Imaging of Peripheral-type Benzodiazepine Receptor in Tumor: Carbon Ion Irradiation Reduced the Uptake of a Positron Emission Tomography Ligand $[^{11}\text{C}]\text{DAC}$ in Tumor

Tomoteru YAMASAKI$^1$, Sachiko KOIKE$^2$, Akiko HATORI$^1$, Kazuhiko YANAMOTO$^1$, Kazunori KAWAMURA$^1$, Joji YUI$^1$, Katsushi KUMATA$^1$, Koichi ANDO$^2$ and Ming-Rong ZHANG$^1$*

PET/CIRT/NFSa/$[^{11}\text{C}]\text{DAC}/\text{PBR}$.

We aimed to determine the effect of carbon ion irradiation on the uptake of N-benzyl-N-$^{11}\text{C}$-methyl-2-(7-methyl-8-oxo-2-phenyl-7,8-dihydro-9H-purin-9-yl)acetamide ($[^{11}\text{C}]\text{DAC}$), a positron emission tomography (PET) ligand for the peripheral-type benzodiazepine receptor (PBR), in tumor cells and tumor-bearing mice. Spontaneous murine fibrosarcoma (NFSa) cells were implanted into the right hind legs of syngeneic C3H male mice. Conditioning irradiation with 290 MeV/u carbon ions was delivered to the 7- to 8-mm tumors. In vitro uptake of $[^{11}\text{C}]\text{DAC}$ was measured in single NFSa cells isolated from NFSa-bearing mice after irradiation. In vivo biodistribution of $[^{11}\text{C}]\text{DAC}$ in NFSa-bearing mice was determined by small animal PET scanning and dissection. In vitro autoradiography was performed using tumor sections prepared from mice after PET scanning. In vitro and in vivo uptake of $[^{11}\text{C}]\text{DAC}$ in single NFSa cells and NFSa-bearing mice was significantly reduced by carbon ion irradiation. The decrease in $[^{11}\text{C}]\text{DAC}$ uptake in the tumor sections was mainly due to the change in PBR expression. In conclusion, $[^{11}\text{C}]\text{DAC}$ PET responded to the change in PBR expression in tumors caused by carbon ion irradiation in this study. Thus, $[^{11}\text{C}]\text{DAC}$ is a promising predictor for evaluating the effect of carbon ion radiotherapy.

INTRODUCTION

Since 1994, heavy ion radiotherapy has been performed at the National Institute of Radiological Sciences (NIRS), Japan, and more than 4400 cancer patients have been treated using carbon ions.11

The carbon ion beam used in heavy ion irradiation has unique physical and biological properties, making it suitable as a charged particle beam with high linear energy transfer (LET).1–4) A carbon ion beam has a precise range, travels in a straight line when penetrating tissues and releases a large amount of energy at the end of its range. This well localized deposition of energy (high-dose peak) at the beam end, called the Bragg peak, is a unique physical characteristic of the charged particle beam and enables the safe delivery of an effective dose of radiation to a bulky primary cancer without damaging normal tissues. Because of its radiobiological and precise dose-localization characteristics, carbon ion radiotherapy (CIRT) may offer advantages in tumor radiotherapy and is a promising way of treating human cancers. X-ray CT MRI and positron emission tomography (PET) have been used to evaluate the therapeutic effect of CIRT on cancer. PET is useful for determining the efficacy of radiotherapy by monitoring the abnormal features (receptors, sugar metabolism and nucleic acid) of tumor cells. At our institute, PET with $[^{11}\text{C}]\text{methionine}$ has been used to monitor CIRT response in patients with bone and soft tissue sarcomas and rectal cancer.5,6) However, $[^{11}\text{C}]\text{methionine-PET}$ directs the false-positive in benign lesions and inflammatory tissue.7,8) Thus, to find an alternative and more specific PET ligand, which could evaluate the therapeutic effect of CIRT against cancer, we have been developing PET ligands for receptors present in tumors.

The peripheral-type benzodiazepine receptor (PBR), an 18-kDa protein located on the mitochondrial membrane,9) was overexpressed in various tumor cells such as glioma,10,11 astrocytoma12) and breast cancer13,14) cells. In some malignant tumors at an advanced stage of progression, PBR was expressed on the nuclear membrane in addition to the mito-
chondrial membrane. Because PBR plays an essential role in the abnormal progression of a tumor, we assumed that a PBR ligand would show high and specific uptake into PBR-rich tumor cells and organs. Therefore, PBR binding in tumors may provide a useful biomarker for monitoring the therapeutic effect of CIRT against cancer.

