An imaging target TGF-β1 for hepatocellular carcinoma in mice

Yiqiu Zhang1,2,3 MD,
Beilei Li1,2,3 MD, PhD,
Xiao Li1,2,3 MD,
Hui Tan1,2,3 MD,
Dengfeng Cheng1,2,3 MD, PhD,
Hongcheng Shi1,2,3 MD, PhD

1. Department of Nuclear Medicine, Zhongshan Hospital, Fudan University, Shanghai, 200032, China
2. Nuclear Medicine Institute of Fudan University, Shanghai, 200032, China
3. Shanghai Institute of Medical Imaging, Shanghai, 200032, China

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Corresponding author:
Hongcheng Shi MD, PhD
No. 180, Fenglin Road, Xuhui District, Shanghai
Tel:+862164041990-2064
Fax:+862164038472
shi.hongcheng@zs-hospital.sh.cn

Abstract
It has been reported that the positive detection rate of Fluorine-18-fluorodeoxyglucose (18F-FDG) metabolism in positron emission tomography (PET) imaging, for the diagnosis of hepatocellular carcinoma (HCC) is only about 50%. In particular, 18F-FDG PET imaging is prone to false negative findings in HCC. Transforming growth factor-beta1 (TGF-β1) shows over expression rates in early HCC liver tissue growth and promotes tumor invasion and metastases. Our aim was to use in this study, we used the feasibility of iodine-125 (125I)-labeled TGF-β1 antibody as a nuclear medicine imaging target in HCC. Materials and Methods: The TGF-β1 antibody was obtained from Bioss Inc. The Huh-7 cell line (Liver Cancer Institute, Zhongshan Hospital, Fudan University) is a HCC cell line with high metastatic potential. Each mouse was subcutaneously injected with 5×10⁶ Huh-7 cells in the right upper flank region for the establishment of a subcutaneous xenograft model. The iodogen method was used to label TGF-β1 antibody with 125I. In this experiment, 100μL of 125I-TGF-β1 antibody solution, which contained approximately 18.5MBq of 125I-liraglutide, was injected into the tail veins of each of three nude mice with Huh-7 HCC. Micro SPET/CT imaging was performed for each mouse using a nano SPET/CT. Results: The average percentage of injected dose per gram of tissue (ID%) was 1.3% and 2.4%. The tumor was strongly positive for TGF-β1. Conclusion: This pilot study provides an experimental basis for further exploration of the feasibility of TGF-β1 receptor as a target in HCC imaging and in other cancers.

Introduction
It has been reported that the positive detection rate of Fluorine-18-fluorodeoxyglucose (18F-FDG) metabolism in positron emission tomography (PET) imaging, for the diagnosis of hepatocellular carcinoma (HCC) is only about 50% [1]. In particular, 18F-FDG PET imaging is prone to false negative findings in HCC [2]. 11C-labeled acetate and choline, are more effective but not better to detect smaller lesions in HCC [3].

Transforming growth factor-beta1 (TGF-β1) shows over expression rates in early HCC liver tissue growth and promotes tumor invasion and metastases by stimulating angiogenesis and by increasing tumor cell adhesion [4] and immune suppression. Furthermore, TGF-β1 does not correlate with HCC tumor size, α-fetoprotein (AFP) level, tumor differentiation, lymph node metastases, or vascular invasion [5].

In this study, we used the feasibility of iodine-125 (125I)-labeled TGF-β1 antibody as a nuclear medicine imaging target in HCC, with single photon emission tomography/computed tomography (SPET/CT) imaging in a nude mouse model of HCC.

Materials and Methods
The TGF-β1 antibody was obtained from Bioss Inc. 125I were obtained from Shanghai GMS Pharmaceutical Co., Ltd, China. Six male athymic Balb/C nude mice, 6 weeks old (obtained from the Shanghai Lab. Animal Research Center, China) housed in laminar-flow cabinets under specific pathogen-free conditions. The mice were kept for 5-7 days as an adaptation period before being subjected to experimental use. Micro SPET/CT imaging were performed in the Cancer Hospital, Fudan University, Shanghai, China by Nano SPET/CT ( Bioscan, USA). All animal experiments were approved by the Animal Care and Use Committee of Zhongshan Hospital, and were conducted in accordance with all state regulations.
The Huh-7 cell line (Liver Cancer Institute, Zhongshan Hospital, Fudan University) is a HCC cell line with high metastatic potential. Cells were maintained in high-glucose Dulbecco’s modified eagle medium (D-MEM; GibcoBRL, Grand Island, New York, USA) supplemented with 10% fetal bovine serum (Hyclone, Utah, USA) in a humidified 5% CO₂ atmosphere at 37°C.

