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Differences in accumulation and the transport mechanism of L- and D-methionine in high- and low-grade human glioma cells☆

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ABSTRACT

Introduction: Although [S-methyl-11C]-labeled L-methionine and D-methionine (^{11}C-L-MET and ^{11}C-D-MET) are useful radiotracers for positron emission tomography imaging of brain tumors, it is not known whether the accumulation and transport mechanisms underlying uptake of ^{11}C-D-MET and ^{11}C-L-MET are the same. ^{11}C-L-MET is mainly taken up by the amino acid transport system L. We evaluated accumulation and the transport mechanism of D-MET in high- and low-grade human glioma cells in vitro.

Methods: The expression of transport system genes in high- (A172 and T98G) and low-grade (SW1088 and Hs683) glioma cells was quantitatively analyzed. Accumulation of [S-methyl-^{3}H]-L-MET (^{3}H-L-MET) and [S-methyl-^{3}H]-D-MET (^{3}H-D-MET) in these cells was compared during 60 min of incubation. The transport mechanism of ^{3}H-L-MET and ^{3}H-D-MET was investigated by incubating the cells with these compounds and examining the effect of the inhibitors 2-amino-2-norbornane-carboxylic acid or α-(methylamino) isobutyric acid.

Results: Absolute expression levels of system L and system alanine–serine–cysteine (ASC) in high-grade glioma cells were higher than in low-grade cells. In high-grade glioma cells, expression of system ASC genes was higher than that of system L genes. ^{3}H-D-MET, which is transported by systems L and ASC, accumulated at higher levels than ^{3}H-L-MET at all incubation times because ^{3}H-D-MET is more sensitive to system ASC than ^{3}H-L-MET. Conversely, in low-grade glioma cells with lower expression of system L and ASC, ^{3}H-D-MET accumulated at higher levels than ^{3}H-L-MET in early incubation times because ^{3}H-D-MET may be more sensitive to system ASC than system L.

Conclusion: ^{3}H-D-MET was mainly transported by systems L and ASC and sensitive to system ASC, whereas ^{3}H-L-MET was transported by system L in human glioma cells. In vitro, the accumulation of ^{3}H-D-MET was significantly higher than that of ^{3}H-L-MET during the entire incubation time in high-grade glioma cells, and in early incubation times in low-grade glioma cells.

1. Introduction

[S-methyl-^{11}C]-L-methionine (^{11}C-L-MET), a natural amino acid, has been used as a positron emission tomography (PET) tracer to detect tumors. ^{11}C-L-MET is transported into cells through the amino acid transport system L and accumulates in tumors due to enhanced protein synthesis and the transmethylation metabolic functions of tumors [1]. Although 2-[^{18}F]-fluoro-2-deoxy-D-glucose (^{18}F-FDG) is used most frequently for PET imaging of tumors, ^{11}C-L-MET is superior to ^{18}F-FDG for diagnosis and follow-up of glioma patients because ^{11}C-L-MET does not accumulate much in normal brain due to low protein synthesis and transmethylation metabolic function and because of the high contrast between gliomas and normal brain compared with ^{18}F-FDG [2,3].

Because [S-methyl-^{11}C]-labeled D-MET (^{11}C-D-MET) is less affected by enzymatic metabolism than ^{11}C-L-MET and therefore has fewer metabolites [4], ^{11}C-D-MET more closely reflects the transport function of the amino acid transport system L and the alanine–serine–cysteine (ASC) system in tumor cells. ^{11}C-D-MET is clinically superior to ^{11}C-L-MET as a PET tracer for imaging of abdominal tumors [4,5]. ^{11}C-L-MET

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mainly accumulates in gliomas due to protein synthesis [2,3]. However, accumulation and the transport mechanism of ^{11}C-D-MET have not been investigated in brain tumor cells. In this in vitro study, we investigated accumulation and the transport mechanism of ^{11}C-D-MET in high- and low-grade human glioma cells. Real-time reverse transcription-polymerase chain reaction (qRT-PCR) was used to measure the expression of neutral amino acid transport system genes. Correlations between brain tumor cell accumulation of ^{3}H-L- and D-MET, which are the same chemical compounds as ^{11}C-L- and D-MET but have a longer half-life, and the expression of neutral amino acid transport system genes were evaluated.

