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Dynamic <sup>11</sup>C-Methionine PET analysis has an additional value for differentiating malignant tumors  
from Granulomas: an experimental study using small animal PET

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## ABSTRACT

**Purpose:** We evaluated whether the dynamic profile of  $^{11}\text{C}$ -MET may have an additional value in differentiating malignant tumors from granulomas in experimental rat models by small-animal PET.

**Methods:** *Rhodococcus aurantiacus* and allogenic rat C6-glioma cells were inoculated respectively into the right and left calf muscles to generate a rat model bearing both granulomas and tumors (n=6). Ten days after the inoculations, dynamic  $^{11}\text{C}$ -MET PET was performed by small-animal PET up to 120 min after injection of  $^{11}\text{C}$ -MET. The next day, after overnight fasting, the rats were injected with  $^{18}\text{F}$ -FDG, and dynamic  $^{18}\text{F}$ -FDG PET was performed up to 180 min. The time-activity curves, static images, and mean standardized uptake value (SUV) in the lesions were calculated.

**Results:**  $^{11}\text{C}$ -MET uptake in the granuloma showed a slow exponential clearance after an initial distribution, while the uptake in the tumor gradually increased with time. The dynamic pattern of  $^{11}\text{C}$ -MET uptake in the granuloma was significantly different from that in the tumor ( $p < 0.001$ ). In the static analysis of  $^{11}\text{C}$ -MET, visual assessment and SUV analysis could not differentiate the tumor from the granuloma in all cases, although the mean SUV in the granuloma ( $1.48 \pm 0.09$ ) was significantly lower than that in the tumor ( $1.72 \pm 0.18$ ,  $p < 0.01$ ). The dynamic patterns, static images, and mean SUVs of  $^{18}\text{F}$ -FDG in the granuloma were similar to those in the tumor ( $p = \text{ns}$ ).

**Conclusions:** Dynamic  $^{11}\text{C}$ -MET PET has an additional value for differentiating malignant tumors from

granulomatous lesions, which deserve further elucidation in clinical settings.

**Key Words:** Dynamic  $^{11}\text{C}$ -MET PET; Granuloma; Inflammation; Tumor; Rat

## INTRODUCTION

Increased fluorine-18-2-deoxy-2-fluoro-D-glucose ( $^{18}\text{F}$ -FDG) uptake is not specific to malignant tumors.  $^{18}\text{F}$ -FDG accumulates not only in malignant tumors but also in various forms of inflammatory lesions, particularly in granulomatous lesions, such as sarcoidosis or active inflammatory processes after chemoradiotherapy [1-5]. Thus, an increased  $^{18}\text{F}$ -FDG uptake level in such inflammatory lesions makes it difficult to differentiate malignant tumors from benign lesions [6]. Therefore, limitations of  $^{18}\text{F}$ -FDG PET have recently been noted and the development of post- $^{18}\text{F}$ -FDG molecular probes is strongly required. It has been suggested that these problems may be overcome using post- $^{18}\text{F}$ -FDG PET probes such as L- $^{11}\text{C}$ -methionine ( $^{11}\text{C}$ -MET) [7].

$^{11}\text{C}$ -MET uptake reflects increased amino acid transport and protein synthesis and is related to cellular proliferation activity.  $^{11}\text{C}$ -MET has been shown to possess a high specificity in tumor detection, tumor delineation, and differentiation of benign from malignant lesions [8], owing to the lower uptake level of  $^{11}\text{C}$ -MET in inflammatory cells than that of  $^{18}\text{F}$ -FDG [9-11]. Several authors have reported on trials of  $^{11}\text{C}$ -MET for the evaluation of brain tumors and other malignant tumors. In the clinical study by Chung et al.,  $^{11}\text{C}$ -MET PET showed high sensitivity and good contrast in the evaluation of brain lesions that are hypo- or isometabolic on  $^{18}\text{F}$ -FDG PET. From the results, they concluded that  $^{11}\text{C}$ -MET PET could provide additional information when used in combination with  $^{18}\text{F}$ -FDG [12]. Moulin-Romsée et al. reported that  $^{11}\text{C}$ -MET PET could be used for the early detection of recurrent brain tumor and for biopsy