The aim of this study was to determine the change in uptake of $^{11}$C-DAC in tumor-bearing mice showed a contrast in radioactivity between tumor and blood or muscle (2 regions with low PBR density). Furthermore, $^{11}$C-DAC uptake in tumors was reduced by pretreatment with PK11195, a standard PBR ligand, indicating that $^{11}$C-DAC uptake was attributable to PBR. $^{11}$C-DAC is a potent and selective PET ligand for PBR imaging in tumor-bearing mice.

In this study, we used $^{11}$C-DAC PET to elucidate the difference in $^{11}$C-DAC uptake in tumor-bearing mice subjected or not subjected to irradiation using a carbon ion beam. We investigated the effect of carbon ion irradiation on $^{11}$C-DAC uptake in the tumors. In vitro and in vivo uptake of $^{11}$C-DAC in tumor cells and tumor-bearing mice was compared between irradiated and non-irradiated mice. To evaluate the utility of $^{11}$C-DAC, we used spontaneous murine fibrosarcoma (NFSa) cells and NFSa-bearing mice. NFSa cells are a standard cell line for assessing the effect of CIRT in mice and have been maintained in culture at our institute for the past 2 decades. Our group recently confirmed the presence of PBR in single NFSa cells.

**MATERIAL AND METHODS**

**Production of $^{11}$C-DAC**

$^{11}$C was produced by $^{14}$N (p, α)$^{11}$C nuclear reaction using a Cypris HM18 cyclotron (Sumitomo Heavy Industries). A dose calibrator (IGC-3R Curiometer; Aloka) was used for all radioactivity measurements unless otherwise stated. $^{[11]}$C was radiosynthesized from cyclotron-produced $^{[11]}$CO$_2$, as described previously. Starting from $^{[11]}$CO$_2$ of 12–15 GBq, $^{[11]}$C-DAC of 700–1380 MBq (n = 26) was obtained with 98% radiochemical purity and $105 \pm 44$ GBq/μmol specific activity at the end of synthesis.

**Evaluation of $^{11}$C-DAC**

Animal experiments were performed according to the recommendations of the Committee for the Care and Use of Laboratory Animals, NIRS.

**Mice and tumors**

Ten- to 16-week-old male C3H/HeMsNrsf mice were used in the present study. The animals were produced and maintained in specific pathogen-free facilities at our institute and housed in groups. The tumor was a syngeneic NFSa preserved at our institute, and 16th generation cells ($10^6$ cells) were implanted subcutaneously into the right hind legs of the mice. Single-cell suspensions were prepared by enzymatically digesting the tumor. In vivo experiments using NFSa-bearing mice were performed on day 7 after implantation, when the tumors reached a diameter of 7–8 mm.

Mice selected for irradiation with carbon ions were transported to the accelerator facility shortly before irradiation and maintained in an associated conventional facility.

**Irradiation**

Carbon ions were accelerated by the Heavy Ion Medical Accelerator in Chiba synchrotron up to 290 MeV/u. Irradiation was performed using horizontal carbon beams with a dose rate of 3 Gy/min. The physical dose decreased to approximately 50% when the irradiation position was changed from the proximal edge of a 6-cm spread-out Bragg peak (SOBP) to the distal edge of the SOBP. LET of the 290 MeV/u carbon beams ranged from 14 keV/μm to greater than 200 keV/μm, depending on the depth. Desired LET beams were obtained by selecting the depth along the beam path using a Lucite range shifter, and the desired irradiation field was obtained by simultaneous use of an iron collimator and a brass collimator. Using pentobarbital anaesthesia (50 mg/kg) and taping, 5 mice were immobilized on a Lucite plate such that the right hind leg was located in a rectangular field of $28 \times 100$ mm, and an irradiation dose of 20 Gy was applied. The foot was excluded from the irradiation field. The tumor diameter was $7.5 \pm 0.5$ mm (mean ± range) before irradiation.

**In vitro uptake into single NFSa cells**

Irradiated NFSa-bearing mice (n = 4) were sacrificed; tumor volume was $0.37 \pm 0.04$ cm$^3$ (mean ± range) compared to $0.39 \pm 0.04$ cm$^3$ for non-irradiated NFSa-bearing mice (n = 4) sacrificed on the 7th day after tumor implantation. Tumors from NFSa-bearing mice of the 2 groups were minced carefully on a dish using surgical scissors. The
chopped tissues were incubated with 0.2% trypsin, 0.03% pancreatin and 1 mg DNase mixing solution in a water bath for 25 min at 37°C. Hanks’ medium (Gibco) was added to the mixture to terminate the reaction. The mixture was then passed through a coarse filter, followed by a syringe filter. After centrifugation, single NFSa cells were purified.