2. Material and methods

2.1. Materials

[S-methyl-^{3}H]-L-MET (^{3}H-L-MET: 2.96 GBq/mmol) and [S-methyl-^{3}H]-D-MET (^{3}H-D-MET: 2.96 GBq/mmol) were purchased from American Radiolabeled Chemicals Co. (St Louis, MO, USA). Because ^{11}C-L-MET and ^{11}C-D-MET have a short half-life, and the tritiated compounds function similarly as ^{11}C-labeled compounds, we used ^{3}H-L-MET and ^{3}H-D-MET instead of ^{11}C-L-MET and ^{11}C-D-MET.

2.2. Tumor cells

The following cultured human glioma cell lines were purchased from American Type Culture Collection (Manassas, VA, USA): high-grade glioblastoma (A172 and T98G) [6], low-grade astrocytoma (SW1088), and low-grade oligodendroglioma (Hs683) [7]. All glioma cells (2.0×10^5 cells/mL/well) were incubated in 24-well plates in Dulbecco's Modified Eagle's Medium (Wako, Osaka, Japan) for A172 and Hs683 cells, Eagle's minimal essential medium (Wako) for T98G cells, and L-15 medium for SW1088 cells. All media were supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 0.1 mg/mL streptomycin. Cells were grown at 37 °C in air (SW1088) or a 5% CO_2 atmosphere (A172, T98G, and Hs683). A cell proliferation curve was prepared to determine the duration of the logarithmic growth phase.

2.3. The expression of amino acid transporter in human glioma cells

The expression of amino acid transporters in human glioma cells was investigated according to our previous study [4]. In brief, total RNA was harvested from each glioma cell line using an RNeasy Mini kit (QIAGEN K. K, Tokyo, Japan). cDNA was synthesized only from high-quality total RNA using an AffinityScript qRT-PCR cDNA Synthesis kit (Agilent Technologies) by incubating the reaction for 5 min at 25 °C, 15 min at 42 °C, and 55 min at 95 °C. The genes analyzed with qRT-PCR using an Mx3005P thermocycler (Agilent Technologies) are summarized in Table 1. Amplification was performed for 2 min at 95 °C for one cycle; 5 s at 95 °C followed by 20 s at 60 °C for 40 cycles; 1 min at 95 °C followed by 30 s at 55 °C and 30 s at 95 °C for one cycle. The expression levels of neutral amino acid transport system genes in each cell line were calculated using a calibration curve for each gene. These experiments were all performed in triplicate.

2.4. Assay of MET transport in human glioma cells

Transport assays were performed according to the method described by Shikano et al. [8]. In brief, the sodium-containing incubation medium used was based on phosphate-buffered saline (Na^+-PBS), whereas in the sodium-free incubation medium (Na^+-free PBS), NaCl and Na_2HPO_4 were replaced with the equivalent concentrations of choline chloride and K_2HPO_4, respectively. After pre-incubation with incubation medium for 10 min, the cells were incubated with 0.5 mL incubation medium containing ^{3}H-L-MET or ^{3}H-D-MET (18.5 kBq) for 2, 5, 10, 15, 30, or 60 min at 37 °C as a control. For the competitive inhibition assay, the cells were incubated for 5 min with ^{3}H-L-MET or ^{3}H-D-MET plus 1.0 mM inhibitor: 2-amino-2-norbornane-carboxylic acid (BCH; Sigma Chemical Co.), a substrate specific to system L transporters, or α-(methylamino) isobutyric acid (MeAIB; Sigma Chemical Co.), a substrate specific to system A transporters. At the end of the incubation, each well was rapidly washed twice with ice-cold incubation medium. The cells were then solubilized in 0.5 mL 0.1 N NaOH mixed with Ultima Gold™ Scintillation Cocktail (PerkinElmer, Waltham, MA, USA), and radioactivity was then measured with a liquid scintillation counter (Aloka, Tokyo, Japan; LSC-5100). These experiments were all performed in quadruplicate.

Table 1
Expression of genes of the neutral amino acid transport systems in human brain tumor cells.