guidance [13], Zhang et al. indicated the usefulness of  $^{11}\text{C}$ -MET PET in the early evaluation of prognosis in patients with bone and soft tissue sarcomas treated by carbon ion radiotherapy [14]. Our clinical study [15] also showed that the  $\text{SUV}_{\text{max}}$  of  $^{11}\text{C}$ -MET was significantly higher in lung cancer than in the pneumoconiotic nodules. Inflammatory processes, however, can result in an increased uptake level of  $^{11}\text{C}$ -MET in certain brain regions after stereotactic radiosurgery and be mistaken for residual tumor tissue [16]. In patients with sarcoidosis,  $^{11}\text{C}$ -MET accumulates in the mediastinum and bilateral hilar lymph nodes [17], although the degree of uptake is relatively low. Thus, the usefulness of  $^{11}\text{C}$ -MET PET is still controversial in the differentiation of tumors from inflammatory lesions.

Recently, we have developed two rat models of intramuscular granulomas, histologically similar to sarcoidosis [18,19]. The rat granulomas showed a high  $^{18}\text{F}$ -FDG uptake level comparable to that in the tumors, indicating the usefulness of our models for studies of differential diagnosis. Our preliminary *ex vivo* biodistribution study in the rat models showed that the  $^{11}\text{C}$ -MET uptake level was significantly lower in granulomas than in tumors, whereas  $^{18}\text{F}$ -FDG was not capable of differentiating granulomas from tumors [20]. These findings indicate that  $^{11}\text{C}$ -MET has a potential for differentiating malignant tumors from granulomas. Our results, however, also indicated that visual assessment and SUV analysis of the static images of  $^{11}\text{C}$ -MET could not differentiate malignant tumors from benign lesions in all cases. It should be noted that information on the kinetics of  $^{11}\text{C}$ -MET obtained by static imaging is limited. These limitations may be overcome by dynamic  $^{11}\text{C}$ -MET PET analysis.

Accordingly, in the present study, we evaluated whether the dynamic profile of  $^{11}\text{C}$ -MET may have an additional value in differentiating malignant tumors from granulomas in comparison with  $^{18}\text{F}$ -FDG in experimental rat models by small-animal PET.

## MATERIALS AND METHODS

### Radiopharmaceuticals

$^{18}\text{F}$ -FDG and  $^{11}\text{C}$ -MET synthesized by standard procedures were obtained from Hokkaido University Hospital Cyclotron Facility.

### Animal Studies

All experimental protocols were approved by the Laboratory Animal Care and Use Committee of Hokkaido University. Eight-week-old male Wistar King Aptekman/hok (WKAH) rats (supplied by Japan SLC, Inc., Hamamatsu) were used in all experiments. The *Rhodococcus aurantiacus* ( $3 \times 10^8$  colony forming units (CFU) of viable *R. aurantiacus* (strain 80005) suspended in 0.2 ml of phosphate buffered saline (PBS) [21]) and allogenic rat glioma cells (C6,  $2 \times 10^6$  cells/0.2 ml) [19] were inoculated respectively into the right and left calf muscles to generate a rat model bearing both granulomas and tumors. The C6 glioma cells were purchased from the European Collection of Cell Cultures (ECACC).

Figure 1 shows the experimental protocols of animal studies.

### PET Studies

Ten days after *R. aurantiacus* and C6 inoculation, the rats (n=6) were anesthetized with