Each mouse was subcutaneously injected with 5×10⁷/0.1 mL Huh-7 cells in the right upper flank region for the establishment of a subcutaneous xenograft model. When the subcutaneous tumor reached 1 cm in diameter (approximately 3-4 weeks after injection), micro SPET/CT images were performed, in 2 mice.

The iodogen method was used to label TGF-β1 antibody with ¹²⁵I (purchased from Shanghai GMS Pharmaceutical Co., Ltd Shanghai, China). In the labeling reaction, 50µL of liraglutide (1mg/mL) and 25µL of Na¹²⁵I in 1×phosphate-buffered saline (PBS), (740MBq/mL) were sequentially added to a reaction tube coated with iodogen and mixed gently. The tube was sealed, and reaction was allowed to proceed for 10 minutes at room temperature. After the reaction was completed, the labeling efficiency was determined using a mixture of methanol and water (volume methanol: volume water = 85:15) as the developing solvent and Whatman 3mm chromatography paper as the stationary phase, with RF values of 0 and 0.8 for ¹²⁵I-TGF-β1 antibody and Na¹²⁵I, respectively.

In this experiment, 100µL of ¹²⁵I-TGF-β1 antibody solution, which contained approximately 18.5MBq of ¹²⁵I-liraglutide, was injected into the tail veins of each of three nude mice with Huh-7 HCC. Micro SPET/CT imaging was performed for each mouse using a nano SPET/CT (Bioscan, USA) in 1 hour and 3 hours after this injection, respectively. The CT images were acquired before each SPET scan using standard settings: 45kVp voltage, 0.15mA current, and 500ms exposure. Images were reconstructed using Nucline 1.02 Software (Mediso, Hungary) for real-time images with simultaneous three-dimensional reconstructions. Micro-SPET imaging parameters were 1.0mm/pixel, 256x256 frame size, 10% energy windows, 28keV energy peak, and 60s per projection with 24 projections. Data were reconstructed into transaxial, coronal, and sagittal slices using InVivoScope 1.44 Reconstruction Software (Bioscan, USA). Three-dimensional ordered subset expectation maximization reconstructions were created with a resolution of 0.4mm/pixel using an algorithm that used four subsets and applied an iterative calculation six times.

All the mice were sacrificed and subcutaneous tumor specimens were fixed and stained by H&E and anti-TGF-β1 antibody (sc146, Santa CruzBiotecnology, Inc., Heidelberg, Germany).

Results

Two of six nude mice successfully completed the entire experiment, three mice failed to establish subcutaneous tumors and a mouse died because of deep anesthesia. The uptake of ¹²⁵I-labeled TGF-β1 antibody 30 minutes after injection was significantly greater in the tumor tissue of the right forelimb than in the surrounding tissue background (Figure 1A) especially 3 hours after injection (Figure 1B). The average percentage of injected dose per gram of tissue (ID%) was 1.3% and 2.4% (Figure 2A magnification ×200). The tumor was strongly positive for TGF-β1 (Figure 2B).

Discussion

TGF-β is a polypeptide for the regulation of cell growth factor exists in at least three isoforms and is secreted by many cell types, including macrophages and controls cellular proliferation, differentiation, embryonic development, wound healing, apoptosis, etc. and also combines with specific receptors on cell membrane surface. TGF-β receptors (TBR) are binding proteins with high affinity with TGF-β1. There are two main receptors, called TGF-b receptor I or activin receptor-like kinase (ALK) (TbRI/ALK), and TGF-b receptor II (TbRII) [6]. The activated TGF-β1 releases dimer, then participates in many activities, such as the regulation of intercellular epithelial-mesenchymal transformation, tumorigenesis, metastasis and cells invasion [7]. Some studies [8-15] have shown that serum TGF-β1 levels elevated in varying degrees in patients with cancer of liver, pancreas, lung, breast cancer, colorectal, cervical cancer, ovarian and other while its presence of TGF-β1 is related with the occurrence, development and prognosis of these tumors.

In the present study TGF-β1 antibody staining showed TGF-
β1 strong expression in HCC cells by 125I-labeled TGF-β1 micro SPET / CT imaging in 2 mice. This pilot study, although performed in a very small number of mice, is limited by its very small sample size and the fact that only one representative HCC cell line was studied.

The TGF-β1 antibody has a relatively high molecular weight and will stimulate the body to produce an immune response. Therefore, we chose the TGF-β receptor as the target for tumor imaging and combined the target with radio-labeled, small-molecule inhibitors. Follow-up studies will focus on synthesizing 18F-labeled TGF-β1 inhibitor to minimize the background tissue radiation in PET imaging. In conclusion: This pilot study provides an experimental basis for further exploration of the feasibility of TGF-β1 receptor as a target in HCC imaging and in other cancers.

The authors declare that they have no conflicts of interest

Bibliography