Single NFSa cells were placed (4 × 10⁵ cells) in Falcon tubes with Hanks’ medium and incubated with [¹¹C]DAC (3.7 MBq/mL) in a water bath at 37°C for 30 min. After incubation, the tubes were cooled on ice and placed in 1-mL microtubes. After centrifugation, the supernatant was removed and pre-chilled PBS was added to the tubes. This operation was repeated 3 times. Washed cells were dissolved in 0.2 N NaOH, and radioactivity was measured with an autogamma scintillation counter (Wizard 3″1480; PerkinElmer, Waltham, MA). After the radioactivity decayed, protein concentration of the lysed cell solution was measured by the DC protein assay (Bio-Rad).

In vivo biodistribution

Non-irradiated and irradiated NFSa-bearing mice (4 or 5 males per group) were selected and separated into 2 experimental groups under the same conditions. Body weights of non-irradiated and irradiated mice were 33.3 ± 3.8 and 29.4 ± 1.2 g, respectively, and tumor volumes were 0.34 ± 0.07 and 0.34 ± 0.04 cm³, respectively. Saline solution of [¹¹C]DAC (7.4 MBq/150 μL) was injected into each mouse through the tail vein. Mice were sacrificed by cervical dislocation 30 min after [¹¹C]DAC injection. Heart, liver, lungs, spleen, stomach, kidneys, small intestine, muscle, tumor and blood samples were removed quickly. Radioactivity in these tissues was measured using an autogamma scintillation counter and expressed as the percentage of the injected dose per gram of wet tissue (% ID/g). All radioactivity measurements were corrected for decay.

Small animal PET imaging

A 12-week-old male non-irradiated NFSa-bearing mouse (body weight: 30.2 g, tumor volume: 0.34 cm³) and a 12-week-old irradiated (30.1 g, 0.37 cm³) NFSa-bearing mouse were selected for imaging. The mice were secured in a custom-designed chamber and placed in a small animal PET scanner (Inveon; Siemens Medical Solutions). Body temperature was maintained with a lamp. The mice were anaesthetized with 1.5% isoflurane during scanning. To inject [¹¹C]DAC, a 29-gauge needle with 12–15 cm of PE 10 tubing was inserted into the tail vein. After transmission scans for attenuation correction using a ⁵⁷Co source for 803 s, a dynamic emission scan in 3D acquisition mode was performed for 90 min (1 min × 4 scans, 2 min × 8 scans and 5 min × 14 scans). A bolus of 18 MBq [¹¹C]DAC in 200 μL saline was injected through the tail vein catheter.

Region of interest (ROI) analysis and image reconstruction were performed using the software IDL Virtual Machine (Research System Inc.). Visual analysis was performed by individuals experienced in PET interpretation using coronal, transverse and sagittal reconstructions. ROIs were manually placed across image planes to produce time-activity curves.

Radioactivity was decay corrected for injection time and expressed as the standardized uptake value (SUV), normalized for injected radioactivity and body weight. SUV was calculated as (radioactivity per cm³ tissue/injected radioactivity) × g body weight.

In vitro autoradiography

After the PET scans, 2 NFSa-bearing mice were sacrificed by cervical dislocation. Tumor tissues were removed and immediately frozen in powdered dry ice. After the radioactivity decayed, tumor sections (10 μm) were prepared using a cryotome (HM560; Carl Zeiss) at a temperature of −20°C and mounted on Matsunami adhesive silane-coated glass slides. The sections were pre-incubated in 50 mM Tris buffer (pH 7.4) at room temperature for 20 min and then incubated in the same buffer containing [¹¹C]DAC (7.4 MBq) at room temperature for 30 min. After incubation, the sections were washed twice for 2 min each in 50 mM cold fresh Tris-HCl buffer and 10 s in distilled water. They were then dried with a warm air current and placed in contact with an imaging plate (BAS-MS 2325; Fuji Photo Film, Tokyo, Japan) for 60 min. Radioactivity concentrations in the sections were measured on autoradiograms, and photostimulated luminescence (PSL) values for each tumor section were determined using the bio-imaging analyzer system (BAS-5000; Fuji). Regional radioactivity concentration in the sections was expressed as PSL/mm².

