Research Article

Identifying G6PC3 as a Potential Key Molecule in Hypoxic Glucose Metabolism of Glioblastoma Derived from the Depiction of $^{18}$F-Fluoromisonidazole and $^{18}$F-Fluorodeoxyglucose Positron Emission Tomography

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Purpose. Glioblastoma is the most aggressive primary brain tumor, characterized by its distinctive intratumoral hypoxia. Sequential preoperative examinations using fluorine-18-fluoromisonidazole ($^{18}$F-FMISO) and fluorine-18-fluorodeoxyglucose ($^{18}$F-FDG) positron emission tomography (PET) could depict the degree of glucose metabolism with hypoxic condition. However, molecular mechanism of glucose metabolism under hypoxia in glioblastoma has been unclear. The aim of this study was to identify the key molecules of hypoxic glucose metabolism. Methods. Using surgically obtained specimens, gene expressions associated with glucose metabolism were analyzed in patients with glioblastoma ($n=33$) who underwent preoperative $^{18}$F-FMISO and $^{18}$F-FDG PET to identify affected molecules according to hypoxic condition. Tumor in vivo metabolic activities were semiquantitatively evaluated by lesion-normal tissue ratio (LNR). Protein expression was confirmed by immunofluorescence staining. To evaluate prognostic value, relationship between gene expression and overall survival was explored in another independent nonoverlapping clinical cohort ($n=17$) and validated by The Cancer Genome Atlas (TCGA) database ($n=167$). Results. Among the genes involving glucose metabolic pathway, mRNA expression of glucose-6-phosphatase 3 (G6PC3) correlated with $^{18}$F-FDG LNR ($P=0.03$). In addition, G6PC3 mRNA expression in $^{18}$F-FMISO high-accumulated glioblastomas was significantly higher than that in $^{18}$F-FMISO low-accumulated glioblastomas ($P<0.01$). Protein expression of G6PC3 was consistent with mRNA expression, which was confirmed by immunofluorescence analysis. These findings indicated that the G6PC3 expression might be facilitated by hypoxic condition in glioblastomas. Next, we investigated the clinical relevance of G6PC3 in terms of prognosis. Among the glioblastoma patients who received gross total resection, mRNA expressions of G6PC3 in the patients with poor prognosis (less than 1-year survival) were significantly higher than that in the patients who survive more than 3 years. Moreover, high mRNA expression of G6PC3 was associated with poor overall survival in glioblastoma, as validated by TCGA database. Conclusion. G6PC3 was affluenty expressed in glioblastoma tissues with coincidentally high $^{18}$F-FDG and $^{18}$F-FMISO accumulation. Further, it might work as a prognostic biomarker of glioblastoma. Therefore, G6PC3 is a potential key molecule of glucose metabolism under hypoxia in glioblastoma.
1. Introduction

Glioblastoma is the most aggressive tumor among diffuse gliomas and is defined as grade IV according to the 2016 World Health Organization (WHO) classification [1]. Recently, the prognosis of patients with glioblastoma has been gradually improved, owing to the development of image-guided surgery, radiotherapy, and new chemotherapeutic drugs; however, the 2-year survival rate remains at 43% only [2]. As one of the mechanisms that make tumors more resistant to therapy, hypoxia, a condition where oxygen requirements in a tissue exceed its supply, is closely related to the characteristics of malignant tumors [3]. Consistent with other malignant neoplasms, glioblastoma frequently occurs in severe hypoxia [4–9]. Since fluorine-18-fluoromisonidazole (18F-FMISO) was developed as a hypoxic radio-labeled tracer using positron emission tomography (PET), 18F-FMISO PET has been useful in detecting hypoxic parts of brain tumors [7].