	Transport system	Gene name	High-grade		Low-grade	
			A172	T98G	SW1088	Hs683
Na+-dependent	A	*SNAT1*	101.86	97.65	54.42	88.68
		SNAT2	249.00	339.75	229.04	200.87
		SNAT4	2.66	0.01	4.60	3.59
	ASC	*ASCT1*	82.96	46.22	44.86	26.50
		ASCT2	351.75	435.82	251.99	106.60
Na+-independent	L	*LAT1*	297.54	350.77	258.42	172.98
		LAT2	3.50	1.91	14.83	2.62
		LAT3	4.86	8.45	3.89	2.62
		LAT4	18.05	1.57	6.35	14.57
	Coupling factor	*4F2hc*	475.48	477.17	393.36	267.88

Data are expressed as copy number per 1000 copies of housekeeping genes.

The contribution of amino acid transport systems to MET transport was evaluated according to our previous study [4]. Briefly, MET accumulation in the absence of inhibitors was considered as a percentage of the control value, which was set at 100%. System A transport was calculated by subtracting MET accumulation in the presence of MeAIB from accumulation in control Na^+-PBS. System ASC transport was calculated by subtracting the value of MET accumulation in control Na^+-free PBS from MET accumulation in the presence of MeAIB. Finally, system L transport was calculated by subtracting MET accumulation in the presence of BCH from MET accumulation in control Na^+-free PBS. The rates of tumor cell accumulation of ^{3}H-L-MET and ^{3}H-D-MET were calculated as % injected dose/100 μg protein. The correlation between tumor cell accumulation of ^{3}H-L-MET and ^{3}H-D-MET and expression of neutral amino acid transport system genes was also examined. These measurements were performed in quadruplicate.

2.5. Statistical analysis

Data are presented as means and standard deviation (SD). *P* values were calculated using a two-tailed paired Student's *t* test for comparison between two groups. A *P* value less than 0.05 was considered significant.

3. Results

3.1. Expression of amino acid transport system genes in human glioma cells

The high quality of the total RNA harvested from each glioma cell line was confirmed using a bioanalyzer (data not shown). In Table 1, genes for transporters of the Na^+-dependent transport systems, system A and system ASC, were highly expressed in T98G, followed by A172, SW1088, and Hs683 cells. Genes for transporters of the Na^+-independent transport system, *LAT1*, and the coupling factor, *4F2hc*, were also highly expressed in T98G and A172 cells, followed by SW1088, and then Hs683 cells. The expression levels of *ASCT2* were higher than those of *LAT1* and *SNAT2* in high-grade cells, and those of *ASCT2* levels were similar to those of *LAT1* in low-grade cells.

