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Molecular Imaging of Pancreatic Duct Adenocarcinoma Using a Type 2 Cannabinoid Receptor-Targeted Near-Infrared Fluorescent Probe

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Abstract

Imaging probes targeting type 2 cannabinoid receptor (CB_2R) overexpressed in pancreatic duct adenocarcinoma (PDAC) tissue have the potential to improve early detection and surgical outcome of PDAC. The aim of our study was to evaluate the molecular imaging potential of a CB_2R-targeted near-infrared (NIR) fluorescent probe (NIR760-XLP6) for PDAC. CB_2R overexpression was observed in both PDAC patient tissues and various pancreatic cancer cell lines. *In vitro* fluorescence imaging indicated specific binding of NIR760-XLP6 to CB_2R in human PDAC PANC-1 cells. In a xenograft mouse tumor model, NIR760-XLP6 showed remarkable 50- (*ex vivo*) and 3.2-fold (*in vivo*) tumor to normal contrast enhancement with minimal liver and kidney uptake. In a PDAC lymph node metastasis model, significant signal contrast was observed in bilateral axillary lymph nodes with PDAC metastasis after injection of the probe. In conclusion, NIR760-XLP6 exhibits promising characteristics for imaging PDAC, and CB_2R appears to be an attractive target for PDAC imaging.

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Introduction

Pancreatic duct adenocarcinoma (PDAC) is the fourth leading cause of cancer-related death in the United States, with an extremely poor 5-year survival rate of less than 10% and a median survival of 5-8 months after diagnosis [1]. Due to the absence of early symptoms and a lack of accurate diagnostic tools for early-stage detection, only 20% of cases are candidates for surgical resection at the time of diagnosis [2]. Preoperative assessment of PDAC margin status is challenging using current technologies, so even if surgical resection can be performed, most patients have residual disease from margin and therefore recur quickly [3–5]. For patients with advanced disease, treatment options are limited to chemotherapy and radiotherapy, but the effectiveness is unsatisfactory. This is because PDAC is a heterogeneous disease, reflected in diverse clinical response patterns to therapy. Additionally, the tumor hypovasular nature and dense

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desmoplastic stroma barrier prevent drug delivery [6–8]. Molecular agents that can specifically target PDAC to allow for early diagnosis and improved therapeutic outcome are therefore in urgent need. To date, though some targets have been explored for PDAC imaging and therapy, such as integrin $\alpha_v\beta_3$, claudin-4, epidermal growth factor receptor, vascular endothelial growth factor receptor, urokinase-type plasminogen activator receptor, carcinoembryonic antigen, and carbohydrate antigen 19-9 (CA19-9), more molecular probes with high PDAC imaging contrast still need to be developed [9–14].

The endogenous cannabinoids system composed of endocannabinoids and two major G protein–coupled receptors (GPCRs), cannabinoid receptor type 1 (CB_1R) and type 2 (CB_2R), plays an important role in various physiological and pathological conditions, making this system an attractive therapeutic target [15–17]. Under basal conditions, CB_1R is highly expressed in the central nervous system and mediates the psychotropic effects of cannabinoids, whereas CB_2R is predominately found in the immune system with high expression only in the spleen and lymph nodes [18–20]. However, many types of cancer, including PDAC, overexpress CB_2R, and the expression levels of CB_2R appear to be associated with tumor aggressiveness [21–25]. Moreover, CB_2R agonists potently inhibited viability, proliferation, adhesion, and migration of various types of cancer cells. Therefore, CB_2R appears to be a promising target for PDAC imaging and therapy.

To date, only a limited number of CB_2R-targeted imaging agents have been reported, which are mainly applied in position emission tomography (PET) imaging [26–28]. Although PET is a great imaging technique for clinical imaging with high sensitivity and deep tissue penetration, it has many limitations, such as relatively low spatial resolution, narrow time window, high instrument cost, and injection of radioactive agents. In contrast, fluorescent imaging is a low-cost imaging method with superior resolution and sensitivity, and is becoming more popularly used in the clinic for diagnostic and surgical navigation purposes. When accompanied with dyes in the near-infrared (NIR) spectrum (650-900 nm) where the interference of high tissue absorption and autofluorescence is minimal, fluorescence imaging can also be used to image deep without any ionizing and radioactive damage [29]. Over the past years, we have developed several CB_2R-targeted fluorescence probes [30–32]. More recently, we reported a new NIR fluorescent probe with high CB_2R-binding affinity and preferential binding selectivity to CB_2R over CB_1R [33]. Here we report the first *in vivo* fluorescence imaging study of PDAC using an NIR CB_2R-targeted exogenous probe.

