In vivo ^{18}F-fluorodeoxyglucose-positron emission tomography/computed tomography imaging of pancreatic tumors in a transgenic rat model carrying the human *KRAS*^{G12V} oncogene

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Abstract. A novel *KRAS*-mediated transgenic rat model has previously been demonstrated, in which animals develop multiple pancreatic ductal adenocarcinoma (PDAC) that is histologically similar to human PDAC within two weeks. Positron emission tomography (PET)/computed tomography (CT) is commonly used for the diagnosis and staging of PDAC in humans, and can be adopted for optimal use in animal experiments. The aim of the present study was to evaluate the carcinogenic process in a rat pancreatic carcinoma model using small-animal multimodality imaging systems. The utility of fluorodeoxyglucose (FDG)-PET/CT in detecting the location and size of PDAC during tumor development in the present transgenic rat model was assessed. A small animal multimodality PET/CT system and contrast-enhanced CT (CECT) system were used for the imaging analysis of *KRAS*^{G12V} male transgenic rats (n=6), which developed pancreatic tumors following the administration of an injection of Cre recombinase (Cre)-carrying adenovirus. Laparotomies performed at six weeks post-treatment revealed that all three (100%) Cre-expressing rats developed pancreatic tumors that were <2 mm in diameter, none of which were detected by ^{18}F-FDG PET/CT or CECT. At eight weeks post-treatment, the pancreatic tumors were heterogeneously visualized by ^{18}F-FDG-PET/CT and CECT in two of the three rats. Furthermore, the autopsies confirmed that all three rats had developed pancreatic tumors. These novel findings provide evidence that the FDG-PET/CT imaging system is a valuable tool for the evaluation of the carcinogenic process, and one which may aid in treatment and preventive methods for pancreatic tumors in mammalian models. A limitation associated with the early detection of PDACs warrants further investigation.

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Introduction

With >250,000 annual mortalities, pancreatic carcinoma is one of the most lethal malignancies, ranking 12th worldwide (1). Mortality resulting from this disease is high even in developed countries, including Japan, the United Kingdom, France and the United States (2,3). Overall, >75% of pancreatic carcinoma cases are histologically characterized as pancreatic ductal adenocarcinoma (PDAC) (4,5). The majority of cases of PDAC are incurable due to the necessity of extensive resection, which is often not feasible, and due to the fact that the disease is rarely identified at an early stage. Furthermore, the majority of patients with advanced PDAC either do not respond, or respond transiently to chemotherapeutic drugs and radiation (6). Typically, the majority of patients with PDAC succumb to the disease within one year of diagnosis, and the overall five-year survival rate is <5% (7). Even in patients with resectable carcinoma, the long-term outcome remains unsatisfactory due to the incidence of early recurrence following surgical resection.

In order to gain an improved understanding of this lethal malignant carcinoma, studies that use animal PDAC models with pancreatic neoplasms that resemble human PDAC are usually desirable. By focusing on human pancreatic adenocarcinomas that express a high frequency of *KRAS* mutation, a transgenic rat model carrying the human *HRAS*^{G12V} or *KRAS*^{G12V} oncogene was established (8,9). The activation of the target transgene is attained by the injection of a Cre recombinase (Cre)-carrying adenovirus into the pancreatic ducts of the animal via the

common bile duct (8,9). In this model, the transgenic rats usually develop pre-neoplastic and neoplastic pancreatic lesions within two weeks of the viral inoculation (10). These lesions in the transgenic rats exhibit morphological similarities to those observed in human pancreatic lesions, including PDAC (11) and intraepithelial neoplasias (PanINs) (9).

Due to the position of the tumors within the abdominal cavity, laparotomy is the only technique that is able to determine the existence and size of pancreatic tumors within the transgenic rats following virus inoculation, as the tumors cannot be visually assessed from surface scans of the affected rats. A previous study determined that in order to serologically detect early-stage PDAC in the rat models, serum N-ERC levels and the levels of several serum miRNAs, which are expressed differentially in PDAC transgenic rats and control rats, could be used (8,12). However, even in the case of elevated levels of high serum biomarkers, the exact location and size of pancreatic tumors is difficult to detect unless exploratory surgery is performed within the abdominal cavity.