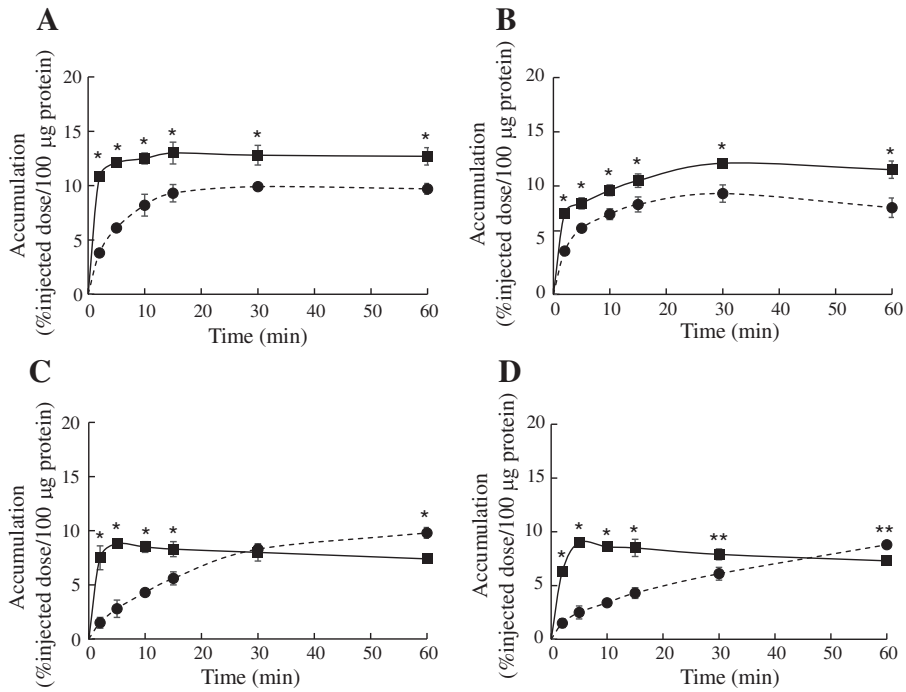

Fig. 1. The contribution of ^{3}H-L-MET (●) and ^{3}H-D-MET (■) in A172 (A), T98G (B), SW1088 (C), and Hs683 (D) cells. ^{3}H-D-MET accumulation was higher than ^{3}H-L-MET accumulation in the high-grade glioma cells, A172 (A) and T98G (B), throughout the entire incubation time, whereas in the low-grade glioma cells, SW1088 (C) and Hs683 (D), ^{3}H-D-MET showed higher accumulation than ^{3}H-L-MET during early incubation times, then ^{3}H-L-MET accumulation gradually increased and became higher than ^{3}H-D-MET at 60 min of incubation. The asterisks and double asterisks indicate $P < 0.01$ and $P < 0.05$, respectively, between the accumulation of ^{3}H-L-MET and ^{3}H-D-MET in each tumor cell line.

3.2. Assay of MET transport in human glioma cells

Accumulation of ^{3}H-L-MET and ^{3}H-D-MET was investigated in human glioma cells for 60 min (Fig. 1). For both tracers, the area under the radioactivity curve in high-grade glioma cells was higher than that in low-grade glioma cells. In the high-grade glioma cell lines, A172 and T98G, accumulation of ^{3}H-D-MET was significantly higher than that of ^{3}H-L-MET throughout the entire incubation time. In the low-grade glioma cells, SW1088 and Hs683, accumulation of ^{3}H-L-MET was significantly lower than that of ^{3}H-D-MET during the early incubation times and then became higher than ^{3}H-D-MET after 60 min of incubation.

3.3. The contribution of each amino acid transport system for accumulation of MET in human glioma cells

The contribution of each amino acid transport system to tumor cell accumulation of ^{3}H-L-MET and ^{3}H-D-MET was analyzed using specific inhibitors and was measured at 5 min after ^{3}H-L-MET or ^{3}H-D-MET incubation (Fig. 2). For accumulation of ^{3}H-L-MET in all cell lines, system L was mainly involved and mediated over 65% of the total accumulation of ^{3}H-L-MET in control conditions. For accumulation of ^{3}H-D-MET, system L mediated over 54% of the total ^{3}H-D-MET accumulation in each of the tumor cell lines in control conditions. We observed involvement of not only system L but also system A and/or system ASC. System ASC was more involved in the low-grade glioma cell lines, SW1088 and Hs683, than the high-grade glioma cell lines, A172 and T98G. Involvement of system A was relatively lower than that of systems L and ASC in both ^{3}H-L-MET and ^{3}H-D-MET accumulation.

3.4. Correlation between MET accumulation and transporter expression in human glioma cells

The correlation between tumor accumulation of MET and the expression of amino acid transport system genes is shown in Table 2. When we evaluated correlations between ^{3}H-L-MET accumulation

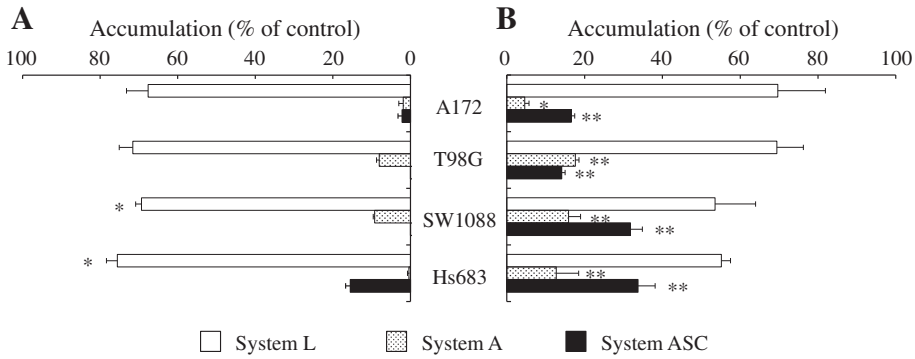

Fig. 2. Contribution of each amino acid transport system with ^{3}H-L-MET (A) and ^{3}H-D-MET (B) accumulation in human glioma cells at 5 min of incubation. System L was involved in tumor cell accumulation of ^{3}H-L-MET, whereas both systems L and ASC were involved in tumor cell transport of ^{3}H-D-MET. The asterisks and double asterisks indicate $P < 0.01$ and $P < 0.05$, respectively, between the accumulation of ^{3}H-L-MET and ^{3}H-D-MET in each tumor cell line. These data were calculated using inhibitors as shown in Fig. 1.