Materials and Methods

Synthesis of NIR760-XLP6

NIR760-XLP6 was synthesized using our previously reported method [33].

Human Normal and PDAC Tissues

The paired PDAC and normal pancreatic tissues obtained adjacent to the tumors were collected from PDAC patients during surgery at Ruijin Hospital, Shanghai, China. The tissues were frozen immediately in liquid nitrogen and stored in a −80°C freezer until further study. The use of human tissues for the analysis was approved by the local ethical committee (Ruijin Hospital, Shanghai, China), and written informed consent was obtained from the patients.

Reagents

The CB_2R selective ligand 4-quinolone-3-carboxamide (4Q3C) was purchased from Cayman (Ann Arbor, MI). IMDM, DMEM, fetal bovine serum, and penicillin-streptomycin were all purchased from Gibco (Waltham, Ma).

Human PDAC Cell Lines

The human PDAC cell lines CAPAN-1, MIA PaCa-2, BxPC3, PANC-1, and CFPAC-1 were all purchased from ATCC (Manassas, VA). CFPAC-1 cells were cultured in IMDM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. The other four cell lines were cultured in DMEM containing 10% fetal bovine serum and 1% penicillin-streptomycin. All cells were grown in an incubator with a constant temperature of 37°C and a humidified atmosphere of 5% CO_2.

Animal Tumor Models

All animal experiments were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of Shanghai Jiao Tong University School of Medicine. The xenograft tumor mouse model was developed by injecting 5×10^6 PANC-1 cells into the right flank of 5- to 6-week-old male BALB/c nude mice. The PDAC lymph node metastasis model was induced by the subcutaneous injection of 1×10^6 PANC-1 cells into the hind footpad in nude mice.

Real-Time PCR Analysis

We performed real-time PCR to evaluate CB_2R expression in human PDAC and normal pancreatic tissues, and 5 PDAC cell lines (CAPAN-1, MIA PaCa-2, BxPC3, PANC-1, CFPAC-1). Total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA). The total RNA was reverse transcribed into first-strand cDNA using M-MLV reverse transcriptase (Invitrogen, Carlsbad, CA). The primer sequences set for PCR were as follows: CB_1R (ATGAAGTCGA TCCTAGATGGCCTT and ATGAAGTCGATCCTAGATG GCCTT), CB_2R (CCATGGAGGAATGCTGGGTG and ATCAG ATAGAGCACAGCCACG), and GAPDH (ATGGGGAAGGTGA AGGTCGGAG and GATGACAAGCTTCCCGTTCTCA). GAPDH was used as a housekeeping gene to normalize the relative expression levels. Real-time PCR amplification was performed with SYBR Green PCR Master Mix (Applied Biosystems, Carlsbad, CA) on an ABI 7900 Sequence Detection System using the following conditions: 95°C for 3 minutes and 45 cycles at 95°C for 15 seconds and 60°C for 15 seconds.

Cell Fluorescent Imaging of NIR760-XLP6

PANC-1 cells were divided into three groups: 1) cells treated with 5 μM of NIR760-XLP6 at 37 °C for 30 minutes; 2) cells treated with 5 μM of NIR760-XLP6 at 37°C for 30 minutes after 30 minutes of pretreatment with 10 μM of 4Q3C as the blocking agent; and 3) cells treated with 5 μM of NIR760 at 37°C for 30 minutes. After the incubation, cells were washed three times with PBS and fixed with 4% paraformaldehyde/PBS for 20 minutes at room temperature. The cell nucleus was stained with 1 μg/ml DAPI for 15 minutes at room temperature. Cells were mounted and then imaged using the Zeiss Axio Observer. Z1 fluorescent microscope equipped with the ApoTome 2 imaging system (Carl Zeiss Microimaging Gmbh, Jena, Germany). NIR760-XLP6 or NIR760 fluorescent images were captured using an NIR camera with an ICG filter (excitation/emission: 750-800/820-875 nm). Nuclear images were obtained with a DAPI filter (excitation/emission: 335-383/420-470 nm). Differential interference contrast (DIC) images were obtained through Trans light DIC.