Malignant gliomas are primary brain tumors associated with a seriously short life expectancy for patients. They consist of several different pathological diseases, such as glioblastoma, anaplastic astrocytoma, and oligodendroglioma. When these lesions are imaged using MRI, they usually present as gadolinium-enhanced lesions on T1-weighted images surrounded by hyperintense lesions on T2-weighted images that are thought to represent tumor-infiltrative or vasogenic brain edema around the tumor core. Detailed and precise radiological determination of the extent of tumor cell aggregation or infiltration within the brain tissue is not only a crucial step for treatment planning but also for accurate evaluation of therapeutic response of the disease. Introduction of antiangiogenic therapy to treat malignant gliomas has made the latter objective more challenging than ever, as those agents strongly modify key radiological features of the tumor, including the magnitude of contrast enhancement of the tumor and the extent of tumor-associated brain edema.

Comparison of diffusion tensor imaging and $^{11}$C-methionine positron emission tomography for reliable prediction of tumor cell density in gliomas

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OBJECTIVE Diffusion MRI is attracting increasing interest for tissue characterization of gliomas, especially after the introduction of antiangiogenic therapy to treat malignant gliomas. The goal of the current study is to elucidate the actual magnitude of the correlation between diffusion MRI and cell density within the tissue. The obtained results were further extended and compared with metabolic imaging with $^{11}$C-methionine (MET) PET.

METHODS Ninety-eight tissue samples from 37 patients were stereotactically obtained via an intraoperative neuronavigation system. Diffusion tensor imaging (DTI) and MET PET were performed as routine presurgical imaging studies for these patients. DTI was converted into fractional anisotropy (FA) and apparent diffusion coefficient (ADC) maps, and MET PET images were registered to Gd-administered T1-weighted images that were used for navigation. Metrics of FA, ADC, and tumor-to-normal tissue ratio of MET PET along with relative values of FA ($rFA$) and ADC ($rADC$) compared with normal-appearing white matter were correlated with cell density of the stereotactically obtained tissues.

RESULTS $rADC$ was significantly lower in lesions obtained from Gd-enhancing lesions than from nonenhancing lesions. Although $rADC$ showed a moderate but statistically significant negative correlation with cell density ($p = 0.010$), MET PET showed a superb positive correlation with cell density ($p < 0.0001$). On the other hand, $rFA$ showed little correlation with cell density.

CONCLUSIONS The presented data validated the use of $rADC$ for estimating the treatment response of gliomas but also caution against overestimating its limited accuracy compared with MET PET.

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KEY WORDS diffusion tensor imaging; DTI; fractional anisotropy; FA; apparent diffusion coefficient; AD; cell density; $^{11}$C-methionine positron emission tomography; oncology
Diffusion MR imaging techniques combined with mathematical and statistical analyses were proposed to solve this challenge with the hope that the reconstructed images could be used to visualize the treatment response in situ and reveal the regions that are unresponsive to treatment to minimize delays in intervention.\textsuperscript{5,8,9} The theoretical background supporting this technique is founded on the hypothesis that cell density within a tissue and the apparent diffusion coefficient (ADC) are negatively correlated. Higher cell density within a region hypothetically should more strongly restrict water diffusion within that region, leading to a decrease in ADC. Although this technique is appealing, the fact that ADC may be affected by necrosis\textsuperscript{10} and conflicting reports regarding the correlation between cell density and ADC\textsuperscript{11,15,22} complicate the above hypothesis. As more and more attempts are being made to use ADC as an imaging biomarker to evaluate the treatment response, understanding the actual magnitude of the correlation between ADC and cell density within the tissue is crucial.

This research was conducted to address this question by comparing glioma tissue samples that were stereotactically obtained during image guidance with presurgical ADC and fractional anisotropy (FA) maps calculated following diffusion tensor imaging (DTI). The investigation was further extended to compare DTI and metabolic imaging with \textsuperscript{11}C-methionine (MET) PET regarding the magnitude of the correlation between imaging and cell density to identify the most reliable imaging modality to evaluate glioma tissue characteristics.