Fig. 2. Tumor growth after a single irradiation dose of 20 Gy administered with 74 keV/μm carbon ions in NFSa-bearing mice (n = 4–8).
RESULTS

Tumor growth
To determine the optimal period for the present experiments, we evaluated tumor growth in the irradiated NFSa-bearing mice by measuring tumor volume after irradiation. A single irradiation dose of 20 Gy was used to irradiate the tumor. A previous study indicated that this dose would cause sub-lethal damage to the tumor.18) Figure 2 shows the growth curve of tumors in NFSa-bearing mice subjected to 20-Gy carbon ion irradiation. Tumor volume remained constant for 5–7 days after irradiation, after which the tumors started to grow again. We selected mice at day 7 after irradiation as a target group to directly compare the effect of carbon ion irradiation in the absence of intra-tumoral hypoxia because their tumor volume was similar to that of non-irradiated mice used as a control group. Tumor volumes of the irradiated and non-irradiated mice were $0.44 \pm 0.04$ cm$^3$ and $0.38 \pm 0.06$ cm$^3$, respectively. From 10 days after irradiation, tumor volume increased significantly and became indefinite. Marked necrosis occurred in the tumor tissue.

In vitro uptake into single NFSa cells
Figure 3 shows in vitro uptake of $[^{11}C]$DAC in single NFSa cells isolated from non-irradiated and irradiated mice. The uptake of radioactivity in NFSa cells after 30-min incubation was $51.9 \pm 2.8$ and $36.3 \pm 4.6\%$ incubation dose/mg cell protein (% ICD/mg protein) for non-irradiated and irradiated mice, respectively. $[^{11}C]$DAC uptake by NFSa single cells isolated from the irradiated mice was about 30% less than that by cells from the non-irradiated mice. Statistical difference in uptake between the 2 cell groups was identified (P < 0.01), and statistical significance of each group was identified by t-test.

Biodistribution study in mice
Figure 4 shows the biodistribution of $[^{11}C]$DAC in the main organs of irradiated and non-irradiated NFSa-bearing mice with or without irradiation and immediately incubated with $[^{11}C]$DAC (3.7 MBq/mL) at 37°C for 30 min. Error bars show the SD (n = 4). **P < 0.01; t-test.
mice (n = 4) examined by the dissection method. The radioactivity in all organs was expressed as decay-corrected % ID/g values 30 min after a bolus injection (Fig. 4). High concentration of radioactivity (> 10% ID/g in the non-irradiated groups) was found in the heart, lungs and kidneys, which are organs with high PBR densities.15,21–23) [11C]DAC uptake in tumor tissue, the target tissue in this study, was 4.50 ± 0.80% ID/g for non-irradiated mice and 2.44 ± 0.24% ID/g for irradiated mice. Uptake in the PBR-enriched tissues (heart, lungs and kidneys) in the irradiated group was significantly lower than that in the non-irradiated group. Using muscle, which is a low PBR content tissue, as a reference tissue, the tumor-to-muscle ratios in the non-irradiated and irradiated groups were 1.73 ± 0.35 and 1.39 ± 0.07 (Table 1), respectively. This difference was significant (P < 0.05), although the ratios of kidney and heart with high [11C]DAC uptake to muscle were similar. This indicates that tumor tissue as an irradiation target was affected not only by metabolic disorder but also by decline of PBR expression.

### Small animal PET imaging

Figure 5 shows 0- to 90-min summation images from non-irradiated and irradiated mice in coronal and transverse views. A marked decrease in [11C]DAC uptake in the tumor was observed in the irradiated mouse compared to the non-irradiated mouse. [11C]DAC uptake in the main organs (liver, kidney and muscle) of the irradiated mouse was also lower compared to that in the corresponding organs of the non-irradiated mouse.

Figure 6 shows time-activity curves for the tumor, muscle, liver, kidney and bladder as well as the tumor-to-muscle ratios. Radioactivities of [11C]DAC in the tumor and muscle in the irradiated mouse declined from 0.8 to 0.5 and from 0.4 to 0.3 SUV, respectively, compared to that in the non-irradiated mouse. The accumulation rate of radioactivity in the liver of the irradiated mouse was slower than that in the liver of the non-irradiated mouse. In particular, marked reduction in uptake was observed in the kidneys. Uptake into the kidneys of the irradiated mouse was two-thirds of that in

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<th>Irradiated</th>
<th>Decline%**</th>
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*Mean ± SD (n = 5 in the non-irradiated mice; n = 4 in the irradiated mice)  
**Decline% = (Irradiated ratio/non-irradiated ratio) \times 100  
# P < 0.05 (t-test; non-irradiated vs. irradiated)