Fluorine-18-fluorodeoxyglucose (18F-FDG) is a common radiotracer that can visualize intracellular glucose metabolism. Since glycolysis is highly activated in cancer cells (Warburg effects), 18F-FDG uptake is increased in cancer cells. Hence, 18F-FDG PET has been useful in detecting cancer cells [10]. Similarly in glioma, 18F-FDG is accumulated in proportion to tumor malignancy [11]. 18F-FDG uptake is determined by multiple factors, such as glucose transporter (GLUT), hexokinase (HK), or glucose-6-phosphatase (G6Pase) based on “metabolic trapping” as an 18F-FDG kinetic principle [12, 13]. The molecular mechanisms of glucose metabolism in 18F-FDG PET were reportedly associated with 18F-FDG uptake by GLUT and metabolism by HK and G6Pase in Figure 1 [14]. As GLUT and HK play important roles in glycolysis, G6Pase acts as a gluconeogenic enzyme and is categorized into three subtypes: glucose-6-phosphatase catalytic unit (G6PC) 1–3 [15, 16].

We previously showed that semiquantitative evaluation by 18F-FDG and 18F-FMISO PET could demonstrate metabolically active hypoxic volume, a significant predictor for progression-free survival and overall survival (OS) in glioblastoma [9]. However, the underlying mechanism remains unclear. Based on the fact that hypoxia can induce glycolysis [17], we hypothesized that any glycolysis-associated molecules induced by hypoxia might influence the prognosis of patients with glioblastoma. We aimed to identify the key molecules associated with active glucose metabolism under hypoxia, and the expression of these glucose- and hypoxia-related genes in glioblastoma tissues obtained from the patients who underwent preoperative 18F-FDG and 18F-FMISO PET was analyzed. Then, we investigate whether the identified molecule is associated with the prognosis of patients with glioblastoma.

2. Materials and Methods

2.1. Surgical Excision of Glioblastoma Specimens. Patients who suffered from glioblastoma and underwent both 18F-FMISO and 18F-FDG PET preoperatively between 2008 and 2016 in Hokkaido University Hospital (Sapporo, Japan) were included. The surgical procedure was performed in the same manner as described previously [18]. We used an intraoperative neuronavigation system (Stealth Station S7®, Medtronic, Louisville, CO, USA). Gadolinium-enhanced T1-weighted imaging (Gd-T1WI) with three-dimensional spoiled gradient echo (3D-SPGR) magnetic resonance and PET images were transferred to the navigation system. The surgical procedure was either biopsy or tumor resection. In both procedures, the tissue showing the highest FDG tracer uptake was carefully resected and cryopreserved at −80°C.

2.2. Sample Selection and Genetic Analysis of IDH Mutation. To perform the gene expression analysis of glucose metabolism and related molecules, surgical specimens obtained from the patients were selected according to the following criteria: (1) obtained during primary tumor resection without any adjuvant therapy, including anticancer chemotherapy or radiation therapy, and (2) histologically diagnosed as glioblastoma based on the WHO classification [1]. DNA/RNA extraction from the frozen tumor tissue and analysis of the mutational status of isocitrate dehydrogenase (IDH) 1/2 were performed as previously described [19].

2.3. Image Acquisition and Reconstruction. For each patient, 18F-FDG and 18F-FMISO PET were performed in random order within 1 week. PET imaging protocols for both 18F-FDG and 18F-FMISO were consistent with our previous studies [7, 9]. PET scans were conducted using one of following three PET scanners: an ECAT EXACT HR+ PET scanner (Siemens, Munich, Germany), a Biograph 64 PET computed tomography (CT) scanner (Siemens, Munich, Germany), and Gemini TF64 PET-CT scanner (Philips, Amsterdam, Netherlands). All scanners were operated in a three-dimensional reconstruction mode. On the ECAT
EXACT HR+ PET scanner, the emission acquisition was performed after a 3 min transmission scan with a $^{68}$Ge/$^{68}$Ga retractable line source. On the Biograph 64 PET/CT and Gemini TF64 PET-CT scanners, CT scanning was performed after an emission acquisition. Transmission images from HR+ and CT images from Biograph 64 PET/CT and Gemini TF64 PET-CT were used to estimate attenuation maps. Attenuation-corrected radioactivity images were reconstructed using the filtered back-projection (ECAT EXACT HR+ and Biograph 64) or the ordered subset expectation maximization method (Gemini TF64) with a Hann filter of 4 mm full width at half maximum. For $^{18}$F-FDG PET, patients had fasted for >6 h before the scheduled tracer injection. $^{18}$F-FDG was intravenously injected, and 10 min emission scanning was performed at 1.23 ± 0.3 h post-tracer injection. The actual injected dose was 323.3 ± 90.6 MBq. For $^{18}$F-FMISO PET, 10 min static PET scanning was performed at 4.06 ± 0.3 h post-tracer injection, according to our previously investigation [20]. The actual injected dose was 413.9 ± 28.2 MBq.