^{18}F-fluorodeoxyglucose-positron emission tomography ([^{18}F-FDG-PET) is commonly used during the diagnosis of pancreatic tumors (13,14). Due to a high sensitivity and penetration depth, PET is considered to be more accurate for the detection and identification of metastases in humans and animal models than other imaging systems (15,16).

The objective of the present study was to evaluate the carcinogenic process in a mammalian model using imaging modalities, such as PET/computed tomography (CT), which are applicable for the study of human PDAC.

Materials and methods

Animals. In total, six male *KRAS*^{G12V} oncogene transgenic rats were used in the present study. Routine genotyping was performed as previously described (8). The rats were kept in plastic cages in an air-conditioned room at 24±2˚C and 60±5% humidity with a 12-h light/12-hour dark cycle. A basal diet (Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water were available *ad libitum* throughout the experiment. All experiments were approved by the Animal Care and Use Committee of Nagoya City University Graduate School of Medical Sciences and the National Institute of Radiological Sciences (Tokyo, Japan).

Procedure of adenovirus inoculation. The preparation and inoculation of the adenoviruses was performed as previously described (9). In brief, a Cre-recombinase expressing adenovirus was amplified in HEK293 cells and then purified using the Vivapure AdenoPACK (Vivascience, Hannover, Germany) (17). The titer of the adenovirus was then determined using an Adeno-X rapid titer kit (Clontech, Mountain View, CA, USA). The virus was prepared to a concentration of 4.0×10^9 plaque-forming units/ml. The virus (300-400 μl) was injected using a small syringe into the pancreatic duct of the rats as previously described (9).

[^{18}F-FDG-PET and CT procedures, and image analysis. The time course of the experimental protocol is shown in Fig. 1. For the present study, 10-week-old male *KRAS*^{G12V} transgenic rats were used. The rats were divided into two groups, with three rats per group. The rats in groups 1 and 2 were administered with the Cre-expressing adenovirus vector or an empty vector (negative control), respectively. A small-animal multimodality PET system (Inveon; Siemens Healthcare Inc., Malvern, PA, USA) was used for PET data acquisition. Following an overnight fast, each rat (body weight, 403-583 g) was injected with 15 MBq (14.6±1.6 MBq) ^{18}F-FDG (Nihon Medi-Physics Co., Ltd., Tokyo, Japan) via the tail vein, whilst the rat was under isoflurane anesthesia. The PET data acquisition was conducted for 10 min, beginning 50 min after the ^{18}F-FDG injection. Using a lamp, the body temperature of the rats was maintained at between 36 and 37˚C during the scan. The images were reconstructed using a 3D maximum *a posteriori* (18 iterations with 16 subsets; β=0.2), without attenuation correction. The tracer uptake was expressed as the standardized uptake value (SUV).

Subsequent to PET scanning, plain or contrast-enhanced CT (CECT) was conducted with an X-ray source set at 90 kVp and 200 μA, using a small-animal CT system (R_mCT2; Rigaku, Tokyo, Japan). For CECT, the rats were intravenously injected with 10 ml Iopamiron 370 contrast medium (Bayer Yakuhin Ltd., Osaka, Japan) using an infusion pump (NE-1000; Neuroscience Inc., Tokyo, Japan) at the rate of 2 ml/min, whilst the rats were under isoflurane anesthesia. The CECT images were acquired 5 min subsequent to the injection. In order to reduce the motion artifacts caused by respiratory and peristaltic movement during the CT scan, a respiratory gating system was used whilst the rats under inhalable isoflurane anesthesia. The ^{18}F-FDG-PET scanning was conducted at two, three, four, five and eight weeks subsequent to administration of the Cre-expressing adenovirus or the empty vectors. In order to confirm the results of the PET analysis, the CECT scan was also conducted at five and eight weeks subsequent to the virus injection. For the quantitative analysis, the PET and CT data sets were imported and the fused images were then obtained using ASIPro VM software (CTI Concorde Microsystems, Knoxville, TN, USA). Laparotomy was performed six weeks subsequent to the injection in order to confirm the location and size of the pancreatic tumors, which were visible to the naked eye. The experimental rats were sacrificed eight weeks subsequent to the injection.

