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Hand-held high-resolution fluorescence imaging system for fluorescence-guided surgery of patient and cell-line pancreatic tumors growing orthotopically in nude mice

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ABSTRACT

Background: In this study, we investigated the advantages of fluorescence-guided surgery (FGS) in mice of a portable hand-sized imaging system compared with a large fluorescence imaging system or a long-working-distance fluorescence microscope.

Methods: Mouse models of human pancreatic cancer for FGS included the following: (1) MiaPaCa-2-expressing green fluorescent protein, (2) BxPC3 labeled with Alexa Fluor 488-conjucated anti-carcinoembryonic antigen (CEA) antibody, and (3) patient-derived orthotopic xenograft (PDOX) labeled with Alexa Fluor 488-conjugated anti-carbohydrate antigen 19-9 antibody.

Results: Each device could clearly detect the primary MiaPaCa-2-green fluorescent protein tumor and any residual tumor after FGS. In the BxPC3 model labeled with Alexa Fluor 488-conjugated anti-CEA, each device could detect the primary tumor, but the MVX10 could not clearly detect the residual tumor remaining after FGS whereas the other devices could. In the PDOX model labeled with Alexa Fluor 488-conjugated anti-carbohydrate antigen 19-9, only the portable hand-held device could distinguish the residual tumor from the background, and complete resection of the residual tumor was achieved under fluorescence navigation.

Conclusions: The results described in the present report suggest that the hand-held mobile imaging system can be applied to the clinic for FGS because of its convenient size and high sensitivity which should help make FGS widely used.

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1. Introduction

The complete detection of tumors at the time of surgery is vital in optimizing surgical resection. The means to make tumors glow offers great advantages for tumor detection enabling fluorescence-guided surgery (FGS) and a number of different approaches have been attempted to label and image tumors during surgery [1–8].

We have previously demonstrated the enhanced visualization and detection of primary and metastatic lesions with the use of telomerase-dependent adenovirus (OBP-401) that express the green fluorescent protein (GFP) gene only in cancer cells for use in FGS [9–11]. We have also demonstrated the use of fluorescent-labeled antibodies [12–17] administered to the tumor-bearing mice for successful FGS of metastatic cancer in mouse models.

However, the FGS studies described previously have used large complex imaging systems, such as the OV100 (Olympus Corporation, Tokyo, Japan) and the MVX10 Macro View (Olympus Corporation, Center Valley, Pennsylvania), which would not be useful in the clinic. What is currently needed for clinical application of FGS is a simpler and more convenient imaging system to be used in the operating room (OR).

In the present study, we compared a hand-held completely mobile fluorescence imaging system with the conventional *in vivo* imaging systems for the detection of pancreatic cancer in mouse models, labeled with fluorescent proteins or fluorescent antibodies, to test the effectiveness of FGS.

2. Materials and methods

2.1. Establishment of green fluorescent protein labeled cancer cell line

The MiaPaCa-2 human pancreatic cell line was stably transfected with GFP as previously described [18–20]. In brief, cells were incubated with a ratio of 1:1 precipitated mixture of retroviral supernatants of PT67-GFP cells and RPMI 1640 (Irvine Scientific, Santa Ana, CA) containing 10% fetal bovine serum (Hyclone Laboratories, Logan, UT) for 72 h. Fresh medium was replenished at this time. Cells were harvested with trypsin-ethylenediaminetetraacetic acid at 72 h after transduction and subcultured at a ratio of 1:15 into a selective medium, which contained 200 μg/mL of G418. The level of G418 was increased stepwise up to 800 μg/mL [18–22].

2.2. Cell culture

MiaPaCa-2-GFP and BxPC3 human pancreatic cancer cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum. The cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. The cells were collected after trypsinization and stained with trypan blue (Sigma-Aldrich, St. Louis, MO). Only viable cells were counted with a hemocytometer (Hausser Scientific, Horsham, PA).

2.3. Animals

Athymic NCR nude mice (nu/nu) (AntiCancer, Inc, San Diego, CA), aged 4–6 wk, were used in this study. The mice were kept in a barrier facility under high efficiency particulate air (HEPA) filtration. The mice were fed with autoclaved laboratory rodent diet. All surgical procedures and imaging were performed with the animals anesthetized by intramuscular injection of 0.02 mL of a solution of 50% ketamine, 38% xylazine, and 12% acepromazine maleate. All animal studies were conducted in accordance with the principals and procedures outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals under Public Health Service Assurance Number A3873-1.

2.4. Subcutaneous tumor cell implantation

MiaPaCa-2-GFP and BxPC3 cells were harvested by trypsinization and washed twice with serum-free medium. Cells (2×10^6 in 100 μL serum-free media) were injected subcutaneously, within 30 min of harvesting, over the right and left flanks in male nu/nu mice aged between 4 and 6 wk. Subcutaneous tumors were allowed to grow for 2–4 wk until large enough to supply adequate tumor to harvest for subsequent orthotopic implantation [23].

2.5. Establishment of patient-derived orthotopic xenograft (PDOX®) of pancreatic cancer

Pancreatic cancer patient tumor tissue was obtained at surgery and cut into 3-mm^3 fragments and transplanted subcutaneously in non-obese diabetic-severe combined immune deficient mice (NOD-SCID) [24–26]. The patient tumors were then harvested from the NOD-SCID mice and passed orthotopically in nude mice [21–24,27]. All patients provided informed consent, and the study was conducted under the approval of the Institutional Review Board of the UC San Diego Medical Center.

2.6. Orthotopic tumor implantation

A small 6- to 10-mm transverse incision was made on the left flank of the mouse through the skin and peritoneum. The tail of the pancreas was exposed through this incision and a single 3-mm^3 tumor fragment from subcutaneous tumors was sutured to the tail of the pancreas using 8-0 nylon surgical sutures (Ethilon; Ethicon Inc, NJ). On completion, the tail of the pancreas was returned to the abdomen, and the incision was closed in one layer using 6-0 nylon surgical sutures [21–24,27].

2.7. Antibody conjugation

Monoclonal antibodies specific for carcinoembryonic antigen (CEA) or carbohydrate antigen 19-9 (CA19-9) were purchased from RayBiotech, Inc (Norcross, GA) or Abcam, Inc (Cambridge, MA). The antibodies were labeled with the AlexaFluor 488 Protein Labeling Kit (Molecular Probes Inc, Eugene, OR) according to the manufacturer's instructions and as previously described [12].