2.4. Imaging Analysis. The lesion-to-normal cerebellum ratio (LNR) was calculated for semiquantitative analysis as previously described [8]; the maximum standardized uptake value (SUV) of the tumor was divided by the average cerebellar SUV and LNR.

2.5. Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) Analysis. As a control reference, two sets of commercially available human brain total RNA were obtained (Life Technologies; Clontech). The glioma cDNA was synthesized from 1 mg of the total RNA using the Pri-meScript™ II 1st Strand cDNA Synthesis Kit (Takara Biotechnology Co., Ltd., Kusatsu, Japan). Several genes associated with hypoxia and glucose metabolism were selected, including GLUT1, GLUT3, G6PC1, G6PC2, G6PC3, HK1, HK2, vascular endothelial growth factor (VEGF), and proliferation cell nuclear antigen (PCNA). The oligonucleotide primers are listed in Table S1. qRT-PCR analysis was performed using FastStart Essential DNA Green Master with LightCycler 96 (Roche Diagnostics, Basel, Switzerland), and the PCR product specificities were confirmed by melt curve analysis. PCR experiments were run in triplicate. The relative target gene mRNA expression levels compared to 18S rRNA were measured by qPCR using the 2$^{-\Delta\Delta CT}$ method [21]. The relationship between mRNA expression and LNR of $^{18}$F-FDG PET was explored. Moreover, each mRNA expression was evaluated in two groups of specimens categorized by the degree of hypoxia in a 2.0 threshold of FMISO LNR, as has been described previously [22].

2.6. Immunofluorescence Analysis. To evaluate the protein expression of G6PC3 and GLUT1, triple staining was performed in a glioblastoma sample with antibodies against G6PC3 (1:100, ab221647, Abcam plc, Cambridge, UK) and GLUT1 (1:400, 66290-1-IG, Proteintech, Manchester, UK). A formalin-fixed paraffin-embedded (FFPE) block was used for antigen retrieval as previously described [23] and then blocked with Block Ace (UKB80, DS Pharma Biomedical, Osaka, Japan). Diluted with 1%BSA-PBC were added followed by overnight incubation at 4°C. As secondary antibodies, Alexa Fluor goat anti-mouse-488 (1:400, A11001, Invitrogen, Massachusetts, USA) and goat antirabbit-594 (1:400, A11012, Invitrogen,). All nuclei were stained with ProLong Diamond antifade reagent (P36962, Invitrogen, Massachusetts, USA). All images of Immunofluorescence staining was obtained using a fluorescent microscope (BZ-X710, KEYENCE, Osaka, Japan).

2.7. Association between Candidate Gene Expression and Survival. To analyze the association between candidate gene expression and prognosis in glioblastoma, we subjectively selected patients with glioblastoma independent of the discovery cohort. Among the patients with glioblastoma who underwent gross total resection of primary tumors prior to the standard treatment [24], eight patients with >3 years of survival after the primary surgery were selected as the good prognosis group and 9 patients with <1 year of survival as the poor prognosis group. RNA extraction from each frozen tumor tissues and gene expressions of candidate molecule were performed as the same manner as discovery cohort.

2.8. Data Mining in TCGA. Clinical information, gene expression, and gene mutation status were obtained from The Cancer Genome Atlas (TCGA, https://portal.gdc.cancer.gov/) database for patients with glioblastoma. Patients aged <16 years at the time of diagnosis and those without survival data were excluded. The raw count data of RNA-seq were obtained from TCGA and then normalized using the edgeR package (version 3.26.1) in R (version 3.5.3). The OS of patients with glioblastoma in TCGA was evaluated according to mRNA expression of candidate gene.