Histopathological examination. The rats in groups 1 and 2 survived until the end of the experimental period. The pancreatic tumors and the normal pancreatic lobes were removed from the abdomen of the rats, fixed with 10% buffered formalin and then processed for histopathological examination using hematoxylin and eosin stain (9). The pancreatic lesions were diagnosed histopathologically based upon previously described criteria (8,9).

Results

PET/CT findings and histopathological examination. The six rats were euthanized eight weeks subsequent to the injection of the Cre-expressing viral or empty vectors. All three Cre-expressing transgenic rats in group 1 (100%) developed orthotopic pancreatic tumors without distant metastasis. By contrast, no tumors were identified in the negative control rats of group 2. Upon macroscopic analysis, the tumors appeared nodular and solid in shape, and were ochre yellow in color. The

Table I. Mean SUV of the tumor and each organ in the experimental rats.

Rat	Tumor (SUV_{max})	GI tract (SUV_{max})	Liver	Kidney (cortex and medulla)
1	0.7-1.2 (1.4)	0.5-1.0 (3.2)	0.4-0.5	0.8-0.9
2	0.9-2.0 (3.0)	0.5-1.8 (2.7)	0.5-0.6	0.9-1.1
3	ND	0.6-1.4 (4.2)	0.6-0.7	0.9-1.1

The rats were inoculated with a Cre recombinase-expressing vector as described in the Materials and methods section. The values were calculated using the scanning data obtained at eight weeks post-inoculation. SUV_{max}, maximum standardized uptake value; GI, gastrointestinal; ND, Not detected.

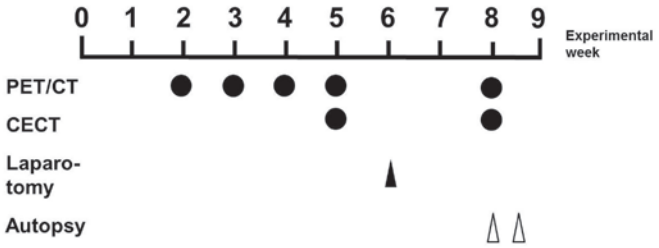

Figure 1. Experimental time course. Closed circle indicates the time of scanning. Closed triangle indicates the time of laparotomy. Open triangle indicates the time of autopsy. Laparotomy and autopsy were performed on the three rats from group 1 at eight weeks post-viral inoculation. Viral inoculation was performed at week zero. PET, positron emission tomography; CT, computed tomography; CECT, contrast-enhanced computed tomgraphy.

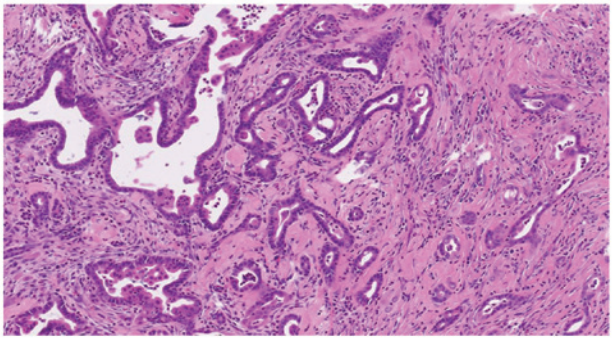

Figure 2. Representative microscopic image of a pancreatic ductal adenocarcinoma, revealing papillotubular growth of the tumor cells and abundant fibrous tissue proliferation (hematoxylin and eosin staining; magnification, x100).

PET/CT images obtained eight weeks subsequent to the viral injection revealed the majority of tumor tissues to be distinguishable from the adjacent organs, but the tissues were challenging to distinguish from the normal intestinal tissues, depending on the site of the tumor. The pancreatic tumors were of the ductal adenocarcinoma histological type. The coexistence of adenocarcinoma and PanIN lesions surrounded by fibrous tissue with inflammatory cell infiltration was also identified (Fig. 2).

