Clinical Neurology and Neurosurgery 125 (2014) 212–216

Contents lists available at ScienceDirect

Clinical Neurology and Neurosurgery

journal homepage: www.elsevier.com/locate/clineuro

^{11}C-methinine uptake correlates with *MGMT* promoter methylation in nonenhancing gliomas

Yoshiko Okita[a], Masahiro Nonaka[a,*], Tomoko Shofuda[b], Daisuke Kanematsu[c], Ema Yoshioka[b], Yoshinori Kodama[d], Masayuki Mano[d], Shin Nakajima[a], Yonehiro Kanemura[a,c]

[a] *Department of Neurosurgery, Osaka National Hospital, National Hospital Organization, 2-1-14 Hoenzaka, Chuo-ku, Osaka City 540-0006, Japan*
[b] *Division of Stem Cell Research, Institute for Clinical Research, Osaka National Hospital, National Hospital Organization, 2-1-14 Hoenzaka, Chuo-ku, Osaka City 540-0006, Japan*
[c] *Division of Regenerative Medicine, Institute for Clinical Research, Osaka National Hospital, National Hospital Organization, 2-1-14 Hoenzaka, Chuo-ku, Osaka City 540-0006, Japan*
[d] *Department of Central Laboratory and Surgical Pathology, Osaka National Hospital, National Hospital Organization, 2-1-14 Hoenzaka, Chuo-ku, Osaka City 540-0006, Japan*

ARTICLE INFO

Article history:
Received 7 July 2014
Received in revised form 29 July 2014
Accepted 3 August 2014
Available online 10 August 2014

Keywords:
^{11}C-methionine positron emission tomography
Gliomas
MGMT promoter methylation

ABSTRACT

Objectives: Several studies have aimed to detect biomarkers in glioma using noninvasive imaging techniques. However, few studies have been able to image 1p/19q deletion by ^{11}C-methionine positron emission tomography (^{11}C-methionine PET) or 2-hydroxyglutarate (2HG) by proton magnetic resonance spectroscopy (MRS). This study examines the correlation between ^{11}C-methionine uptake and *MGMT* promoter methylation in grade II and grade III nonenhancing gliomas.
Patients and methods: Data was collected from 20 patients with grade II and III nonenhancing gliomas who underwent both MRI and ^{11}C-methionine PET as part of their pre-surgical examination. We examined *MGMT* promoter methylation by quantitative methylation-specific PCR.
Results: The mean *MGMT* promoter methylation for tumors with T/N ratios $\geq$1.6 was 28.0 $\pm$ 26.3, and that for tumors with T/N ratios <1.6 was 0.68 $\pm$ 0.89. The *MGMT* promoter methylation for tumors with T/N ratios $\geq$1.6 was significantly higher than that for tumors with T/N ratios <1.6 ($P < 0.05$).
Conclusions: A higher uptake in ^{11}C-methionine PET may reflect increased *MGMT* promoter methylation. ^{11}C-methionine PET could be a useful tool to detect *MGMT* promoter methylation in nonenhancing glioma.

1. Introduction

A strong predictive and prognostic role for promoter methylation in the O_6-methylguanylmethyltransferase (*MGMT*) gene in newly diagnosed glioblastoma patients treated with temozolomide has been suggested [1,2]. *MGMT* promoter methylation predicted a favorable outcome in patients with WHO grade III and grade IV tumors who were treated with either alkylating agent chemotherapy or radiotherapy [1,3,4].

Several studies have investigated biological image features of gliomas by using a noninvasive imaging modality. Few studies have been able to detect and visualize 1p/19q deletion [5,6] or 2-hydroxyglutarate (2HG) [7–9] within the tumor. ^{11}C-methionine positron emission tomography (^{11}C-methionine PET) is a noninvasive imaging method that can be used to image brain tumors [10,11]. This ^{11}C-methionine tracer is used to measure protein synthesis rates in tissues [12]. In the rat brain and in tumors, the tracer is incorporated into nucleic acids by transmethylation via S-adenosylmethionine (SAM) and reflects the enhanced transmethylation processes in the tumors [13,14].

