Association of ADC of hyperintense lesions on FLAIR images with TERT promoter mutation status in glioblastoma IDH wild type

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Research Article

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Abstract

Purpose: Although mutations in telomerase reverse transcriptase (TERT) promoter (TERTp) are the most common alterations in glioblastoma, predicting TERTp mutation status by preoperative imaging is difficult. We determined whether tumor-surrounding hyperintense lesions on fluid-attenuated inversion recovery (FLAIR) were superior to those of contrast-enhanced lesions (CEL) in assessing TERTp mutation status using magnetic resonance imaging (MRI).

Methods: This retrospective study included 114 consecutive patients with primary isocitrate dehydrogenase (IDH)-wild-type glioblastoma. Apparent diffusion coefficient (ADC) and volume of CELs and FHLs were determined, and the correlation between MRI features and TERTp mutation status was analyzed. In a subset of cases, FHLs were histopathologically analyzed to determine the correlation of tumor cell density and ADC.

Results: TERTp mutations were present in 77 (67.5%) patients. The minimum ADC of FHLs was significantly lower in the TERTp-mutant group than in the TERTp-wild-type group (mean, 961.0 × 10⁻³ and 1159.9 × 10⁻³ \text{m²/s}, respectively; \( P < 0.01 \)). However, other MRI features such as CEL and FHL volumes, minimum ADC of CELs, and FHL/CEL ratio, were not significantly different between the two groups. Histopathologic analysis indicated high tumor cell density in FHLs with low ADC.

Conclusion: The ADC of FHLs was significantly lower in IDH-wild-type glioblastoma with TERTp mutations, suggesting that determining the ADC of FHLs on preoperative MRI might be helpful in predicting TERTp mutation status and surgical planning.

Introduction

Glioblastoma (GBM) is the most common primary malignant tumor of the central nervous system in adults [1]. In patients with GBM, the primary aim of surgical resection is complete disappearance of contrast-enhanced lesions (CELS) on magnetic resonance imaging (MRI) to achieve gross total resection [2, 3]. However, median survival remains approximately 24 months despite gross total resection in combination with concomitant chemoradiation therapy based on temozolomide [4, 5].

Several genetic markers such as methylguanine methyltransferase promoter unmethylation, phosphatase and tensin homolog gene mutations, epidermal growth factor receptor gene amplification are associated with poor survival in patients with GBM [6, 7]. In addition, recent reports indicate that 70%–80% of GBMs harbor either the C228T or C250T mutation in the promoter region of TERT (telomerase reverse transcriptase). [8, 9] Although several studies reported the prognostic significance of TERT promoter (TERTp) mutations in patients with GBM, their clinical and pathologic impact remains unclear [10, 11].

Necrosis detected by magnetic resonance imaging (MRI) has been reported to indicate the presence of TERTp mutations [12]. However, predicting TERTp status by preoperative imaging studies alone remains difficult.
We recently reported that *TERTp* mutations were strongly associated with poor prognosis and multifocal/distant lesions in *IDH* (isocitrate dehydrogenase)-wild-type GBMs [13]. Another study demonstrated that GBM cells infiltrated the tissue surrounding CELs on T1-weighted MRI scans [14]. Fluid-attenuated inversion recovery (FLAIR) images are considered to represent such invasive cells as well as cerebral edema. However, discriminating tumor invasion from cerebral edema in hyperintense lesions on FLAIR (FHLs) remains a challenge. We previously reported that apparent diffusion coefficient (ADC) calculated using diffusion-weighted imaging was useful in evaluating tumor invasion in FHLs [15]. This finding raises the possibility that *IDH*-wild-type GBMs with *TERTp* mutations might be invasive and that MRI might aid in the detection of tumor cells infiltrating the tissue beyond CELs. However, to our knowledge, no study to date has examined the relationship between the *TERTp* mutation status and FHLs on MRI.

Therefore, we aimed to determine if the *TERTp* mutation status correlated with MRI features, with a focus on the ADC of FHLs.

**Materials And Methods**

**Patients and samples**

This retrospective study was conducted with the approval of the Ethics Committee of the Yamagata University Faculty of Medicine, and written informed consent using the optout approach was obtained from all patients or their families. Among 115 consecutive patients treated between January 2009 and October 2021, 114 patients who met the following inclusion criteria were included: 1) diagnosis of grade 4 *IDH*-wild-type GBM according to the 2021 World Health Organization classification [1]; 2) no history of former lower-grade tumors; 3) available genomic DNA; 4) available neuroradiological examinations including contrast-enhanced MRI and FLAIR images on preoperative MRI.

In all patients, tumor specimens were obtained from lesions that exhibited enhancement on gadolinium-enhanced MRI scans and immediately stored at −80°C until DNA extraction.