[^{18}F-FDG-PET imaging prior to experimental week five. The representative maximal-intensity projection images from ^{18}F-FDG-PET are shown in Fig. 3. PET scanning was performed four times prior to the five experimental weeks. A marginal or very high uptake in the gastrointestinal tract and urinary bladder, which was considered to be physiological FDG uptake, was observed in all three Cre-expressing transgenic rats and three control rats. At five weeks post-treatment, the tumors were not clearly visualized by the indicated imaging system. Following the laparotomy at six weeks post-treatment, a few small nodules measuring between 1 and 2 mm in diameter, indicative of a carcinoma, were identified in the pancreas of all Cre-expressing transgenic rats. No metastases were identified in the rats of the negative control group.

Analysis of CECT, PET and PET/CT images at eight weeks post-treatment. Representative slices of the ^{18}F-FDG-PET/CT fusion images obtained from the Cre-expressing transgenic rats are shown in Figs. 4-6. The SUV_{max} and SUV_{mean} of the pancreatic tumors and each organ are shown in Table I. In rat 1 (body weight, 503 g), the CECT images revealed a heterogeneous lesion measuring 17 mm in the maximum sagittal diameter in the left side of the abdomen. In addition, the PET and PET/CT fusion images revealed moderately increased ^{18}F-FDG uptake in the lesion located in the left side of the abdomen, with a SUV_{max} of <1.5. Physiological FDG uptake was observed in the gastrointestinal tract, and the SUV_{max} of this organ site was 3.2, as shown in Table I. Autopsy revealed a large tumor in the splenic lobe, which was detected by CECT and PET/CT. However, multiple tumors that were present in the duodenal lobe of the pancreas were not revealed by CECT and PET/CT (Fig. 4). In rat 2 (body weight, 470 g), the CECT images revealed a heterogeneously-enhanced pancreatic tumor, measuring 20 mm in the maximum sagittal diameter, in the left side of the abdomen. PET/CT fusion images revealed moderate uptake of ^{18}F-FDG (SUV_{max}, 3.0) in the pancreatic tumor. In addition, physiological FDG uptake in the gastrointestinal tract (SUV_{max}, 2.7) was observed. During autopsy, a large tumor in the splenic lobe was identified by the imaging analysis. In addition, multiple tumors were identified in the duodenal lobe of the pancreas, but these tumors were not visualized on CECT and PET/CT (Fig. 5). In rat 3 (body weight, 564 g), the presence of a tumor was not observed on either CECT or PET scans (Fig. 6). Next, an incision was made in the abdomen of the rat, and a number of nodules, which were smaller in size than those observed in rats no. 1 and 2, were identified in the