![Fig. 5.](image)

**Fig. 5.** PET images of NFSa-bearing mice with (irradiated) or without (non-irradiated) carbon ion irradiation. These images are 0- to 90-min summations on coronal (left) and transverse (right) views. The mouse was anaesthetized with 1.5% isoflurane during the scan. [11C]DAC (18 MBq) was injected through the tail vein. PET scanning was performed for 90 min (1 min × 4 scans, 2 min × 8 scans, 5 min × 14 scans). SUV = (radioactivity per cubic centimetre tissue/injected radioactivity) × grams body weight. Tumor positions are indicated as dotted red circles. Transverse views are slices of the coronal views at positions indicated by white dotted lines.
the non-irradiated mouse. In contrast, radioactivity uptake in the bladder of the irradiated mouse continued to increase until the end of the PET scan. The SUV value in the irradiated mouse was 1.5 times higher than that in the non-irradiated mouse, and the tumor-to-muscle ratio in the irradiated mouse was about 20–30% of that in the non-irradiated mouse.

In vitro autoradiography

After the PET scan, tumor sections for in vitro autoradiography were prepared from the 2 NFSa-bearing mice. Figure 7 shows autoradiographic images of tumor sections from non-irradiated and irradiated mice. The radioactivity values in the sections from non-irradiated and irradiated mice were 315 ± 32 and 253 ± 17 PSL/mm³, respectively. [11C]DAC uptake in tumor sections from irradiated mice was about 20% less than that in sections from non-irradiated mice.

DISCUSSION

In this study, we demonstrated that the uptake of [11C]DAC into NFSa cells and NFSa-bearing mice was reduced in terms of the suppression of tumor growth caused by carbon ion irradiation.
Reduce of $[^{11}C]D$AC Uptake in Tumor by CIRT

PBR is highly expressed in tumors, and its expression level is closely related to tumor progress. Therefore, using a PBR ligand to evaluate the effect of CIRT in the clinical treatment of cancer appears to be promising. We had previously developed $[^{11}C]D$AC as a novel PET ligand specific for PBR (Fig. 1) and reported the PET imaging of NFSa-bearing mice. NFSa has been used to assess the effect of CIRT at our institute for 2 decades and shown to express PBR abundantly. If a difference in specific binding and $[^{11}C]D$AC uptake in NFSa cells and NFSa-bearing mice is confirmed between non-irradiated and irradiated mice, $[^{11}C]D$AC PET could be a useful tool for evaluating the effect of CIRT on cancer treatment. In the present study, we used high-LET radiation provided by a carbon ion beam, which forms a SOBP at a given depth that depends on beam energy. A previous study found only a small difference between a fractionated and single dose of high-LET carbon ions with respect to tumor growth in the NFSa cell. We therefore performed single-dose irradiation at 290 MeV/u, 6-cm SOBP and 74 keV/μm LET. This condition of irradiation was more aggressive with respect to the tumor than the other LET condition. The much higher dose of radiation delivered using high-LET irradiation resulted in complete destruction of tumors, because this form of irradiation caused apoptosis by activation of caspase-9 following mitochondrial damage. Consequently, we selected the 20-Gy dose for the carbon ion irradiation beam in this study. In a previous study, 20-Gy carbon ion irradiation caused sublethal damage in tumors, and tumor growth was suspended temporarily in NFSa-bearing mice. As expected, in the present study, tumor growth in mice subjected to 20-Gy carbon ion irradiation was inhibited until about 7 days after irradiation, when the tumor started to grow again (Fig. 2).

We first demonstrated a change in the in vitro uptake of $[^{11}C]D$AC using single NFSa cells isolated from tumor-bearing mice subjected or not subjected to carbon ion irradiation. The results of the in vitro cell assay showed that carbon ion irradiation reduced the uptake of $[^{11}C]D$AC into NFSa cells (Fig. 3). In a previous study, we confirmed the presence of PBR in the NFSa cell line using an immunohistochemical assay. Moreover, $[^{11}C]D$AC uptake was reduced by unlabelled DAC in a dose-dependent manner. The present study indicated that the uptake value of $[^{11}C]D$AC was related to the level of PBR expression in NFSa cells and that carbon ion irradiation might reduce PBR expression in NFSa cells by damaging cellular DNA.