**MRI**

All patients underwent preoperative MRI within 7 days prior to surgery with a 1.5- or 3.0-T Achieva MRI scanner (Philips Medical Systems, Amsterdam, Netherlands). Diffusion-weighted imaging was conducted with b-values of 0 and 1000 sec/mm² to generate ADC maps. Sequences were obtained using 30 slices, with a slice thickness of 5 mm. Impax EE picture archiving and communication system (Agfa Healthcare, Bonn, Germany) was used for data evaluation. In FLAIR examination, the following settings were used: set repetition time, 9000 msec; echo time, 125 msec; inversion time, 2500 msec; and slice thickness, 6 mm. First, FLAR images were used to set several regions of interests (ROIs) in almost all FHLs surrounding the CEL; the ROIs were placed at least 3 mm away from the CEL. Next, ADCs of corresponding ROIs on FHLs were determined (Fig. 1).
Tumor and FHL volumes were measured after image fusion using iPlan 3.0 (BrainLab AG). On gadolinium-enhanced and FLAIR images, CEL and FHL segmentations based on differences in signal intensity were created using the “smartbrush” tool. Next, volumetric evaluations of CELs and FHLs were completed using multiple ROIs (Fig. 1). Additional neuroradiological features, such as necrosis, multifocal lesions, and lesions crossing midline, were also evaluated in MRI scans.

**Molecular analysis**

Genomic DNA was extracted using the QIAamp DNA mini kit (Qiagen), according to the manufacturer’s instructions. Mutation status for *IDH1*, *IDH2*, and *TERTp* was analyzed using Sanger sequencing, as described previously [16].

**Statistical analysis**

Statistical analyses were performed using SPSS ver. 26 (IBM Japan, Tokyo, Japan). Relationships between two variables were evaluated using the Mann–Whitney *U* test. Pearson’s correlation coefficient analysis was used to evaluate the association between ADC and tumor cell density.

**Histopathological analysis of FHLs**

For the comparison of tumor cell density and ADC of biopsied FHLs, navigation-guided multiple tumor sampling of FHLs was performed in 10 patients with GBM who had available tissue samples. Tumor cell counts were determined in three high-powered fields of view (400×) in each sample, as previously described [17]. All cells left after the exclusion of normal cells, such as vascular cells and hematogenic cells, were counted.

**Results**

**Patient characteristics**

Among 115 cases with the histological features of GBM, one patient with *IDH1* mutation was excluded from the study. Therefore, a total of 114 patients with *IDH*-wild type GBM, including 56 male and 58 female patients, with a median of age of 68 years (range, 39–86 years) were included in the present study. The tumors were located in the frontal, temporal, parietal, and occipital lobes in 40, 36, 26, and 4 patients, respectively, and in insula, basal ganglia, and corpus callosum in 5, 2, and 1 patient, respectively. Gross total surgical resection was performed in 69 (60.5%) patients. *TERTp* mutations were found in 77 of the 114 (67.5%) patients; of these, 89 patients were previously reported [13]. There were no significant differences in age, sex, preoperative Karnofsky performance scale score, tumor localization, and removal rate between the patients with and without *TERTp* mutations (Table 1).

**ADC volume and value of CELs and FHLs**
As shown in Fig. 1, the ROIs were set around the CELs and FHLs and minimum ADCs of ROIs in both lesions were calculated. Total CEL and FHL volumes were also measured using volumetric analysis (Fig. 1). The results are summarized in Table 2. Briefly, the mean CEL and FHL volumes were 29.8 and 50.3 cm³, respectively; these values were not significantly different between the TERTp-wild type and TERTp-mutant groups (CEL, 33.7 vs. 31.6 cm³, respectively, \( P = 0.74 \); FHL, 50.3 vs. 54.6 cm³, \( P = 0.51 \), respectively). Similarly, the FHL/CEL ratio was larger in the TERTp-mutant group than in the TERTp-wild-type group, although the difference was not statistically significant (1.82 vs. 1.11, \( P = 0.39 \)).

The minimum ADC of CELs was not significantly different between the TERTp-wild type and TERTp-mutant groups (668.5 \( \times 10^{-3} \) and 681.2 \( \times 10^{-3} \) mm²/s, respectively; \( P = 0.49 \)) (Fig. 2A). However, the minimum ADC of FHLs was significantly lower in the TERTp-mutant group than in the TERTp-wild-type group (mean, 961.0 \( \times 10^{-3} \) and 1159.9 \( \times 10^{-3} \) mm²/s, respectively; \( P < 0.01 \)) (Fig. 2B). Furthermore, the frequency of multifocal lesions was significantly higher in the TERTp-mutant group than in the TERTp-wild-type group (\( P = 0.03 \)), as we previously reported [11], whereas the other MRI features were not significantly different between the two groups.

**Correlation between the tumor cell density and ADC of FHLs**

As shown in Fig. 3, sparse tumor cells (Fig. 3A) were found in FHLs with high ADCs (1534.9 \( \times 10^{-3} \) mm²/s) whereas tumor cell density was higher in FHLs with low ADC (1272.5 \( \times 10^{-3} \) mm²/s). There was a trend toward a negative correlation between ADC and tumor cell density; however, a statistically significant correlation could not be observed due to the small number of tumor samples (Fig. 3).