1.0-1.5% isoflurane inhalation. The rats were placed in a small-animal PET scanner (Inveon; Siemens Medical Solutions USA Inc., Knoxville, TN) in a supine position and intravenously injected with  $^{11}\text{C}$ -MET (on average 86 MBq/rat) in the lateral tail vein. Dynamic PET (list-mode acquisition) was performed for 120 min. CT was also performed for attenuation correction. Then, the rats were allowed to recover from the anesthesia. The next day, after overnight fasting, the rats were anaesthetized again, injected with  $^{18}\text{F}$ -FDG (on average 37 MBq/rat), and dynamic PET (list-mode acquisition) was carried out for 180 min. CT was performed for attenuation correction. The injection doses of  $^{11}\text{C}$ -MET and  $^{18}\text{F}$ -FDG were determined, considering the half-lives of  $^{11}\text{C}$  (20 min) and  $^{18}\text{F}$  (110 min) [15,16] and tumor accumulation levels of  $^{11}\text{C}$ -MET and  $^{18}\text{F}$ -FDG. During the PET scanning, body temperature was maintained using cotton bedding that we prepared. The data were reconstructed and corrected for attenuation and scatter using 2D filtered back-projection (FBP). The image matrix was  $256 \times 256 \times 159$ , resulting in a voxel size of  $0.385 \times 0.385 \times 0.796$  mm. For visual grading assessment, images were viewed in the coronal, axial, and sagittal slices. The degree of  $^{11}\text{C}$ -MET or  $^{18}\text{F}$ -FDG uptake in the granuloma and tumor was visually classified from grade 0 to 3 in comparison with the tracer uptake in the normal testis: grade 0, no uptake; grade 1, mild uptake; grade 2, moderate uptake and grade 3, intense uptake. For quantitative analysis, images were analyzed by drawing volume of interests (VOIs) centered over the granulomas and tumors without correction for partial volume effects. The time-activity curves, static images (30-40 min for  $^{11}\text{C}$ -MET, 110-120 min for  $^{18}\text{F}$ -FDG), and the mean standardized uptake

value (SUV) in the lesions were determined. The SUV was calculated using the following equation;  $SUV = \text{Activity in a VOI (MBq/cc)} / [\text{Injected dose (MBq)} / \text{Body weight (g)}]$ . The SUV was used in order to normalize the differences in the injected doses (radioactivity) with the body weight of rats. To further evaluate the dynamic pattern of  $^{11}\text{C}$ -MET uptake, we determined the ratios of SUVs at a peak level (2 min postinjection) to that at a plateau level (15 min postinjection) in the granulomas. The ratios of SUVs were similarly determined in the tumors (SUV ratios of 2 and 15 min). In the evaluation of the dynamic pattern of  $^{18}\text{F}$ -FDG uptake, we determined the ratios of SUVs in the early image (60 min postinjection) to that in the delayed image (120 min postinjection) instead of the peak-to-plateau SUV ratios [22,23], since no peaks were observed in the time-activity curves of the granulomas and tumors within 120 min after the injection. Using tissue samples from the tumors and granulomas, we prepared formalin-fixed, paraffin-embedded specimens for subsequent histological staining.

### **Histochemical Studies**

Formalin-fixed, paraffin-embedded tumor and granuloma tissue sections of 3  $\mu\text{m}$  thickness were stained with hematoxylin and eosin (H&E). Immunohistochemical staining was also carried out using the adjacent sections, in accordance with a standard immunostaining procedure [24]. Macrophages were immunohistochemically stained using a monoclonal antibody (mAb) (mouse IgG1, Clone No. RM-4, TransGenic Inc.). RM-4 recognizes the antigen with a molecular weight of 46 kDa presenting on the

membrane of endosomes and lysosomes in macrophages and dendritic cells. Glucose transporter 1 (Glut-1) was immunohistochemically stained using a polyclonal antibody (rabbit IgG, synthetic peptide, abcam). The antibody reacts with the amino acids at the C-terminal end of the Glut-1 protein (predicted molecular weight: 55 kDa) presenting either on the cell surface or in intracellular sites. A cell proliferation-associated antigen, Ki-67 (a proliferating nuclear antigen, was immunohistochemically stained using a mAb (mouse IgG1, Clone No. MIB-5, DakoCytomation) [25].

Adjacent tissue sections incubated with mouse IgG instead of the primary anti-RM-4 antibody and anti-Ki-67 antibody were used as negative controls for RM-4 and Ki-67. Adjacent tissue section incubated with rabbit IgG instead of the primary anti-Glut-1 antibody was used as a negative control for Glut-1. The sections of rat spleen were used as positive controls for RM-4 (macrophages) and Ki-67 immunohistochemistry. Erythrocytes from the human breast cancer tissue were immunohistochemically stained as a positive control for Glut-1.

