An imaging target TGF-β1 for hepatocellular carcinoma in mice

Yiqiu Zhang[1,2,3] MD,

Beilei Li[1,2,3] MD, PhD,

Xiao Li[1,2,3] MD,

Hui Tan[1,2,3] MD,

Dengfeng Cheng[1,2,3] MD, PhD,

Hongcheng Shi[1,2,3] 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

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 (^{18}F-FDG) metabolism in positron emission tomography (PET) imaging, for the diagnosis of hepatocellular carcinoma (HCC) is only about 50%. In particular, ^{18}F-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 (^{125}I)-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 5×10^6/0.1mL 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 [125]I. In this experiment, 100μL of [125]I- TGF-β1 antibody solution, which contained approximately 18,5MBq of ^{125}I-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 (^{18}F-FDG) metabolism in positron emission tomography (PET) imaging, for the diagnosis of hepatocellular carcinoma (HCC) is only about 50% [1]. In particular, ^{18}F-FDG PET imaging is prone to false negative findings in HCC [2]. ^{11}C-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, a-fetoprotein (AFP) level, tumor differentiation, lymph node metastases, or vascular invasion [5].

In this study, we used the feasibility of iodine-125 (^{125}I)-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. [125]I 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. Mirco 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% CO_2 atmosphere at 37°C.

Each mouse was subcutaneously injected with 5×10^6/0.1mL Huh-7 cells in the right upper flank region for the establishment of a subcutaneous xenograft model. When the subcutaneous tumor reached 1cm 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 ^{125}I (purchased from Shanghai GMS Pharmaceutical Co., Ltd Shanghai, China). In the labeling reaction, 50µL of liraglutide (1mg/mL) and 25µL of Na^{125}I 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 ^{125}I- TGF-β1 antibody and Na^{125}I, respectively.

In this experiment, 100µL of ^{125}I-TGF-β1 antibody solution, which contained approximately 18.5MBq of ^{125}I-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, 256×256 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 antibodyc (sc146, Santa CruzBiotechnology, 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 up-

take of ^{125}I-labeled TGF-β1antibody 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).
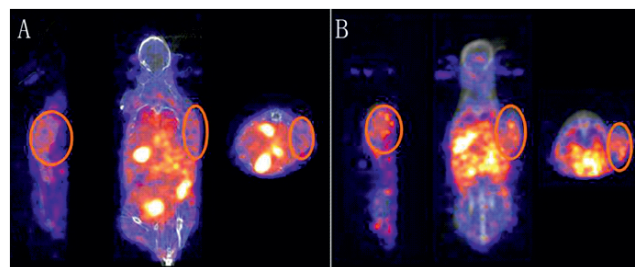

Figure 1. Micro SPET/CT imaging showed that uptake of ^{125}I-labeled TGF-β1 antibody 30 minutes after injection was significantly greater in the tumor tissue of the right forelimb than in the surrounding tissue background (Figure A). The difference in radioactivity between the tumor and the soft-tissue background was even more obvious 3 hours after injection (Figure B).
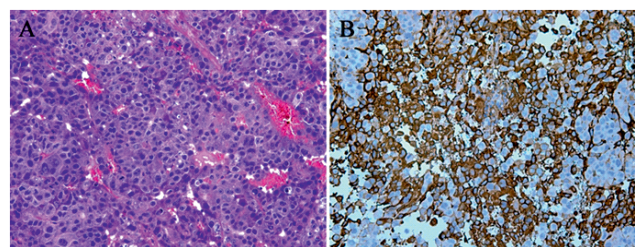

Figure 2. Histology showed a large number of tumor cells within the lesion (H&E×200) (Figure A). The tumor was strongly positive for TGF-β1 (H&E×200) (Figure B).

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 (TβR) 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 ^{125}I-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 radiolabeled, small-molecule inhibitors. Follow-up studies will focus on synthesizing ^{18}F-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

1. Forner A, Vilana R, Ayuso C et al. Diagnosis of hepatic nodules 20 mm or smaller in cirrhosis: proSPETive validation of the noninvasive diagnostic criteria for hepatocellular carcinoma. *Hepatology* 2008; 47: 97-104.
2. Fletcher JW, Djulbegovic B, Soares HP et al. Recommendations on the use of ^{18}F-FDG PET in Oncology. *J Nucl Med* 2008; 49: 480-508.
3. Yamamoto Y, Nishiyama Y, Kameyama R et al. Detection of Hepatocellular Carcinoma Using ^{11}C-Choline PET: Comparison with ^{18}F-FDG PET. *J Nucl Med* 2008; 49:1245-8.
4. Ji GZ, Wang XH, Miao L et al. Role of transforming growth factor-beta1-smad signal transduction pathway in patients with hepatocellular carcinoma. *World J Gastroenterol* 2006; 12: 644-8.
5. Chen Z, Xie B, Zhu Q et al. FGFR4 and TGF-β1 expression in hepatocellular carcinoma: correlation with clinicopathological features and prognosis. *Int J Med Sci* 2013; 10:1868-75.
6. Leask A, Abraham DJ. TGF-beta signaling and the fibrotic response. *FASEB J* 2004; 18: 816-27.
7. Valkov A, Sorbye SW, Kilvaer TK et al. The prognostic impact of TGF-β1, fascin, NF-κB and PKC-ζ expression in soft tissue sarcomas. *PLoS One* 2011; 6:17507.
8. Dituri F, Serio G, Filannino D et al. Circulating TGF-β1-related biomarkers in patients with hepatocellular carcinoma and their association with HCC staging scores. *Cancer Lett* 2014; 353: 264-71.
9. Liu SG, Yuan SH, Wu HY et al. The Clinical Research of Serum VEGF, TGF-β1, and Endostatin in Non-small Cell Lung Cancer. *Cell Biochem Biophys* 2015; 72: 165-9.
10. Zu X, Zhong J, Tan J et al. TGF-β1 induces HMGA1 expression in human breast cancer cells: Implications of the involvement of HMGA1 in TGF-β signaling. *Int J Mol Med* 2015; 35: 693-701.
11. Ma H, Wei Y, Leng Y et al. TGF-β1-induced expression of Id-1 is associated with tumor progression in gastric cancer. *Med Oncol* 2014; 31: 19.
12. Zheng Y. The therapeutic effects of rehmannia oral liquid for the syndrome of heat accumulation with Yin consumption in esophagus cancer patients undergoing radiotherapy-a report of 60 cases. *J Tradit Chin Med* 2007; 27: 248-54.
13. Kim YH, Kim G, Kwon CI et al. TWIST1 and SNAI1 as markers of poor prognosis in human colorectal cancer are associated with the expression of ALDH1 and TGF-β1. *Oncol Rep* 2014; 31: 1380-8.
14. Torres-Poveda K, Bahena-Román M, Madrid-González C et al. Role of IL-10 and TGF-β1 in local immunosuppression in HPV-associated cervical neoplasia. *World J Clin Oncol* 2014; 5: 753-63.
15. Tas F, Karabulut S, Serilmez M et al. Clinical significance of serum transforming growth factor-beta 1 (TGF-β1) levels in patients with epithelial ovarian cancer. *Tumour Biol* 2014; 35: 3611-6.