent an invasion marker that is independent of cell proliferation.

Our study revealed that the remote recurrence rate of ACTC1-positive HGGs was high (87.5% for GBM only vs 90.9% for HGGs); however, ACTC1-negative HGGs did not display remote recurrence, and their recurrence occurred in the primary (local) site. Although HGGs (especially GBMs) have a nature of recurrence, at present there is no way to predict if recurrence will occur only locally or at a remote site. Evaluating ACTC1 expression in HGG tissue may be beneficial for predicting remote recurrence.

Because ACTC1-positive GBM cells, which have a strong capacity to migrate, could exist in high-intensity areas on T2-weighted or fluid-attenuated inversion recovery (FLAIR) MRI images, supratotal resection including the

high-intensity areas on T2-weighted or FLAIR imaging is encouraged.¹⁰ Although the molecular mechanisms for migration should be elucidated in a future study, it is conceivable that ACTC1 might play an important role in the migratory capacity of GBMs.

In addition, the fact that ACTC1-negative GBMs recur locally and not in remote sites could encourage performing multiple surgeries in order to remove recurrent tumors because the ACTC1-negative GBMs may not display remote recurrence. The local control of tumors could be effective for extending mOS. Thus, ACTC1 might be an important molecule for molecular-based surgical decisions for GBM, in the context of a new suggested grading system based on biological molecular markers.¹⁸

Our study, which used FFPE glioma samples, has some advantages. The first is that the extensive retrospective analysis with long-term, clinical follow-up data could be achieved. The second is that the quantitative analysis by qRT-PCR of the mRNA expression associated with glioma-specific biomarkers, such as ACTC1 in this study, could be performed with small samples of FFPE tissue materials, although protein expression could not be measured. The third is that this research approach using FFPE tissue materials can be implemented at multiple institutions, not limited facilities, because it is easy to collect FFPE sample tissues from multiple institutions. Thus, this study approach using FFPE glioma materials is considered to be a simple and effective method for analyzing the relationship among the expression of ACTC1 and various clinical factors.

Conclusions

ACTC1 is expressed in a large subset of GBM tumors, especially high-grade tumors. ACTC1-positive GBMs indicated poorer prognosis compared with ACTC1-negative GBMs. The results of this study shed new light on the potential mechanisms of GBM invasion. ACTC1 may serve as a novel independent prognostic biomarker and a potential therapeutic target for molecular-based surgery for HGGs.

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Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions

Conception and design: Wanibuchi, Ohtaki, Kataoka-Sasaki, Sasaki, Honmou. Acquisition of data: Wanibuchi, Ohtaki. Analysis and interpretation of data: Wanibuchi, Ohtaki, Kataoka-Sasaki, Sasaki, Oka, Noshiro, Akiyama, Mikami, Mikuni, Honmou. Drafting the article: Wanibuchi, Ohtaki, Sasaki, Mikuni, Honmou. Critically revising the article: Wanibuchi, Ohtaki, Kataoka-Sasaki, Sasaki, Honmou. Approved the final version of the manuscript on behalf of all authors: Wanibuchi. Statistical analysis: Wanibuchi, Ohtaki, Kataoka-Sasaki, Oka, Noshiro. Administrative/technical/material support: Oka, Noshiro, Akiyama, Mikami, Mikuni, Kocsis, Honmou. Study supervision: Mikuni, Kocsis, Honmou.

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