### **Statistical Analyses**

Repeated measures ANOVA was used to assess the significance of difference in the dynamic patterns of  $^{11}\text{C}$ -MET and  $^{18}\text{F}$ -FDG uptake between the granulomas and tumors (Figures 4c and 5c). A paired *t* test was performed to evaluate the significance of difference in SUV ratio (Figure 4d and 5d) and SUV (Figures 6c and 6d) between the two types of the lesion (tumors vs granulomas). A two-tailed value

of  $P < 0.05$  was considered significant.

## RESULTS

### Histopathological Findings

In the intramuscular granuloma induced by *R. aurantiacus*, the granulomatous lesions showed mature epithelioid cell granuloma formation and massive lymphocyte infiltration around the granuloma (Fig. 2-1a). The epithelioid cells and macrophages determined by positive immunohistochemical staining with RM-4 were found in the epithelioid cell granulomas and macrophages around epithelioid cell granulomas (Fig. 2-1b). A high expression level of Glut-1 was observed in the granuloma (Fig. 2-1c). The accumulation of Ki-67 positive lymphocytes was seen in the periphery of the granulomas (Fig. 2-1d). In the intramuscular tumor induced by C6 cells, massive viable and proliferating cancer cells were observed by H&E staining (Fig. 2-1e). The immunohistochemical staining with RM-4 showed the presence of macrophages infiltrating the tumor tissue (Fig. 2-1f). A high expression level of Glut-1 was also observed in the tumor cell membrane (Fig. 2-1g). A high expression level of Ki-67 was also observed in the tumor (Fig. 2-1h).

Figure 2-2 shows the positive and negative controls for RM-4, Glut-1 and Ki-67 staining. Macrophages in red pulp (RP) of the spleen from a normal rat were positively stained (Fig. 2-2a). Strong staining of erythrocyte membranes present in a human breast cancer tissue section was observed with Glut-1 immunostaining and used as a positive control (Fig. 2-2b). Ki-67 positive cells were seen in the

white pulp (WP) and in red pulp (RP) of the spleen from a normal rat (Fig. 2-2c). Negative controls used for immunohistochemistry did not show any immunoreactivity (Figs. 2-2d-i).

### **PET image analysis**

Figure 3 shows representative dynamic  $^{11}\text{C}$ -MET (Fig. 3a) and  $^{18}\text{F}$ -FDG (Fig. 3b) images of rats bearing granulomas and tumors.  $^{11}\text{C}$ -MET (Fig. 3a) and  $^{18}\text{F}$ -FDG (Fig. 3b) distributions in the granuloma and tumor were clearly visualized by PET.  $^{11}\text{C}$ -MET dynamic images showed a maximal tracer uptake level in the granuloma in the first few minutes postinjection, while the uptake level in the tumor gradually increased with time (Fig. 3a). The distributions of  $^{18}\text{F}$ -FDG in the granuloma and tumor gradually increased, and the level in the granuloma was visually similar to that in the tumor for each time point (Fig. 3b).

Figures 4a-4c show the time-activity curves of  $^{11}\text{C}$ -MET in the granulomas and tumors. The uptake of  $^{11}\text{C}$ -MET in the granuloma peaked at 2 min, showed a slow exponential clearance after the initial distribution, and reached a plateau level at around 15 minutes postinjection. The uptake level in the tumor gradually increased with time. The dynamic pattern of  $^{11}\text{C}$ -MET uptake in the granuloma was significantly different from that in the tumor ( $p < 0.001$ ). The peak-to-plateau SUV ratio in the granuloma ( $1.20 \pm 0.07$ ) was significantly higher than that in the tumor (SUV ratios of 2 and 15 min:  $0.93 \pm 0.06$ ,  $p < 0.001$ ) (Fig. 4d). The peak-to-plateau SUV ratio analysis (SUV ratios of 2 and 15 min) in  $^{11}\text{C}$ -MET PET

enabled the differentiation of the tumor from the granuloma in all rats (granuloma > 1.10; tumor < 1.03).