\section*{Methods}

\section*{Patient Selection}

This study was approved by the local ethics committee and was found to conform to generally accepted scientific principles and ethical standards. We collected data from all glioma patients who underwent DTI and/or MET PET for presurgical examination, as well as intraoperative navigation-guided stereotactic tissue sampling between 2007 and 2011. There were 5 patients with low-grade glioma (1 pleomorphic xanthoastrocytoma, 2 diffuse astrocytomas, and 2 oligoastrocytomas) and 32 patients with high-grade glioma (8 anaplastic astrocytomas, 3 anaplastic oligoastrocytomas, and 21 glioblastomas). In total, 98 lesions were obtained from this patient population.

\section*{MRI}

All patients were studied using a 3.0-T MRI scanner (Signa, GE Medical Systems) prior to surgery. T1-weighted images with and without gadolinium enhancement, T2-weighted images, and FLAIR images were acquired in all cases for delineation of tumors. DTI was performed in 27 of the 37 patients included for analysis. As DTI was performed primarily for surgical planning focusing on white matter fiber tracking, DTI was not performed in 10 patients in whom DTI was considered unnecessary as presurgical imaging. Images were acquired using a single-shot echo-planar imaging technique, with TE = 80 msec and TR = 10,000 msec. Diffusion gradient encoding in 25 directions, with \(b = 2000\) sec/mm\(^2\) and an additional measurement without the diffusion gradient (\(b = 0\) sec/mm\(^2\)) were performed.\textsuperscript{26} A parallel imaging technique was used to record data with a spatial resolution of 128 \(\times\) 128 and a field of view of 260 \(\times\) 260 mm. A total of 50 sections were obtained, with a section thickness of 3 mm and no intersection gap. ADC and FA maps were processed using Diffusion Toolkit (Martinos Center for Biomedical Imaging, Massachusetts General Hospital; \url{http://www.trackvis.org/dtk/}).

\section*{PET}

PET studies were performed using the Eminence instrument (Shimazu). MET (111–222 MBq; 3–6 mCi), synthesized according to the method of Berger et al.,\textsuperscript{3} was injected intravenously. Tracer accumulation was recorded over 12 minutes in 99 transaxial slices of the entire brain. Total activity from 20 to 32 minutes after tracer injection was used for image reconstruction. A 3-minute transmission scan in which \textsuperscript{137}Cs was used as the line source preceded data acquisition. Images were stored in 256 \(\times\) 256 \(\times\) 99 anisotropic voxels, with 1 \(\times\) 1 \(\times\) 2.6 mm for each voxel. MET PET was performed in all 37 patients included for analysis.

\section*{Image Fusion and Registration}

After DTI and PET images were obtained, images were registered onto contrast-enhanced T1- or T2-weighted standard anatomical images using normalized mutual information with the Vinci image-analyzing software from the Max-Planck Institute for Neurological Research Cologne (http://www.nf.mpg.de/vinci/). Registration of the images was confirmed visually. The reported registration error for normalized mutual information is less than 1 mm.\textsuperscript{24} After image registration was completed, all image sets, including the standard anatomical MR images, ADC map, FA map, and MET PET, were converted to 256 \(\times\) 256 \(\times\) 256 isotropic, 1 \(\times\) 1 \(\times\) 1-mm images, to enable further voxel-wise analysis (Fig. 1).

\section*{Stereotactic Tissue Sampling}

Thin-slice contrast-enhanced T1-weighted images were transferred to the neuronavigation system, and biopsy targets were planned for histopathological examination. Either the Stryker surgical navigation system (Stryker) or the VectorVision neuronavigation system (Brainlab) was used for image guidance. Tissue samples were obtained randomly from the tumor core for histological diagnosis or from the tumor periphery considered safe for resection for clinical purposes to evaluate viable tumor cells at the resection margin. To minimize the effects of brain shift during resection, biopsies were performed at the earliest stages of surgery. The accuracy of the navigation system was verified by visual confirmation of anatomical landmarks, such as cortical veins and the sulcus. To minimize the influence of brain shift, a Nelaton catheter was inserted under navigation guidance, aiming at the planned biopsy site to “anchor” the target of interest. Subsequently, the targeted area was biopsied by accurately tracing the catheter. Real-time navigation at each biopsy site in the tumor was performed to confirm the biopsy position.
As previous exploratory investigation showed that cell density, rather than MIB-1 activity, showed stronger correlation with MET PET,
19 cell density was chosen as the reference for evaluating tissue characterization capability of the images. Formalin-fixed specimens were embedded in paraffin for histopathological analysis. Hematoxylin and eosin–stained specimens were evaluated to calculate cell density. Cell counting was performed at \( \times 400 \) magnification under light microscopy (Nikon), and all cells were counted, except those that were apparently different from tumor cells, such as endothelial cells or lymphocytes. The area for the tumor cell count was 0.0497 mm\(^2\), and data for cell density were recorded as the mean from 3 different locations within each specimen.