We then demonstrated that carbon ion irradiation reduced the in vivo uptake of $[^{11}C]D$AC in NFSa-bearing mice. As shown in Fig. 4, radioactivity uptake in tumor tissue was lower in NFSa-bearing mice subjected to carbon ion irradiation than non-irradiated mice. However, the uptake of $[^{11}C]D$AC in the irradiated mice was lower not only in the tumor but also in organs such as the heart, lungs, liver, kidneys, spleen and muscle (Fig. 4). This decrease in $[^{11}C]D$AC uptake in the whole body may have been due to a homeostatic disorder resulting from an overflow of inflammatory cytokines after radiation injury. In fact, the skin of the irradiated field showed marked inflammation, which showed increased progression from 10 days after irradiation (data not shown). We used muscle, which has a low PBR density, as a reference and calculated uptake ratios of the tumor, heart and kidney to muscle in order to study changes in PBR binding, independent of blood flow and metabolic disturbance (Table 1). Compared to non-irradiated mice, irradiated mice showed significant reduction in the tumor-to-muscle ratio (P < 0.05), whereas uptake ratios of heart and kidney to muscle were similar in irradiated and non-irradiated mice. These results are consistent with those of our in vitro cell assay.

In small animal PET imaging (Figs. 5 and 6), $[^{11}C]D$AC radioactivity in tumors in irradiated mice was less, as observed visually, in both coronal and transverse views, compared to non-irradiated mice (Fig. 5). The time-activity curve for $[^{11}C]D$AC showed less uptake in tumor, muscle, liver and kidney, also found using the dissection method. In contrast to the decreased radioactivity in most organs, accumulation of radioactivity in the bladder of irradiated mouse remained high (Fig. 6). This result indicates that uptake of $[^{11}C]D$AC into peripheral organs in irradiated mouse was inhibited as a result of a homeostatic disorder caused by radiation injury, leading to rapid elimination of $[^{11}C]D$AC through the bladder after a bolus injection.

We further discovered that in vivo uptake of $[^{11}C]D$AC was related to PBR expression in tumor sections. To determine whether decline in in vivo uptake was related to change in PBR expression in tumor sections, we performed in vitro autoradiography using tumor sections prepared from a mouse used in the PET experiment (Fig. 7). This in vitro experiment directly reflected the change in $[^{11}C]D$AC uptake in tumor sections after irradiation and was not affected by blood flow and re-uptake of radioactivity from other organs or regions. As shown in Fig. 7, $[^{11}C]D$AC binding in tumor sections prepared from the irradiated mouse was about 20% lower than that in sections prepared from the non-irradiated mouse. This reduction rate was consistent with the PET imaging results and in vitro cell assay. Thus, although the uptake of $[^{11}C]D$AC revealed by its in vivo biodistribution and PET imaging was reduced by radiation injury, most of the reduction in radioactivity in the tumor tissue was related to a difference in PBR expression. The high potency of DAC for PBR and its high selectivity for other receptors support the specificity of $[^{11}C]D$AC uptake for PBR.

In the present study, we have demonstrated a decline in $[^{11}C]D$AC uptake during suspension of tumor progression produced by carbon ion irradiation in NFSa cells and NFSa-bearing mice. Furthermore, the decline in $[^{11}C]D$AC uptake was related to decrease in PBR expression in tumor sections. However, much work remains to be done before $[^{11}C]D$AC...
PET can be used for evaluating and monitoring the effect of CIRT. Because of the rigid time schedule of irradiation, a comparative experiment was performed only at a single time point after irradiation in the present study. Examination of \([11C]\)DAC uptake should be performed at different time points; for example, just after irradiation and after 10–20 days, a period that would include tumor re-growth, as shown in Fig. 2. Longitudinal monitoring of the same irradiated mice would be useful in evaluating the response to carbon ion irradiation, as observed by \([11C]\)DAC PET. Furthermore, although the functions of PBR in retarding tumor progression have been reported frequently, the mechanism of PBR overexpression in some malignant cancers remains unresolved. Therefore, basic molecular biological investigation of changes in PBR expression caused by carbon ion irradiation and the relationship between the decline in PBR expression and tumor progression is still needed.

In conclusion, using \([11C]\)DAC, we confirmed that carbon ion irradiation reduced PBR expression in NFSa cells and NFSa-bearing mice. Moreover, the change in \([11C]\)DAC uptake in vivo was also identified by PET scanning. The present results may be useful in clarifying the molecular biological mechanism of interaction between PBR expression and damage to the cell nucleus. Although much work remains to be done, \([11C]\)DAC uptake appears to be a promising new index of the clinical effect of CIRT.

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