Figures 5a-5c show the time-activity curves of  $^{18}\text{F}$ -FDG in the granulomas and tumors. The uptake level of  $^{18}\text{F}$ -FDG in the granulomas increased with time. Both the levels and time-courses of  $^{18}\text{F}$ -FDG uptake in the granulomas were comparable to those in the tumors. The dynamic pattern of  $^{18}\text{F}$ -FDG uptake (Fig. 5a-c) and the early-to-delayed SUV ratio (Fig. 5d) in the granuloma was similar to that in the tumor ( $p = \text{ns}$ ).

Figure 6 shows representative static  $^{11}\text{C}$ -MET and  $^{18}\text{F}$ -FDG images (Figs. 6a and 6b) and mean SUVs of  $^{11}\text{C}$ -MET and  $^{18}\text{F}$ -FDG (Figs. 6c and 6d) in rats bearing granulomas and tumors. In the static analysis of  $^{11}\text{C}$ -MET,  $^{11}\text{C}$ -MET accumulation level in the granuloma appeared to be lower than that in the tumor (Fig. 6a). There was a significant difference in the visual grading of  $^{11}\text{C}$ -MET between the granulomas and tumors ( $1.83 \pm 0.75$  vs.  $2.67 \pm 0.52$ ,  $p < 0.01$ ). Visual and SUV assessment of the static images did not enable the differentiation of the tumor from the granuloma in all cases owing to several overlaps of  $^{11}\text{C}$ -MET uptake between the granuloma and the tumor, although the mean SUV in the granuloma ( $1.48 \pm 0.09$ ) was significantly lower than that in the tumor ( $1.72 \pm 0.18$ ,  $p < 0.01$ ) (Fig. 6c). The static images and the mean SUVs of  $^{18}\text{F}$ -FDG in the granuloma were similar to those in the tumor (tumor vs granuloma:  $2.83 \pm 0.41$  vs  $2.83 \pm 0.41$  for visual grading;  $5.73 \pm 0.49$  vs  $6.04 \pm 0.63$  for SUV,  $p = \text{ns}$  for both) (Figs. 6b and 6d).

## DISCUSSION

In this study,  $^{11}\text{C}$ -MET uptake in the granuloma showed a slow exponential clearance after an initial distribution, while the uptake in the tumor was gradually increased with time. The dynamic pattern of  $^{11}\text{C}$ -MET uptake in the granuloma was significantly different from that in the tumor (Figs. 3a and 4a-4d). In contrast, the dynamic pattern of  $^{18}\text{F}$ -FDG uptake in the granuloma was comparable to that in the tumor. These findings suggest that dynamic  $^{11}\text{C}$ -MET PET has an additional value for differentiating malignant tumors from granulomatous lesions.

The results of this study showed that there was a difference in the kinetics of early  $^{11}\text{C}$ -MET uptake in the granuloma and tumor. The kinetics of  $^{11}\text{C}$ -MET uptake can be expected to be dependent on both increased local blood flow and the transporters of amino acids.  $^{11}\text{C}$ -MET was taken up faster and at a higher level in the granuloma than in the tumor, which may be related to local blood flow increased by tissular inflammatory reaction. At later time points, the washout of  $^{11}\text{C}$ -MET from the granuloma and continuous accumulation of  $^{11}\text{C}$ -MET in the tumor were observed. It is also considered that the pathophysiological explanation for different  $^{11}\text{C}$ -MET uptake patterns over time between inflammatory and malignant lesions is based on differences in the amino acid transporter or enzyme activity in the tumor and granuloma tissues [13,26,27].