**Discussion**

TERTp mutations are the most common genetic abnormality in GBM. Previous studies suggested that TERTp mutations were involved in multiple lesions, remote recurrence, and poor prognosis in GBM [8-11, 13]. Furthermore, TERTp mutations were shown to be associated with invasiveness and metastasis in other carcinomas, such as urothelial cancer, thyroid cancer, melanoma, non-small cell lung cancer, and squamous cell carcinoma [18-22].

ADC, a well-known parameter associated with tumor cell density and infiltration, has been used to predict prognosis and to evaluate recurrent lesions in patients with GBM [23-28]. Additionally, we recently reported that FHLs with lower ADC were associated with future relapse sites [15], suggesting the presence of infiltrating tumor cells around CELs in FHLs with low ADC.

In the present study, we could not detect differences in the MRI features of CELs and FHLs between the TERTp-wild type and TERTp-mutant groups. These results confirm previous studies suggesting that predicting TERTp mutation status by preoperative MRI alone was difficult [12, 29, 30].
In the present study, we also confirmed the findings of a previous study which reported that the ADC of CELs did not differ between $TERT_p$ wild type and $TERT_p$-mutant GBMs [30]. However, we found that the minimum ADC of FHLs was significantly lower in the $TERT_p$-mutant group than in the $TERT_p$-wild-type group, suggesting that GBMs with $TERT_p$ mutations were more aggressive and that the number of tumor cells infiltrating the FHL was high. In the present study, we histologically confirmed aggressive tumor invasion in areas with low ADC in FHLs.

Several recent studies reported the clinical utility of the additional removal of FHLs beyond gross total resection [31-33]. However, complete removal of FHLs without inducing morbidity is difficult [31, 32]. In such cases, resection of areas with low ADC in FHLs might be considered as a new resection approach in GBM surgery. Particularly, GBMs with $TERT_p$ mutations harboring low-ADC lesions in FHLs might be good candidates for this approach.

This study has several limitations. First, this was a single-center study including the analysis of retrospectively collected datasets. Second, there were relatively small cases in which tumor cell density was determined with histological evaluation.

**Conclusion**

ADC of FHLs was significantly lower in GBMs with $TERT_p$ mutations than in those with wild-type $TERT_p$. These results suggest that the ADC of FHLs on preoperative MRI might be helpful in predicting the $TERT_p$ mutation status and surgical planning.

**Declarations**

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**Compliance with ethical standards**

**Conflict of interest:** All authors declare that they have no conflicts of interest.

**Ethical approval:** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed consent:** Informed consent was obtained from all participants included in the study.

**Author Contributions**
Yukihiko Sonoda contributed to the study conception and design. Material preparation, data collection and analysis were performed by Ken-ichiro Matsuda, Yuta Mitobe, Yonehiro Kanemura, Misturu Futakuchi and Masafumi Kanoto. The first draft of the manuscript was written by Ken-ichiro Matsuda and Yukihiko Sonoda and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

References


**Tables**

Tables 1-2 is available in the Supplementary Files section.

**Figures**
Figure 1

Image analysis of preoperative MRI scans. Several regions of interests (ROIs) of hyperintense lesions on fluid-attenuated inversion recovery (FLAIR) (FHLs) at least 3 mm away from the contrast-enhanced lesion (CEL) (A) on FLAIR (B). Next, apparent diffusion coefficients (ADCs) of corresponding ROIs in FHLs were calculated on the ADC map (C). CEL and FHL volumes were measured after image fusion of contrast-enhanced T1-weighted and FLAIR images. Segmentations of CEL and FHL were based on differences in signal intensity using the semiautomatic “smartbrush” tool (D). Finally, 3D volumetric evaluations of CELs and FHLs were performed (E).

MRI, magnetic resonance imaging
Figure 2

Bar graphs showing the comparison of FHL and minimum ADC values between the groups with and without *TERT* promotor mutations. (A) Note that the FHL volume is not significantly different between the two groups. (B) Minimum ADC in ROIs on preoperative MRI scans was significantly lower in patients with *TERT* promotor mutations (light bar) than in those without *TERT* promotor mutations (dark bar).
**Figure 3**

Histological features of FHLs. (A) Note sparse tumor cells in a tissue sample collected from a lesion with high ADC ($1534.9 \times 10^{-3} \text{ mm}^2/\text{s}$). (B) Note high tumor cell density in a tissue sample collected from a lesion with low ADC ($1272.5 \times 10^{-3} \text{ mm}^2/\text{s}$). (C) There was a trend toward a negative correlation between ADC and tumor cell density, although a statistically significant correlation could not be observed due to the small number of tumor samples.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- Table12023030.xlsx
- Table22023130.xlsx