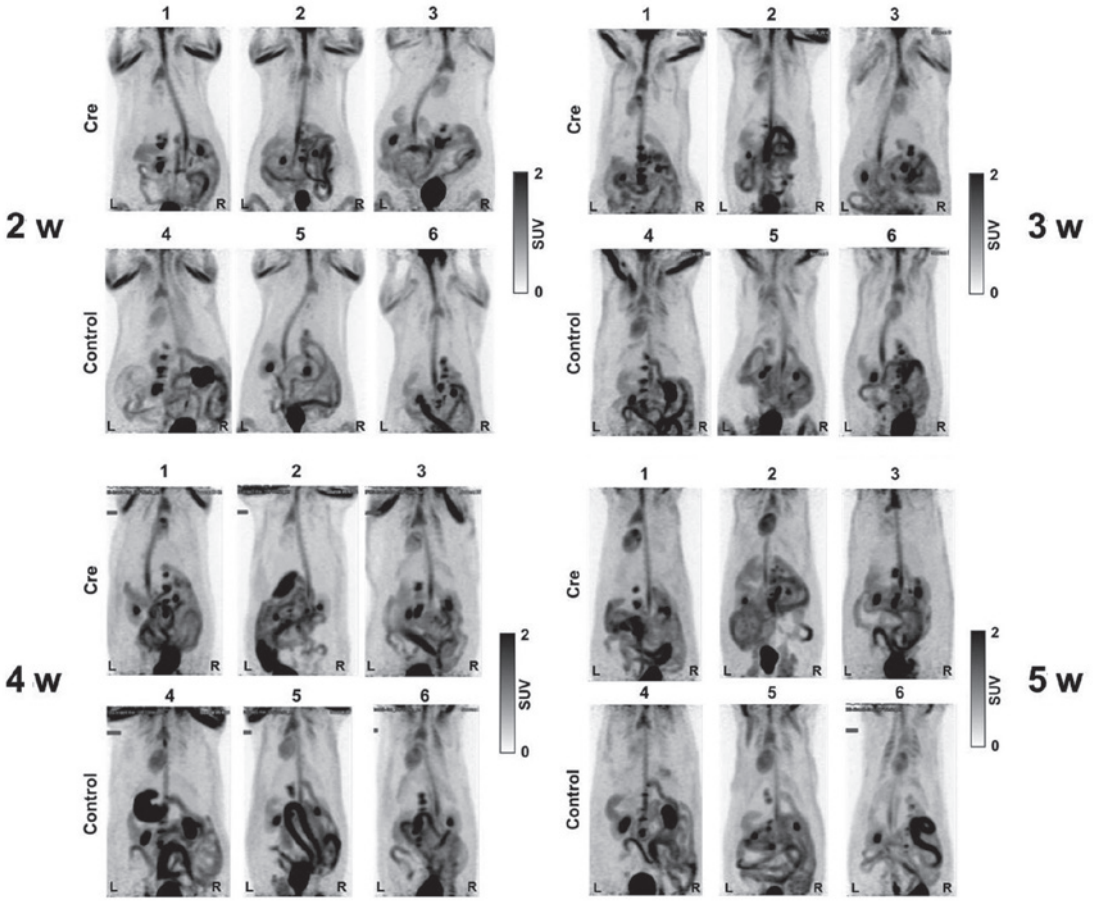

Figure 3. Representative positron emission tomography (PET) images of the transgenic rats at a maximum intensity projection. Images were obtained from Cre recombinase (Cre)-expressing transgenic rats (1-3) and control rats (4-6). Scanning was performed at two, three, four and five weeks post-viral inoculation. Subsequent to fasting, the rats were injected under isoflurane anesthesia with ~15 MBq ^{18}F-fluorodeoxyglucose (FDG) via the tail vein. PET data was acquired 50 min post-injection. Physiological FDG uptake was observed in the intestines, kidney and urinary bladder, but no tumor masses were identified in any of the images. L, left side; R, right side. SUV, standardized uptake value; w, weeks.

duodenal lobe of the pancreas. No tumor was identified in the splenic lobe of the pancreas. No macroscopic metastasis was identified in rats 1-3.

Discussion

A limited number of the documented studies that involve imaging analysis of pancreatic tumors in animal models used the FDG-PET/CT system (18,19). In the present study, a *KRAS*-mediated transgenic rat model was used to develop multiple pancreatic tumors that resembled the developmental and histological features of human PDAC within two weeks (8). In living rats at eight weeks post-treatment, the pancreatic tumors were clearly enhanced in the CECT images following the administration of a contrast media, and were distinguishable from the gastrointestinal tract. In the absence of imaging analysis, calipers are used to determine the location and size of a pancreatic tumor following a laparotomy or autopsy of an animal. Imaging analysis therefore allows each animal to be scanned sequentially in a sectional plane of interest, such as transverse, coronal or sagittal, and be monitored over time without the need to be sacrificed. In addition, PET/CT enables the accurate measurement of irregularly-shaped tumors in a pancreatic tumor model. According to the Three Rs principle (20), which aims to replace existing experimental methods with those that do not use animals, reduce the number of test animals used and refine methods in order to minimize the suffering of test animals, the PET/CT system reduces the number of animals required for experimental treatment and control groups. This indicates that imaging systems should be recommended for use in animal experiments. Recently, inoculation efficacy has been improved by clamping the common bile duct and increasing the amount of virus that is administered. Using this technique, studies may be able to control the size of the pancreatic tumor quantitatively within an appropriate time period, a factor that demonstrates the usefulness of this model.

