An approach of imaging technique using MRI & ^{18}F-fludeoxyglucose (^{18}F-FDG) PET/CT for longitudinal monitoring of mouse hepatocellular carcinoma model

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1. Introduction

Hepatocellular carcinoma (HCC) is the most common cancers with growing incidence around the world. Some researchers have developed preclinical models in which tumors arise in a background that resembles the naturally developing HCC in human. There are genetically modified mouse models to mimic pathophysiological and molecular features of HCC (1) as well as chemical carcinogen-treated mouse models (2).

For the detection of tumor lesions, among various imaging modalities, computed tomography (CT) and magnetic resonance imaging (MRI) provide for anatomical information and positron emission tomography (PET) supply functional information of disease (3-5).

The purpose of the present work is to evaluate non-invasive and reliable monitoring method for HCC models developed by the treatment with diethylnitrosamine (DEN) as a chemical carcinogen or Hepatitis B virus (HBV) X gene expressing transgenic mice (HBx-Tg model) using ^{18}F-FDG PET/CT and 3.0 T MRI.

2. Methods and Results

2.1 Mouse HCC models

C57BL/6 female and male mice were purchased from The Central Lab. Animal Inc., Seoul, Korea and the HBx-Tg model was previously described (6). For chemical carcinogen-induced mouse HCC model (DEN-model), 3 and 5 weeks old male mice were intraperitoneally injected once with 20 mg/kg body weight of DEN (Sigma Chemical Co., MO, USA). The care, maintenance and treatment of animals in these studies followed protocols approved by the Institutional Animal Care and Use Committee of Korea Institute of Radiological and Medical Sciences.

2. 2 MRI acquisition

MRI scanning was performed with a 3.0 Tesla (T) MR unit (Magnetom Tim Trio, Siemens Medical solution, Erlangen, Germany) with a wrist coil and the mice was fixed prone position. Fourteen DEN-models (19~21 months after induction of DEN) and thirteen HBx-Tg models (11~17 months old) were scanned. Before scanning, the animals were anesthetized with 2% isoflurane in oxygen.

2. 3 ^{18}F-FDG-PET/CT image acquisition

All mice, harboring HCC detected by 3.0 T MRI, were kept fasting overnight before ^{18}F-FDG PET/CT scanning. The mice were anesthetized by 2% isoflurane in oxygen and then injected with 7.4 MBq of ^{18}F-FDG into the tail vein. After injection, mice were kept in a chamber with 0.5% isoflurane to maintenance for 1 h. PET/CT images were acquired using an Inveon small animal PET/CT system (Siemens Medical Solutions, Erlangen, Germany). The CT scan was conducted first, followed by PET acquisition. During the acquisition of PET image, the mice were placed under 2% isoflurane anesthesia. ^{18}F-FDG was provided by Korea Institute of Radiological and Medical Sciences.

2.4 MRI and ^{18}F-FDG PET/CT imaging of mouse HCC model

The HCC from DEN-model or HBx-Tg model could be detected by 3.0 T MRI as small as 2 mm of long diameter. The multiple tumors of various sizes in an identical model were developed. In case of DEN-model, seven of the fourteen mice developed HCC at 19 to 21 months of ages and one to four nodules in a model mouse were detected by MRI. We also detected five HCC-bearing mice of thirteen HBx-Tg models at 11 to 17 months of ages by MRI. Fifteen and eight tumors larger than 2 mm were diagnosed in DEN-models and HBx-Tg models through MRI imaging, respectively. In an identical mouse, ^{18}F-FDG PET/CT images were obtained and the patterns of ^{18}F-FDG PET/CT coincided also with those of MR images. All multiple nodules detected by ex-vivo examination could not be visualized on MRI or ^{18}F-FDG PET/CT.

2.5 Longitudinal monitoring of HCC of HBx-Tg models

We monitored HCC progression longitudinally and non-invasively with HCC-bearing HBx-Tg models. As shown in Figure 1, tumors of HBx-Tg models were

appeared larger as time progresses judged by ^{18}F-FDG PET/CT. Most of tumor nodule became increasingly large during hepatocarcinogenesis.

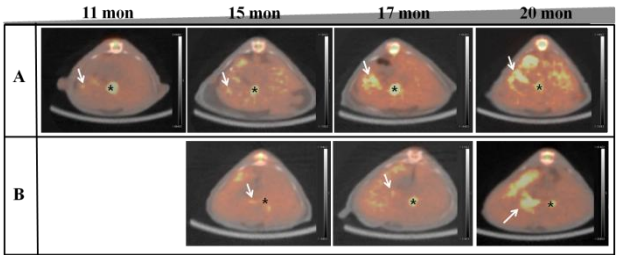

Fig. 1. Longitudinal monitoring of HCC in HBx-Tg model according to tumor growth over time. The HBx-Tg mice were serially imaged after birth 11 to 20 months by PET/CT with ^{18}F-FDG. A asterisk indicates gallbladder.

2.6 Histology

To evaluate histopathological changes of the liver after in vivo imaging, the mice were euthanized and the liver tissues with visible cancerous masses were dissected. The specimens were washed with cold PBS, fixed in 10% neutral buffered formalin and then processed for paraffin embedding. The tissue blocks were cut in 5 μm thickness and then underwent routine hematoxylin and eosin (H&E) staining for light microscopic examination. All HCC-induced models showed hepatic tumor nodules in various sizes. The tumor nodules were either encapsulated by connective tissue or not well circumscribed (Fig. 2). In both models, hepatocellular adenoma and carcinoma were developed. Hepatocellular adenoma developed in both DEN-models and HBx-Tg models showed adenoma characteristics including irregular hepatic cord and vacuolated cytoplasm (Fig. 2B, arrows). In addition, hepatocellular carcinomas were evident in both models. In case of carcinoma, the cytoarchitecture of the hepatocytes appeared to be quite irregular and the tumor nodules contained pleomorphic cells (Fig. 2C).

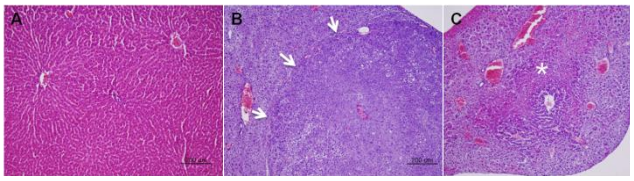

Fig. 2. Representative photomicrographs of the liver of normal (A), DEN-model (B), HBx-Tg model (C) stained with hematoxylin and eosin. Note that tumor nodules are either encapsulated (B, arrows) or diffuse (C, asterisk) within the liver tissue. Scale bar=200um.

3. Conclusions

The imaging methods including MRI and ^{18}F-FDG PET might be required for longitudinal monitoring of tumor growth in an internal organ like as HCC. In this study, primary monitoring about a location, boundary, number and volume of multiple tumor nodules could be analyzed from 3.0 T MRI and confirmed by ^{18}F-FDG PET/CT images based on the MR images of HCC models. We could detect easily and precisely HCC of mouse model by this combination imaging method MRI followed by ^{18}F-FDG PET/CT. The non-invasive and reliable combination imaging methods for HCC models were evaluated with MRI and ^{18}F-FDG-PET/CT. This observation is the first evaluation about spontaneously developing HCC by MRI and ^{18}F-FDG PET /CT.

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