Table 2
Correlation coefficients (R^2) between tumor uptake of ^{3}H-L-MET and ^{3}H-D-MET at 5 min of incubation and the expression of amino acid transport system genes in human brain tumor cells.

System	Correlation between MET and gene expression	
	3H-L-MET	3H-D-MET
L	0.90	0.81
L + A	0.74	0.88
L + ASC	0.86	0.97
L + ASC + A	0.87	0.91

and expression of system L genes, and between ^{3}H-D-MET accumulation and expression of both systems L and ASC genes in glioma cells, the highest correlation was found between both ^{3}H-L-MET accumulation and system L ($R^2 = 0.90$), and ^{3}H-D-MET accumulation and systems L and ASC genes ($R^2 = 0.97$) (Fig. 3).

4. Discussion

In this in vitro study, we investigated accumulation and the transport mechanism of ^{3}H-L-MET and ^{3}H-D-MET into high- and low-grade human glioma cells in comparison with qRT-PCR evaluation of the expression levels of neutral amino acid transport system genes in the glioma cells. In our previous study of three different human cancer cell lines—lung, breast, and colon cancer cells—^{3}H-L-MET was mainly transported via system L, and ^{3}H-D-MET was mainly transported via systems L and ASC [4].

Our current in vitro study basically shows just transport function and not metabolism of L- and D-MET. In Fig. 1, significantly higher accumulation of ^{3}H-D-MET than ^{3}H-L-MET was seen in the high-grade glioma cell lines, A172 and T98G. Although ^{3}H-D-MET was transported through systems L, A, and ASC in the A172 and T98G cell lines, the involvement of systems A and ASC was significantly higher for ^{3}H-D-MET transport (Fig. 2). We previously reported that ^{3}H-D-MET was transported via systems L and ASC in human abdominal tumor cells [4]; thus, we here examined the correlation between ^{3}H-D-MET accumulation and the expression levels of genes for systems L and ASC in human glioma cells. ^{3}H-D-MET uptake showed the highest correlation with systems L and ASC combined ($R^2 = 0.97$, Table 2 and Fig. 3).

Expression levels of system ASC genes (*ASCT1, 2*) in high-grade glioma cells were higher than those of system L (*LAT1–4*, Table 1). Therefore, system ASC was responsible for higher accumulation of ^{3}H-D-MET than ^{3}H-L-MET in high-grade glioma cells. In the low-grade glioma cells, SW1088 and Hs683 (Fig. 1), the accumulation of ^{3}H-D-MET was higher than that of ^{3}H-L-MET at early incubation times; but ^{3}H-L-MET accumulated at higher levels than ^{3}H-D-MET at late incubation times. In Table 1, the expression levels of system L genes (*LAT1–4*) were nearly equal to those of system A (*SNAT1*, *2*, *4*) and ASC (*ASCT1*, *2*) genes in SW1088 cells, and were higher than those of system ASC genes (*ASCT1*, *2*) but lower than those of system A genes (*SNAT1*, *2*, *4*) in Hs683 cells. Therefore, the involvement of systems ASC and/or A allowed ^{3}H-D-MET accumulation at higher levels than ^{3}H-L-MET in glioma cells, whereas system L may cause significant ^{3}H-L-MET accumulation in glioma cells (Figs. 1 and 2). If there is also higher in vivo expression of system ASC in high-grade gliomas than in low-grade gliomas, ^{11}C-D-MET may accumulate more in the high-grade gliomas and may provide brain images with high contrast because of its high metabolic stability. Although ^{11}C-D-MET may be useful for diagnosis and staging of these gliomas, its usefulness for studies in human patients needs to be confirmed. ^{11}C-L-MET is taken up through system L not only in brain tissue but also in gliomas, and accumulates in gliomas due to protein synthesis and transmethylation in tumors during human clinical examination. Therefore, future in vivo results in human may be different.