2.9. Statistical Analysis. The mRNA expression levels in patients with glioblastoma were compared by the Mann-Whitney U test between groups. Categorical variables were expressed as frequency and compared using the chi-square test. Correlation between mRNA expression and LNR was tested using Spearman’s rank correlation test. The candidate gene expression level higher than the median value was

<table>
<thead>
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<th>Parameters</th>
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<tr>
<td>Age (years)</td>
<td>Median (±SD)</td>
</tr>
<tr>
<td></td>
<td>61.0 (±15.6)</td>
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<tr>
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<td>Female</td>
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<tr>
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<td>16</td>
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<tr>
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<tr>
<td></td>
<td>IDH mutant without 1p/19q codeletion</td>
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considered high expression, whereas the gene expression level lower than the median value was considered low expression. The OS was presented in Kaplan–Meier curves. The Kaplan–Meier survival curves with the log-rank test were calculated and then plotted using GraphPad Prism version 8. \( P < 0.05 \) was considered to indicate a statistically significant difference.

3. Results

3.1. Patient Characteristics. For gene expression analysis, 33 glioblastoma patients who underwent preoperative \(^{18}\text{F}-\text{FMISO}\)/\(^{18}\text{F}-\text{FDG}\) PET were included. As shown in Table 1, study participants consisted of 16 female (48.4%) and 17 male (51.6%) patients (age \(61.0 \pm 15.6\) years). \( IDH \) wild type in 32 patients and \( IDH1\)-R132H mutation in one patient were confirmed with retained 1p19q.

3.2. The Relationship between Hypoxia- and Glucose-Related Gene Expression and FDG PET Value. The LNR of \(^{18}\text{F}-\text{FDG}\) uptake did not correlate with \(^{18}\text{F}-\text{FMISO}\) LNR (\( P = 0.43 \)). (b, c) Hexokinase 2 and glucose-6-phosphatase catalytic unit 3 mRNA levels significantly correlated with FDG LNR (\( P = 0.03, Y = 1.52X - 0.24, R^2 = 0.14 \) and \( P = 0.02, \log_2 Y = 3.02X - 1.40, R^2 = 0.16 \), respectively). These were tested by Spearman’s rank correlation coefficient.

![Figure 2: Relationship between glucose metabolism-related molecule mRNA levels and fluorine-18-fluorodeoxyglucose (\(^{18}\text{F}-\text{FDG}\)) uptake lesion-to-normal brain ratio (LNR). (a) Glucose transporter 1 (GLUT1) mRNA levels did not correlate with \(^{18}\text{F}-\text{FDG}\) LNR (\( P = 0.43 \)). (b, c) Hexokinase 2 and glucose-6-phosphatase catalytic unit 3 mRNA levels significantly correlated with \(^{18}\text{F}-\text{FDG}\) LNR (\( P = 0.03, Y = 1.52X - 0.24, R^2 = 0.14 \) and \( P = 0.02, \log_2 Y = 3.02X - 1.40, R^2 = 0.16 \), respectively). These were tested by Spearman’s rank correlation coefficient.

3.3. Comparison between Hypoxia- and Glucose-Related Gene Expressions at the Intensity of FMISO Accumulation. Next, we investigated the correlation between the mRNA expression of each gene and the intensity of FMISO accumulation. As described above, glioblastomas were divided into high and low FMISO accumulation in a 2.0 threshold of FMISO LNR. In this series, \(^{18}\text{F}-\text{FDG}\) uptake LNR in high \(^{18}\text{F}-\text{FMISO}\)-accumulated tissues was higher than in low \(^{18}\text{F}-\text{FMISO}\)-accumulated tissues (Figure 3(a)). mRNA levels of GLUT1 and G6PC3 in samples from patients with high \(^{18}\text{F}-\text{FMISO}\) accumulation were significantly higher than those from patients with low FMISO accumulation (\( GLUT1: P = 0.04; G6PC3: P < 0.01 \); Figures 3(b) and 3(c)). In contrast, there is no difference in HK2 mRNA expression depending on \(^{18}\text{F}-\text{FMISO}\) accumulation (\( P = 0.79 \); Figure 3(d)). Other molecule mRNA expression did not have significant difference depending on \(^{18}\text{F}-\text{FMISO}\) accumulation (Figure S2).