In this study, we investigated the correlation between ^{11}C-methionine uptake and DNA methylation at the *MGMT* promoter of grade II and grade III nonenhancing gliomas. This is the first report to demonstrate the methylation status of *MGMT* in gliomas using the noninvasive ^{11}C-methionine PET imaging technique, and would contribute for understanding of detailed biological properties of gliomas and the future clinical diagnosis.

* Corresponding author. Tel.: +81 6 6942 1331; fax: +81 6 6943 6467.
E-mail address: nonakamasa65@gmail.com (M. Nonaka).

http://dx.doi.org/10.1016/j.clineuro.2014.08.004
0303-8467/

2. Materials and methods

2.1. Subjects and glioma tumor samples

This study was carried out in accordance with the principles of the Helsinki Declaration, and approval was obtained from the ethical committee of Osaka National Hospital (No. 94, IRB No. 0713). Clinical data was collected from 20 patients with grade II or III glioma who underwent both MRI and ^{11}C-methionine PET as part of their pre-surgical examination from 2009 to 2012. Tumor tissue specimens from each patient were also obtained with written informed consent. A portion of the tumor samples were fixed in 10% formalin and embedded in paraffin wax using routine processing. In each case, hematoxylin and eosin (H&E)-stained sections were examined to classify the tumors according to the World Health Organization (WHO) International Histological Classification of Tumours.

2.2. Genomic DNA extraction

Tumor samples were immediately frozen in liquid nitrogen and stored at −80 °C. Genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA), according to manufacturer's protocol.

2.3. MGMT promoter methylation analysis

MGMT promoter methylation status was determined by quantitative methylation-specific PCR (qMSP). DNA extracted from tumor tissue was subjected to bisulfite modification by an EZ DNA Methylation-Gold Kit (Zymo Research Corporation, Irvine, CA), according to manufacturer's instructions. Bisulfite-modified DNA was analyzed by qMSP using the Applied Biosystems®7300 Real-Time PCR System (Applied Biosystems, Foster City, CA) with POWER SYBR® Green PCR Master Mix (Applied Biosystems). Methylated and unmethylated DNA molecules were amplified separately using specific primers (Supplemental Table 1) [15]. The quantification of methylated and unmethylated sequences was performed by employing the standard curve method using serial dilutions of bisulfite-modified EpiScope® Methylated HCT116 gDNA (TaKaRa-Bio, Shiga, Japan), which was highly methylated by CpG methylase, and EpiScope® Unmethylated HCT116 DKO gDNA (TaKaRaBio), which is genetically lacking both of methyltransferases DNMT1 (DNA methyltransferase 1) and DNMT3B (DNA methyltransferase 3B). The percentages of methylation and standard deviations (S.D.) were calculated from triplicate PCRs.

Supplementary Table 1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.clineuro.2014.08.004.

2.4. ^{11}C-methionine positron emission tomography

PET images were obtained using a SET-3000 GCT/X scanner (Shimadzu Corp.) with gadolinium oxyorthosilicate crystals as emission detectors. ^{11}C-methionine tracer was synthesized according to the method described by Berger et al. [16] and injected intravenously at a dose of 111–222 MBq (3–6 mCi). Tracer accumulation was recorded over 15 min in 99 transaxial slices, spanning the entire brain. The summed activity 20–35 min after tracer injection was used for image reconstruction. Images were stored in 256 × 256 × 99 anisotropic voxels, with a voxel size of 1 mm × 1 mm × 2.6 mm. The tumor/normal tissue (T/N) ratios were calculated non-stereotactically by dividing the maximum standard uptake value (SUV) for the tumor by the mean SUV of the contralateral normal frontal cortex. The tumor maximum SUVs were selected at the location of highest accumulation. We chose this measurement because it has been previously used for similar analysis [17,18].