With regard to studies that have used small animal models, Kitahashi *et al* (21) used micro-CT to detect chemically-induced pancreatic tumors of >4 mm in diameter in Syrian hamsters. Another study by Fendrich *et al* (18) detected precursor pancreatic adenocarcinoma lesions with an activity of 9.6±0.5 MBq in a five-month-old transgenic mouse model by FDG-PET/CT. Kayed *et al* (22) measured the anticancer effects of cyclopamine in a pancreatic carcinoma xenograft with an activity of 7.4 MBq model using ^{18}F-PET/CT. The study examined the size and SUV of each

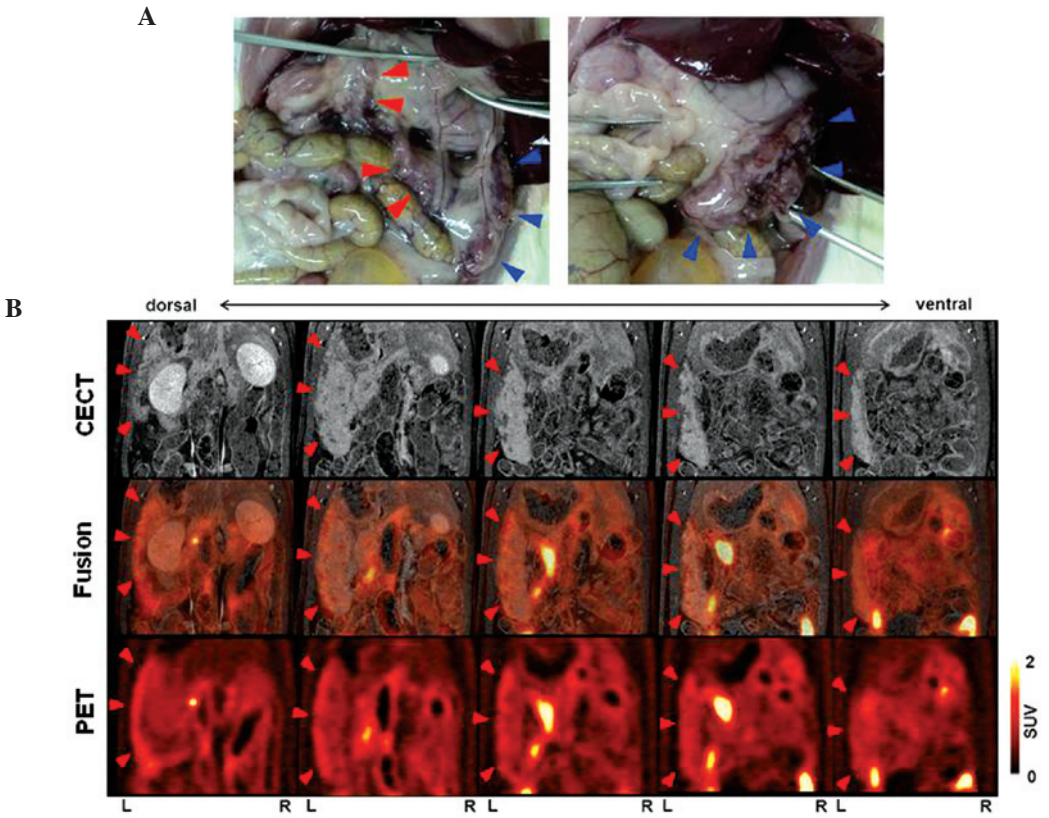

Figure 4. Autopsy view, positron emission tomography (PET)/contrast-enhanced CT (CECT) and PET/CECT fusion images of rat 1. (A) Tumors were observed in the duodenal lobe (left panel, red arrowheads) and the in splenic lobe (left and right panel, blue arrowheads) of the pancreas. (B) PET, CECT and PET/CECT fusion images revealed a large mass on the left side of the body (arrowheads). Physiological fluorodeoxyglucose uptake was also observed in the kidneys and intestines. L, left side; R, right side; SUV, standardized uptake value.