In a distribution study with abdominal tumor-bearing mice (n = 4) injected with ^{14}C-L-MET and ^{3}H-D-MET, the mice were sacrificed at 5, 10, 30, and 60 min after MET injection, and the brains were excised [4]. The cerebral accumulation of ^{3}H-L-MET was 1.31 ± 0.18, 1.99 ± 0.28, 1.63 ± 0.17, and 1.30 ± 0.11% injected dose (ID)/g tissue at 5, 10, 30, and 60 min after injection. On the other hand, ^{3}H-D-MET accumulation in the brain was 1.20 ± 0.14, 1.89 ± 0.21, 1.92 ± 0.14, and 1.80 ± 0.18%ID/g tissue at 5, 10, 30, and 60 min after injection, respectively. Specifically, not much difference was observed in normal brain accumulation between ^{3}H-L-MET and ^{3}H-D-MET during early injection times as input functions. Therefore, we suggest that accumulation of ^{3}H-L-MET and ^{3}H-D-MET in brain tumors in mice may be similar to the accumulation observed in the in vitro study in Fig. 1 because brain tumors induce the same disruption in the blood–brain barrier in mice whether injected with ^{3}H-L-MET or ^{3}H-D-MET. Unfortunately, we could not use glioma-bearing mice to evaluate accumulation of ^{3}H-L-MET and ^{3}H-D-MET because injection of our four human-derived glioma cell lines into normal mouse brain was difficult. An in vivo study of glioma-bearing mice or patients with glioma should be performed to determine if glioma imaging with ^{11}C-D-MET is superior to that of ^{11}C-L-MET, and to investigate glioma diagnosis and staging. In addition, the transport mechanism of both METs in glioma cells could be clarified with an in vitro study.

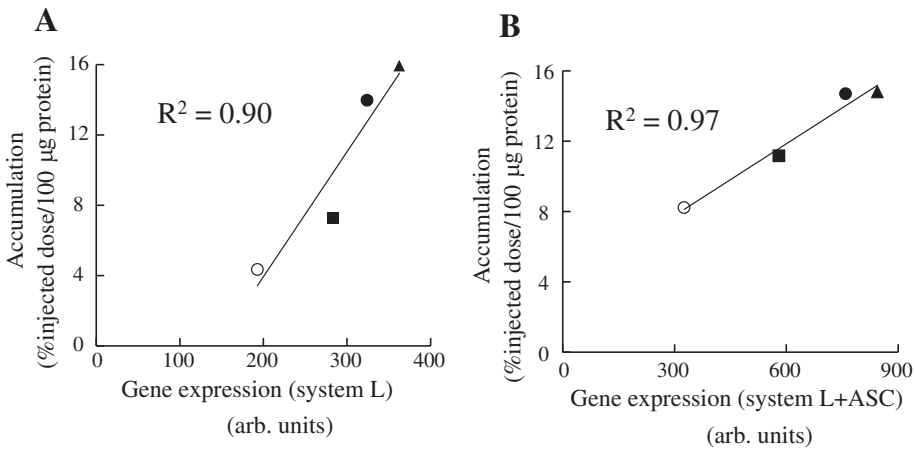

Fig. 3. Correlation between tumor cell accumulation of ^{3}H-L-MET and ^{3}H-D-MET at 5 min of incubation and the expression of amino acid transport system genes in human glioma cell lines, H172 (●), T98G (▲), SW1088 (■), and Hs683 (○). The graphs show the highest correlation between ^{3}H-L-MET accumulation and expression of system L genes (A), and that between ^{3}H-D-MET accumulation and expression of system L and system ASC genes (B).

5. Conclusion

The transport mechanisms of ^{3}H-L-MET and ^{3}H-D-MET differed in human glioma cells. ^{3}H-D-MET was mainly transported by systems L and ASC and more sensitive to system ASC than system L, whereas ^{3}H-L-MET was transported by system L. Based on these transport mechanisms in our in vitro study, the accumulation of ^{3}H-D-MET was significantly higher than that of ^{3}H-L-MET throughout the entire incubation time in high-grade glioma cells, and during early incubation times in low-grade glioma cells.

Conflicts of interest

There are no conflicts of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.nucmedbio.2016.09.003.

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