A

Correlation between methionine uptake and *MGMT* promoter methylation

N=20	*MGMT* promoter methylation ≧3.0(%)	*MGMT* promoter methylation < 3.0(%)
T/N ratio ≧ 1.6	10	4
T/N ratio < 1.6	0	6
	Fisher's exact test p=0.01	

B

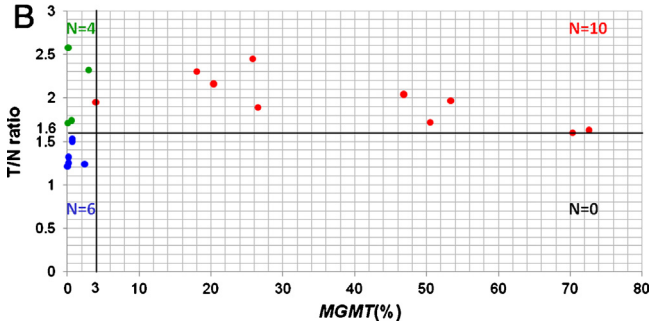

Fig. 1. (A) Correlation between *MGMT* promoter methylation and methionine uptake ($P = 0.01$, Fisher's exact test). (B) Plot to demonstrate the correlation between T/N ratio and *MGMT* promoter methylation. Ten patients with T/N ratio ≥1.6 and *MGMT* promoter methylation ≥3% were plotted with red dots. Four patients with T/N ratio ≥1.6 and *MGMT* promoter methylation <3% were plotted with green dots. Six patients with T/N ratio <1.6 and *MGMT* promoter methylation <3% were plotted with blue dots. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

2.5. Statistical analysis

T/N ratio and *MGMT* promoter methylation expressed as means ± standard deviation (SD). Correlation between the T/N ratio and *MGMT* promoter methylation was analyzed using Fisher's exact test and unpaired *t*-test. The study data were collected prospectively and analyzed using JMP version 8 (SAS Institute Inc., Cary, NC, USA). A *P* value <0.05 indicated a statistically significant difference.

3. Results

Tissue specimens from each patient were obtained, and post-surgical histological examination revealed 15 grade II and 5 grade III glioma patients. Five patients were recurrent cases, and only one case received radiotherapy and chemotherapy prior to ^{11}C-methionine PET examination. Detailed information on all 20 patients is listed in Table 1.

Nine patients were diagnosed with diffuse astrocytoma, 2 with oligoastrocytoma, 4 with oligodendroglioma, 4 with anaplastic astrocytoma, and 1 with anaplastic oligodendroglioma. All patients had nonenhancing lesions by MRI (Table 1 and Fig. 2). The mean T/N ratios were 1.63 ± 0.38, 2.01 ± 0.35, and 2.02 ± 0.42, for patients with diffuse astrocytomas, oligoastrocytomas or oligodendrogliomas, and anaplastic astrocytomas, respectively. These results were not statistically significant.

A threshold T/N ratio value of 1.6 significantly correlated with a quantitative threshold *MGMT* methylation status of 3%. Ten patients had *MGMT* promoter methylation of at least 3%. For statistical analysis, the T/N ratio (≥1.6 vs <1.6) and the *MGMT* promoter methylation (≥3% vs <3%) were compared using Fisher's exact test, and the *P* value was calculated to be 0.01, which was significant (Fig. 1A). Tumor samples with T/N ratio ≥1.6 tend to show *MGMT* promoter methylation ≥3% and those with T/N ratio <1.6 tend to show *MGMT* promoter methylation <3%. There were no cases with T/N ratio <1.6 and *MGMT* promoter methylation ≥3% (Fig. 1B). The mean *MGMT* promoter methylation in samples with a T/N ratio ≥1.6 was 28.0 ± 26.3, and the promoter methylation in samples with a T/N