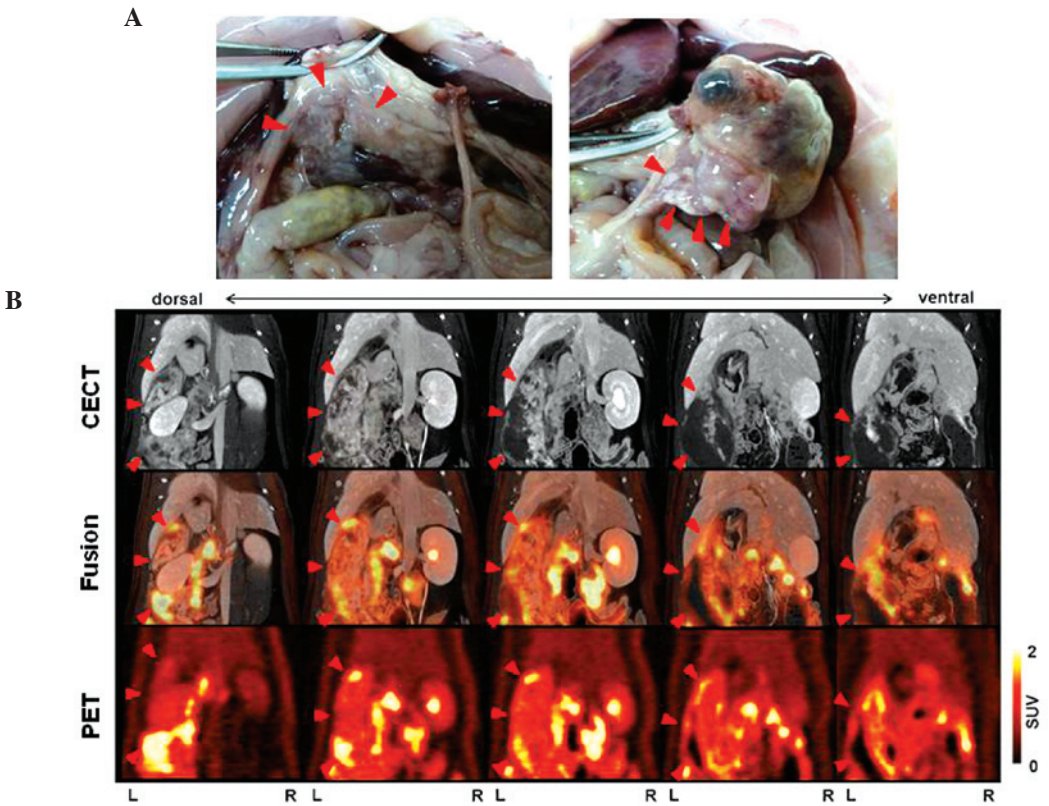

Figure 5. Autopsy view, positron emission tomography (PET), contrast-enhanced CT (CECT) and PET/CECT fusion images of rat 2. (A) Tumors were observed in the duodenal lobe (left and right panels, arrowheads) of the pancreas. The liver was intact. (B) PET, CECT and PET/CECT fusion images revealed multiple tumor masses in the left side of the body (arrowheads). L, left side; R, right side; SUV, standardized uptake value.