3.4. The Correlation between G6PC3 Gene and Protein Expression in Glioblastoma. To confirm whether protein synthesis was regulated by mRNA regarding G6PC3 expression, immunofluorescence staining was performed. As
shown in Figure 4, merged images showed that G6PC3 was strongly expressed in cytoplasm of all samples with high mRNA expression while samples with G6PC3 low expression had faintly stained cytoplasm. Anti-GLUT1 staining had similar tendency of anti-G6PC3.

3.5. The Correlation between G6PC3 Expression and Overall Survival of Patients with Glioblastoma.

Since G6PC3 might be a key molecule of glioblastoma under hypoxic condition through previous evaluation, prognostic analysis in glioblastoma was performed whether G6PC3 expression is associated with the survival. First, using different cohorts in our archive, the G6PC3 mRNA expression of glioblastoma tissues was investigated in two groups of patients according to their prognosis (Table. S2). This demonstrated that G6PC3 mRNA expression was markedly higher in the poor prognosis group than that in the good prognosis group ($P = 0.027$) (Figure 5(a)). Then, we further attempted to validate the correlation between G6PC3 gene expression and prognosis using TCGA database for a larger population (Table. S3). Patients with high G6PC3 mRNA expression ($n = 84$, median 385.0 days, 95% C.I. 0.46–0.91) had significantly poorer OS than those with low G6PC3 mRNA expression ($n = 83$, median 455.0 days, 95% C.I. 1.11–2.18) ($P = 0.008$) (Figure 5(b)). These results suggested that high G6PC3 expression is associated with poor prognosis in glioblastoma.

4. Discussion

Using $^{18}$F-FDG and $^{18}$F-FMISO PET, we previously demonstrated that the degree of glucose metabolic activation in hypoxic area in glioblastoma was correlated their prognosis [9]. In this exploratory study, we identified the potential key molecule, G6PC3, to highly activate glucose metabolism under hypoxia. G6PC3 expression was elevated in tissues of glioblastoma with higher $^{18}$F-FDG and $^{18}$F-FMISO accumulation, and also, G6PC3 elevation was related to the poor prognosis in another independent cohort as well as TCGA database, indicating that it could become a prognostic marker in glioblastomas.

In malignant tumor cells, especially under severe hypoxic conditions, HIF-1α is often promoted through signal transduction pathways, such as the phosphoinositide-3-kinase/Akt/mTOR pathway, extracellular-regulated protein kinase pathway, and adenosine 5'-monophosphate-activated protein kinase pathway. Hypoxia-induced metabolic reprogramming was essential to satisfy cellular energetic demands...
during acute hypoxic stress via HIF-1α [25, 26]. Tumor cells reduce their dependency on mitochondrial oxidative phosphorylation and switch to the O2-independent glycolytic pathway to promote adenosine triphosphate production to meet their demands [17]. HIF-1α forms a complex of HIF-1α or other proteins and adheres to hypoxia-responsive elements (HRE; i.e., 5′-ACGTG-3′) on the genome line. HIF-1α binds with HRE and stimulates the downstream enzymes in the glycolytic system, such as glucose transporters, hexokinase (HK), pyruvate kinase, and lactate dehydrogenase A [26, 27]. This cascade of response to hypoxia enables tumor cells to metabolize glucose anaerobically and survive under severe hypoxia.

We started this study with a hypothesis that molecular features relating to 18F-FDG and 18F-FMISO accumulation might affect tumor aggressivity. According to previous investigations, 18F-FDG uptake has been reported to reflect GLUT1 [28, 29] and HK2 [30, 31] in gliomas or other
malignant tumors. Therefore, we determined molecules associated with glucose and investigate the relationship between mRNA expression and a lesional semiquantitative value obtained from $^{18}$F-FDG and $^{18}$F-FMISO PET. We could not identify the single parameter as an $^{18}$F-FDG uptake regulator. Not only GLUT1 but also HK2 and G6PC3 were significantly correlated with $^{18}$F-FDG uptake, although GLUT1 also tended to correlate with $^{18}$F-FDG uptake. This was partially not consistent with that of previous studies that reduced G6Pase exhibited a high $^{18}$F-FDG uptake in some tumors [32]. Since glucose metabolism might be determined by multiple factors in complex manners, the exact mechanism remains unclear.