Statistical Analysis
Statistical analysis was performed using JMP (version 8, SAS Institute). The Student t-test was used for 2-group comparisons. A linear regression model using the method for least squares was used for modeling 2 or more independent variables. A \( p \) value < 0.05 was considered statistically significant for both analyses.

Results
Sampling Tissue Characteristics
Because equal sampling of tissues was performed for contrast-enhanced and nonenhanced lesions, characteristics of the obtained tissue were evaluated. Among the 98 obtained samples, 41 samples were from contrast-enhanced and 57 were from nonenhanced lesions. The obtained cohort showed no difference in cell density of the tissue between contrast-enhanced and nonenhanced lesions (Fig. 2A).

DTI Metrics and MET PET for Contrast-Enhanced Versus Nonenhanced Lesions
ADC, rADC (ADC value of the VOI divided by the ADC value contralateral to the VOI), FA, rFA (FA value of the VOI divided by the FA value contralateral to the VOI), and tumor-to-normal tissue ratio (T/Nr) of MET PET. For T/Nr of MET PET, the standardized uptake value of the contralateral tumor-affected gray matter at the axial plane of the thalamus was averaged, and the derived value was used to normalize the standardized uptake value in a voxel-wise manner, enabling calculation of T/Nr of the VOI.

ADC Metrics and Cell Density in Glioma
ADC, rADC, FA, rFA, and T/Nr of MET PET were correlated with cell density within the stereotactically obtained tissues. For ADC and rADC, only rADC showed a moderate but statistically significant negative correlation with cell density (\( p = 0.010 \), Fig. 4C). When the analysis was restricted to high-grade glioma, both metrics showed statistically significant negative correlation with cell density (\( p = 0.010 \) for rADC [Fig. 4D]). FA and rFA both showed little correlation with cell density suitable enough for cell density estimation by using those metrics (Fig. 5 and Supplementary Fig. G and H).

When the analysis was restricted to high-grade glioma, both metrics showed statistically significant negative correlation with cell density and rADC showing stronger correlation (\( p = 0.020 \) for ADC [Fig. 4C] and \( p = 0.010 \) for rADC [Fig. 4D]). FA and rFA both showed little correlation with cell density suitable enough for cell density estimation by using those metrics (Fig. 5 and Supplementary Fig. G and H). Finally, for MET PET, the T/Nr of MET PET showed a superb correlation with cell density (\( p < 0.05 \)).
0.0001, Fig. 6), which was reproducible when analysis was restricted to high-grade glioma (p < 0.0001, Fig. 6 right).

The linear regression fitting was as follows: MET PET T/Nr = 2.0 \times 10^{-4} \times \text{[cell density(cells/mm}^2\text{)]} + 1.0, and as follows for high-grade glioma only-analysis: MET PET T/Nr = 2.1 \times 10^{-4} \times \text{[cell density(cells/mm}^2\text{)]} + 1.0. This was also shown with multiple linear regression analysis of rADC and T/Nr of MET PET for the estimation of cell density. Although T/Nr of MET PET was statistically significant for modeling (p < 0.001), rADC was not (p = 0.06).

**Discussion**

Radiological assessment of brain tumors is one of the most crucial steps in the management of malignant gliomas. Without accurate information about the tumor, one can neither plan surgical and nonsurgical treatment nor perform optimal follow-up of the patient. To make matters more complicated, the introduction of antiangiogenic therapy such as bevacizumab to malignant glioma treatment has altered the way gliomas should be radiologically followed.\textsuperscript{10} As bevacizumab pharmacologically diminishes contrast enhancement of the tumors, which used to be considered one of the hallmarks of tumor recurrence, an alternative and objective imaging biomarker is required for follow-up of patients treated with bevacizumab. Response assessment criteria for high-grade gliomas published by the Response Assessment in Neuro-Oncology Working Group are the most reliable and widely used parameters for radiological response assessment during glioma treatment in real clinical settings.\textsuperscript{25} However, the fact that these criteria rely heavily on information regarding the performance status of the patient and the dosage of steroids prescribed to the patient brings to light the challenges of “pure radiological images” for revealing the treatment response in situ.