Various studies have demonstrated that the increased uptake level is predominantly due to a higher L-amino acid transporter (LAT) activity in the tumors [26]. The transport of  $^{11}\text{C}$ -MET into a cell

can be followed by rapid metabolization and trapping inside the cell. The exact trapping mechanism is not known but this selective uptake pattern results in a very high tumor-to-background (T/B) ratio enabling the clear delineation of tumors [13]. However, the exact mechanism underlying the accumulation of  $^{11}\text{C}$ -MET in granulomas is not fully understood. Stober et al. [27] suggested that methionine uptake may also be affected by a larger inflammatory component. In inflammatory cells, only 50 % of the tracer uptake could be inhibited by specific transport inhibitors for methionine, while in tumor cells, the tracer uptake was reduced to below 10 %. This high level of “unspecific uptake” in inflammatory cells might be explained by the existence of uptake via free diffusion. A increased local blood flow and a higher unspecific uptake level will result in the kinetic pattern that follow the plasma kinetics more closely with an initial faster and higher rise followed by a slow exponential clearance such as those observed in the granulomas (Figs. 3a, 4a and 4c). On the other hand, tumors showed a gradually increasing methionine accumulation level with time (Figs. 3a, 4b and 4c). It indicated that  $^{11}\text{C}$ -MET is predominantly transported by the L-transport system [28,29]. Kubota et al. [30] reported that the uptake level of  $^{11}\text{C}$ -MET is proportional to the amount of viable tumor cells, but is low in macrophages and other non-neoplastic cellular components. The expression level of Ki-67 in the tumor (Fig. 2-1h) was higher than that in the granuloma (Fig. 2-1d). It is indicated that  $^{11}\text{C}$ -MET uptake is also related to cellular proliferation activity. Iuchi et al. [31] reported that  $^{11}\text{C}$ -MET uptake correlated significantly with the Ki-67 proliferative index. Our previous report showed that  $^{11}\text{C}$ -MET uptake in the granuloma was

significantly lower than that in the tumor, which was concordant with the Ki-67 index [32]. The SUV in the granuloma was significantly lower than that in the tumor (Fig. 6c), which is consistent with the previous reports [15,30]. Thus, the dynamic pattern of  $^{11}\text{C}$ -MET uptake was significantly different between tumors and granulomas.

In the static analysis of  $^{11}\text{C}$ -MET, SUV in the granuloma was significantly lower than that in the tumor (Fig. 6c), which is consistent with the report by Kubota et al. [30] and our previous report [15]. Visual assessment, however, could not differentiate the tumor from the granuloma in all cases. This is due to the fact that the  $^{11}\text{C}$ -MET uptake levels partly overlapped between the granuloma and the tumor (Fig. 6c). It should be noted that information on the kinetics of  $^{11}\text{C}$ -MET obtained by static imaging is limited. Our previous clinical study [15] also showed that  $^{11}\text{C}$ -MET accumulates not only in malignant lesions but also in benign pneumoconiotic nodules, although the  $\text{SUV}_{\text{max}}$  of  $^{11}\text{C}$ -MET was significantly higher in lung cancer than in the pneumoconiotic nodules. Taken together, in such clinical situations, dynamic  $^{11}\text{C}$ -MET PET can be more suitable than conventional static  $^{11}\text{C}$ -MET PET for differentiating malignant tumors from granulomatous lesions. Our results also showed that the peak-to-plateau SUV ratio analysis (SUV ratios of 2 and 15 min) of  $^{11}\text{C}$ -MET PET enabled the differentiation of tumors from granulomas in all rats (Fig. 4d). Early-to-late image analysis may be another option for our findings to be applicable in clinical settings.

The uptake level of  $^{18}\text{F}$ -FDG in the granulomas continued to increase with time, and the