In previous reports, G6Pase was thought to be associated with $^{18}$F-FDG6P washout [13, 16, 33]. Generally, it was reported to be inversely correlated with $^{18}$F-FDG uptake in hepatocellular carcinoma [34]. However, a recent analysis for $^{18}$F-FDG kinetics delineated that $^{18}$F-FDG-6P hydrolysis by G6Pase was extremely low to be negligible, whereas $^{18}$F-FDG uptake and glucose consumption were tremendously high [33, 35]. Furthermore, $^{18}$F-FDG release has been found to reflect the expression of the glucose-6-phosphate transporter more than that of G6Pase [13, 14]. Altogether, G6Pase did not function as a negative factor of $^{18}$F-FDG uptake since the glycolysis metabolites might shunt into gluconeogenesis via G6Pase due to increased $^{18}$F-FDG uptake. Although G6PC3 has been less likely known to correlate with $^{18}$F-FDG uptake in glioma, a study reported that the maximum SUV of $^{18}$F-FDG uptake correlated with G6PC3 mRNA expression in intrahepatic cholangiocarcinoma [36].

GLUT1 and G6PC3 expressions were significantly correlated with high $^{18}$F-FMISO accumulation in this study. GLUT1 was a well-known molecule induced by hypoxia [17, 37]. G6Pase was also reported to be increased under hypoxia by the HIF-1α activation [38]. Conversely, G6PC3 was not significantly changed by hypoxia in the mesenchymal stem cells [39]. No study has reported the effects of hypoxia on G6PC3 mRNA expression in glioma. Therefore, the influence of hypoxia on G6PC3 expression should be further explored.

G6Pase catalyzes the hydrolysis of glucose or $^{18}$F-FDG-6-phosphate to $^{18}$F-FDG. It is located in the endoplasmic reticulum [16, 40]. G6Pase was reported to play a protumorigenic role in cell proliferation and migration mainly by regulating oncogenic signaling in glioblastoma. Starving brain tumor-initiating cell was able to survive under glycolytic inhibition, owing to the function of G6Pase as a gluconeogenesis catalyst. Interestingly, surviving cells had more aggressive characteristics after glycolytic inhibition in terms of migration, invasion, and proliferation [41]. G6PC3 is an isoform of G6Pase and was originally known as G6Pase-β. The G6Pase gene family consists of three types of isoforms: G6PC, G6PC2, and G6PC3. G6PC3 is expressed ubiquitously although predominantly in the brain, muscle, and kidney [16, 42]. In our study, G6PC3 protein synthesis was found to be consistent with mRNA expression in glioblastoma. Given that GLUT1 expression had similar tendency, G6PC3 might have an important role in glucose metabolism affecting tumor aggressivity.

Lastly, prognostic analysis was performed using two independent patient cohorts. Both of them proved that higher G6PC3 mRNA expression was associated with a poorer prognosis based on OS. In particular, the Kaplan–Meier survival curve analysis in TCGA dataset revealed that high G6PC3 expression was significantly associated with poor OS. Recently, G6PC3 has been focused on as a candidate prognostic predictor of glioblastoma through big data gene expression analysis [43]. However, no reports have elucidated the molecular mechanism of the relationship between the G6PC3 gene expression and prognosis. Then, G6PC3 induction by hypoxia can be its clue.

Regarding the study limitations, first, mRNA gene expression of frozen samples should be interpreted with tumor heterogeneity. The validity of the tumor sampling site was difficult. Second, the number of samples was small. Although this study could not unravel the underlying mechanism, G6PC3 was found to be a potential candidate of potent prognostic factor.

### 5. Conclusions

G6PC3 was affluently expressed in the tumor with high $^{18}$F-FDG and $^{18}$F-FMISO accumulation. It might be a key molecule of glucose metabolism under hypoxia in glioblastoma. Moreover, it can also work as a prognostic predictor of glioblastoma.

### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>CT:</td>
<td>Computed tomography</td>
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<tr>
<td>$^{18}$F-FMISO:</td>
<td>Fluorine-18-fluoromisonidazole</td>
</tr>
<tr>
<td>$^{18}$F-FDG:</td>
<td>Fluorine-18-fluorodeoxyglucose</td>
</tr>
<tr>
<td>FFPE:</td>
<td>Formalin-fixed paraffin-embedded</td>
</tr>
<tr>
<td>GLUT:</td>
<td>Glucose transporter</td>
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<tr>
<td>G6Pase:</td>
<td>Glucose-6-phosphatase</td>
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<tr>
<td>G6PC:</td>
<td>Glucose-6-phosphatase catalytic unit</td>
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<tr>
<td>HIF:</td>
<td>Hypoxia-inducible factor</td>
</tr>
<tr>
<td>HK:</td>
<td>Hexokinase</td>
</tr>
<tr>
<td>HRE:</td>
<td>Hypoxia-responsive elements</td>
</tr>
<tr>
<td>IDH:</td>
<td>Isocitrate dehydrogenase</td>
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<tr>
<td>LNR:</td>
<td>Lesion-normal tissue ratio</td>
</tr>
<tr>
<td>OS:</td>
<td>Overall survival</td>
</tr>
<tr>
<td>PCNA:</td>
<td>Proliferation cell nuclear antigen</td>
</tr>
<tr>
<td>RT-qPCR:</td>
<td>Reverse transcriptase quantitative polymerase chain reaction</td>
</tr>
<tr>
<td>SUV:</td>
<td>Standardized uptake value</td>
</tr>
<tr>
<td>TCGA:</td>
<td>The Cancer Genome Atlas</td>
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<tr>
<td>VEGF:</td>
<td>Vascular endothelial growth factor</td>
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<td>WHO:</td>
<td>World Health Organization</td>
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### Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.
Ethical Approval

The institutional review board (IRB) of Hokkaido University Hospital for Clinical Research approved this study (no. 018-0363).

Consent

Written informed consent of FMISO-PET was obtained from each patient who participated in the study. And it was permitted by IRB that consent of additional analysis regarding FDG-PET was waived in this study since FDG-PET was performed as one of health insurance treatment.

Disclosure

A preprint has previously been published [44].

Conflicts of Interest

The authors have no relevant financial or nonfinancial interests to disclose.

Authors’ Contributions

MO and SY conceived the ideas and designed the experiments. MO and SE performed the experiments. SY, YI, and HM provided the clinical samples. TT and HK provided clinical information relating to the PET study. MO analyzed the study data. SK and RS contributed to the drafting of the manuscript. MO and SY wrote the paper. MF participated in the critical review and approval of the final version for publication. All authors read and approved the final manuscript.

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Supplementary Materials

Supplementary 1. Figure S1: relationship between glucose metabolism associated with mRNA levels and 18F-FDG uptake LNR. (a) Glucose transporter 3 (GLUT3), (b) glucose-6-phosphatase catalytic subunit 1 (G6PC1), (c) G6PC2, (d) hexokinase 1 (HK1), (e) vascular endothelial growth factor (VEGF), and (f) proliferation cell nuclear antigen (PCNA) mRNA levels were not correlated with 18F-FDG uptake LNR.

Supplementary 2. Figure S2: comparison of mRNA levels with a threshold of 18F-FMISO accumulation; LNR 2.0. (a) Glucose transporter 3 (GLUT3), (b) glucose-6-phosphatase catalytic subunit 1 (G6PC1), (c) G6PC2, (d) hexokinase 1 (HK1), (e) vascular endothelial growth factor (VEGF), and (f) proliferation cell nuclear antigen (PCNA) mRNA levels were changed between 18F-FMISO accumulation.

Supplementary 3. Table S1: the primer sequences of GLUT1, GLUT3, G6PC1, G6PC2, G6PC3, HK1, HK2, PCNA, VEGF, and 18S rRNA.

Supplementary 4. Table S2: patient characteristics and the relationship between G6PC3 mRNA expression and clinicopathological characteristics in glioblastoma patients in another cohort for validation.

Supplementary 5. Table S3: patient characteristics and the relationship between G6PC3 mRNA expression and clinicopathological characteristics in glioblastoma patients based on the TCGA database.

References