The theoretical hypothesis or assumption that increased high cell density restricts water diffusion led to the idea of using ADC to monitor tumor treatment.\textsuperscript{20} A decrease or increase in cell density of the lesion following either successful or unsuccessful treatment could increase or decrease ADC. The concept of a functional diffusion map or a parametric response map was introduced according to this theoretical assumption.\textsuperscript{5,8,9} Based on these theories, it is crucial that imaging parameters such as ADC guarantee precise estimation of cell density within the tissues. However, little histological confirmation has been performed.\textsuperscript{11,15,22} A study that compared autopsy specimens and diffusion images suggested that a decrease in ADC could be attributed to both high cell density and the presence of necrosis.\textsuperscript{10} The fact that contrast-enhancing lesions...
showed a lower rADC than nonenhancing lesions seems to support the above finding (Fig. 2C), as contrast-enhancing lesions are well known to be accompanied by necrotic tissue.

Next, when ADC and rADC were plotted as a function of cell density, a small but statistically significant negative correlation was observed for rADC but not for ADC itself (Fig. 4). This finding does support the use of ADC for analysis such as functional diffusion mapping\(^5,8,9\) or the CIMPLE method\(^6,7\) which claims to independently visualize the proliferation and invasion capability of gliomas. Although these analytical methods use absolute ADC for calculation, the mathematical calculations are shifted toward examining the “relative” changes in ADC, which we have shown to be a valid metric as mentioned above.

Another observation was that FA showed little correlation with cell density (Fig. 5 and Supplementary Fig. G and H). Although a slight positive correlation seems to exist when cell density is greater than 5000 cells/mm\(^2\), it is unlikely that this weak correlation can be clinically use-
ful. This result can be drawn from the fact that FA can be influenced by multiple factors. Not only cell density but also the magnitude of white matter disruption by tumor cell invasion can interfere with FA. FA would initially decrease in relation to disrupted white matter that is invaded by tumor cells, and at a certain point where the white matter is completely destroyed, FA would then be influenced only by the magnitude of cell density, which results in a gradual increase in FA (Fig. 5 and Supplementary Fig. G and H). Finally, the present study showed that MET PET is far more accurate and reliable for estimating cell density of glioma tissue than diffusion-based MR imaging (Figs. 5 and 6). The current study could be criticized for its limitation regarding the accuracy of biopsy sample locations and their image coregistration. Clear differences, however, in the reliability for cell density estimation between diffusion MR and MET PET, as shown in Figs. 4 and 6, elucidates the superiority of MET PET over diffusion MRI as an imaging modality for tissue characterization in gliomas. Positive correlation of MET PET and cell density was previously demonstrated both at the tumor core and tumor periphery. The current investigation is unique in that direct comparison between MET PET and diffusion MRI was performed regarding cell density estimation within glioma tissues. Furthermore, we have previously demonstrated that MET PET could be used as a surrogate imaging modality for glioma treatment response for immunotherapy. Although diffusion MR imaging is attracting more and more interest for tissue characterization of gliomas, our current data demonstrate that amino acid PET is still the de facto standard and that the limitation of diffusion MR imaging in gliomas requires caution. As MET PET is still not widely clinically available in many countries or institutions, it should be mentioned that more efforts should be made to prevail amino acid PET for glioma imaging in daily clinical settings.

Conclusions

Our current study elucidated that rADC showed a mild negative correlation with cell density within glioma tissue, and MET PET demonstrated a more robust and reliable estimation of cell density. We also revealed that FA showed a “J-shaped” curve when correlated with cell density. The presented data suggest that although diffusion MR imaging metrics such as ADC are still valid for estimating the treatment response of glioma tissues in terms of cell density, caution is needed regarding its limited accuracy compared with MET PET.

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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: Kinoshita. Acquisition of data: all authors. Analysis and interpretation of data: all authors. Drafting the article: Kinoshita. Critically revising the article: Kinoshita. Administrative/technical/material support: Kinoshita, Hashimoto, Yoshimine. Study supervision: Kinoshita, Yoshimine.

Supplemental Information

Online-Only Content

Supplemental material is available with the online version of this article.

Supplementary Figure. http://thejns.org/doi/suppl/10.3171/2015.11.JNS151848.

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