dynamic pattern of  $^{18}\text{F}$ -FDG uptake in the granulomas was comparable to that in the tumors. The extensive  $^{18}\text{F}$ -FDG uptake in the granuloma is related to active inflammatory cell infiltrates including lymphocytes, macrophages, and epithelioid cells. Activated inflammatory cells show a markedly enhanced glycolysis. It is of great importance to determine the cause of the difference between  $^{11}\text{C}$ -MET and  $^{18}\text{F}$ -FDG accumulations in granulomas. The cellular uptake of  $^{18}\text{F}$ -FDG in sarcoidosis is considered to be related to inflammatory cell infiltrates, which are composed of lymphocytes, macrophages, and epithelioid cells from monocytes, because  $^{18}\text{F}$ -FDG has been observed in vitro to be accumulated by leukocytes [33,34], lymphocytes, and macrophages [35]. An increased  $^{18}\text{F}$ -FDG distribution level was mainly observed in epithelioid cell granulomas [20], and a high expression level of Glut-1 was observed in the epithelioid cell granulomas and C6 glioma cells (Figs. 2c and 2f). The activities of granuloma formation and granuloma-associated immune cells may be reflected by the accumulation of  $^{18}\text{F}$ -FDG but not that of  $^{14}\text{C}$ -MET, although the detailed mechanisms underlying the accumulation of these tracers in granulomas remain unclarified. The histological similarity of our granuloma rat model to sarcoidosis indicates its potential for providing a biological basis for differentiating sarcoidosis from malignant tumors [18]. As for the accumulation of these tracers in tumors, Kubota et al. [10] have demonstrated by an autoradiographic study that  $^{14}\text{C}$ -MET uptake is achieved largely by viable cancer cells, whereas the uptake levels in macrophages and granulation tissues are low. On the other hand, an increased  $^{18}\text{F}$ -FDG accumulation level in young granulation tissues around a tumor and in macrophages infiltrating the

marginal areas surrounding an extensive tumor necrotic area was observed by autoradiography using  $^{18}\text{F}$ -FDG and  $^3\text{H}$ -DG [35]. The distinctive uptake profiles of  $^{18}\text{F}$ -FDG and  $^{11}\text{C}$ -MET may provide the different roles of these tracers in the diagnosis of tumors and inflammation.

Differential diagnosis of 1) recurrent glioma from radiation-induced necrosis, 2) recurrent lung cancer from radiation-induced necrosis, and 3) hilar and mediastinal lymph node metastasis in lung cancer from lymphadenopathy in sarcoidosis, are the most important concerns that directly affect patient prognosis and therapeutic choices. From the clinical relevance, we used the tumor (Rat C6 glioma) and granuloma (*R. aurantiacus*-induced) models in the present study. The rat C6 glioma cell line is morphologically similar to human glioblastoma multiforme, and has been widely used to elucidate mechanisms underlying the aggressive nature of gliomas. [36]. Granuloma formation is a result of a chronic inflammatory response in many human diseases such as tuberculosis and sarcoidosis. The characteristics of this disease are inflammatory reaction with activated lymphocyte and macrophage infiltration followed by formation of granuloma [17]. Granuloma formation is also induced by various infectious and noninfectious agents. The non-necrotic granulomas resembling granulomatous lesion of patients with sarcoidosis were formed in *R. aurantiacus*-injected mice. *R. aurantiacus* have recently been recognized as human and animal pathogens that caused granuloma formation in brain, lung, liver and spleen [37,38]. Therefore, rat C6 glioma and *R. aurantiacus*-induced granuloma models would mimic those observed in human patients with glioma and granuloma.

As mentioned above, granuloma formation is a result of a chronic inflammatory response. In the present study, however, mature epithelioid cell granuloma-formation and massive lymphocyte-infiltration were already observed in the calf muscle 10 days after the intramuscular inoculation of *R. aurantiacus*, (Fig. 2-1a). Asano et al. [38] reported that granuloma formation in the lung, liver and spleen of *R. aurantiacus*-injected mice proceeded in parallel. Small granulomas or clusters of a few macrophages surrounded with lymphocytes were observed at 1 week. The granulomas composed of histiocytes or of epithelioid cells surrounded with lymphocytes began to appear at 2 weeks and developed to the maximum at 3 weeks after tail vein injection of *R. aurantiacus*. The histopathological characteristics in the present study (10 days after inoculation) were similar to those at 2-3 weeks after intravenous inoculation reported by Asano et al. [38]. Accordingly, the present model may represent a chronic phase of granuloma formation. The granuloma formation in the calf muscle (10 days after intramuscular inoculation) appear to be slightly faster than that in the lung, liver and spleen (2-3 weeks after intravenous inoculation) reported by Asano et al [38]. However, the further studies are necessary including dynamic <sup>11</sup>C-MET PET in more chronic phases of granuloma (induced by *R. aurantiacus*) to confirm our present results.