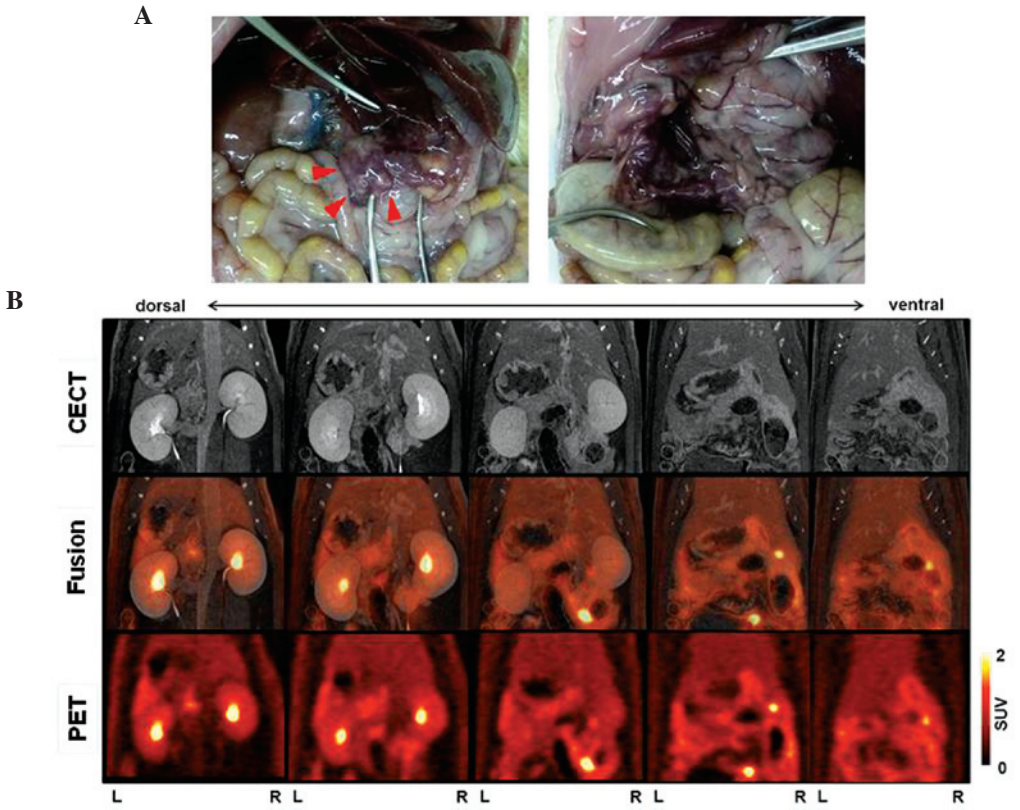

Figure 6. Autopsy view, positron emission tomgraphy (PET), contrast-enhanced CT (CECT) and PET/CECT fusion images of rat 3. (A) Tumors were observed in the duodenal lobe (left panel, arrowheads), but not in the splenic lobe of the pancreas. (B) No tumor-like lesions were revealed in the PET/CT and CECT images. SUV, standardized uptake value; R, right side; L, left side.

tumor that was grown in the hip region of nude mice during a seven-day treatment period. A technical limitation in baseline calibration occurred, but the system was believed to be suitable for practical use. The SUV and SUV_{max} parameters have been demonstrated in a previous study to be potential predictors of early recurrence following the curative resection of lung carcinomas (23).

It was hypothesized that the amount of FDG uptake into the pancreatic tumors would be higher compared with other abdominal organs. However, the results shown in Table I indicate that each value was not necessarily specific to the region of interest. This indicates a limitation in the use of this parameter for differential diagnoses that are based upon imaging techniques. To avoid bias during the measurement of the SUV, representative areas of tumor tissues that demonstrated moderate FDG uptake were selected. Therefore, the potential limitations of this methodology should be considered when calculating the SUV or SUV_{max}. This aspect warrants further investigation.

PET images did not identify tumor masses in any organ of the Cre-expressing rats until five weeks post-treatment (Fig. 3). When the laparotomy was performed six weeks subsequent to the viral inoculation, multiple tumors measuring <2 mm in diameter were identified in the pancreas of all three Cre-expressing transgenic rats. This indicated that the transgenic rats developed macroscopically visible tumors by six weeks post-treatment. At eight weeks post-treatment, the PET/CT images revealed pancreatic tumor masses in two of the three rats, which indicated a potential limitation in the detection of tumors prior to the eighth week by current imaging techniques. Pancreatic tumors were primarily identified in the splenic lobe of the pancreas by PET/CT. Tumors in the duodenal lobe, however, could not be detected by such imaging analyses, even if these tumors were confirmed by laparotomy. In addition, the presence of smaller tumors, and a specific anatomical location within the gastrointestinal tract, may affect the visibility of the tumor. In the PET and PET/CT fusion images, the pancreatic tumors were visible, but the physiological ^{18}F-FDG uptake in the intestine reduced the appearance of the lesions.

In conclusion, the present study demonstrated that pancreatic tumors can be detected in rats using imaging modalities eight weeks after viral inoculation. The FDG-PET/CT imaging system is a valuable approach for the evaluation of the carcinogenic process and potential treatment or prevention methods for pancreatic tumors in mammalian models. Therefore, it is proposed that this experimental system can also be applied to studies that examine cases of human PDAC.

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