## CONCLUSION

The dynamic pattern of <sup>11</sup>C-MET uptake was markedly and significantly different between the

granulomas and tumors. In addition, the distinctive uptake profiles of  $^{11}\text{C}$ -MET and  $^{18}\text{F}$ -FDG may provide the different roles of these tracers in the diagnosis of tumors and inflammation. Thus, dynamic  $^{11}\text{C}$ -MET PET has an additional value for differentiating malignant tumors from granulomatous lesions, which deserves further elucidation in clinical settings.

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## **CONFLICT OF INTEREST STATEMENT**

I have no conflict of interest to declare.

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## FIGURE LEGENDS

**FIGURE 1** Experimental protocols of this study.

**FIGURE 2-1** Microscopy images (x 400) of H&E staining (granuloma [a], tumor [e]) and immunostaining of RM-4 (granuloma [b], tumor [f]), Glut-1 (granuloma [c], tumor [g]) and Ki-67 (granuloma [d], tumor [h]).

White arrow head, epithelioid cell granuloma; black arrow head, viable cancer cell; red arrow head, proliferating cancer cell; white arrow, macrophage infiltration; black arrow, lymphocyte infiltration; red arrow, proliferating lymphocyte.

**FIGURE 2-2** Microscopic images (x 400) of positive [a-c] and negative [d-i] controls used for immunohistochemistry.

a, c: positive controls (rat spleen) for RM-4 [a] and Ki-67 [c]. b: positive control (erythrocytes from human breast cancer) for Glut-1. d-i: negative controls for RM-4 (granuloma [d], tumor [g], Glut-1 (granuloma [e], tumor [h]) and Ki-67 (granuloma [f], tumor [i]). White arrow head, epithelioid cell granuloma; black arrow head, viable cancer cell; red arrow head, proliferating cancer cell; white arrow, macrophage infiltration; black arrow, lymphocyte infiltration; blue arrow, red blood cells; red arrow, proliferating lymphocyte. WP,

white pulp; RP, red pulp.

**FIGURE 3** Dynamic  $^{11}\text{C}$ -MET [a] and  $^{18}\text{F}$ -FDG [b] images.

White arrows, locations of granuloma (right) and C6 tumor (left). R, right; L, left.

**FIGURE 4** Time-activity curves of  $^{11}\text{C}$ -MET [a-c] and peak-to-plateau SUV ratios (SUV ratios of 2 and 15 min) [d] in rats bearing granuloma and tumor.

a, b: Individual values for granuloma (a) and tumor (b) (n=6). c: Mean value for granuloma (solid line) and tumor (dotted line). Error bars indicate standard deviation for each time point. d: Individual values and mean  $\pm$  SD.

**FIGURE 5** Time-activity curves of  $^{18}\text{F}$ -FDG [a-c] in rats bearing granuloma and tumor. a, b: Individual values for granuloma (a) and tumor (b) (n=6). c: Mean value for granuloma (solid line) and tumor (dotted line). Error bars indicate standard deviation for each time point. d: Individual values and mean  $\pm$  SD.

**FIGURE 6** Static  $^{11}\text{C}$ -MET [a] and  $^{18}\text{F}$ -FDG [b] images, and standard uptake values (SUVs) of  $^{11}\text{C}$ -MET [c] and  $^{18}\text{F}$ -FDG [d] in rats bearing granuloma and tumor.

a, b: Transverse, axial, and sagittal images of  $^{11}\text{C}$ -MET (30-40 min) and  $^{18}\text{F}$ -FDG (110-120

min). White arrow, locations of granuloma (right) and C6 tumor (left). c, d: Individual values and mean  $\pm$  SD of SUVs for  $^{11}\text{C}$ -MET (30-40 min) and  $^{18}\text{F}$ -FDG (110-120 min). *NS*, not statistically significant.













