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Precise Non-invasive Imaging Mouse Model of Pancreatic Cancer: Very Narrow Band-width Laser Fluorescence Excitation of Green Fluorescent Protein Provides Ultra-bright Tumor Images With no Skin Autofluorescence

YUTARO KUBOTA[1,2,3], APRIL WANG[4], NEIL CHANG[4], SAMANTHA TARANTINO[4], SEAN GALLAGHER[4], YUSUKE AOKI[1,2], NORIYUKI MASAKI[1,2], KOYA OBARA[1,2], SEI MORINAGA[1,2], TAKUYA TSUNODA[3] and ROBERT M. HOFFMAN[1,2]

[1]*AntiCancer Inc., San Diego, CA, U.S.A.;*
[2]*Department of Surgery, University of California, San Diego, CA, U.S.A.;*
[3]*Division of Internal Medicine, Department of Medical Oncology, Showa University School of Medicine, Tokyo, Japan;*
[4]*Analytik Jena, Upland, CA, U.S.A.*

Abstract. *Background/Aim: Pancreatic cancer is a recalcitrant disease with 5-year survival of only 12%. Improved mouse models of pancreatic cancer are critical for discovery of effective therapeutics. Materials and Methods: Orthotopic mouse nude-mouse models of pancreatic cancer were established with the human pancreatic-cancer cell line Panc-1 expressing green fluorescent protein (GFP) by transplanting tumor fragments into the pancreas, using the procedure of surgical orthotopic implantation (SOI). Four weeks after establishment of the orthotopic models, the mice were imaged with the Analytik Jena UVP Biospectrum Advanced with a very-narrow-band-width excitation at 487 nm and peak emission at 513 nm. Results: Non-invasive fluorescence imaging of the mice implanted with Panc-1-GFP showed a very bright tumor in the area of the pancreas and peritoneal cavity. The skin background autofluorescence was absent. When a laparotomy was performed on the mouse for open imaging, the tumor on the pancreas was clearly imaged. There was very clear concordance of the non-invasive image and the image obtained during laparotomy. Conclusion: A precise orthotopic mouse model of pancreatic cancer was developed in which there was high concordance between non-invasive and invasive fluorescence imaging due to the ultra-bright signal and ultra-low background using very-narrow-band-width laser fluorescence excitation. This model can be used for high-throughput in vivo screening for improved therapeutics for pancreatic cancer.*

Correspondence to: Robert M. Hoffman, Ph.D., AntiCancer Inc, 7917 Ostrow St, San Diego, CA, 92111, U.S.A. Tel: +1 6198852284, Fax: +1 6198852284, e-mail: all@anticancer.com

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Pancreatic cancer is a recalcitrant disease with a 5-year survival rate of 12% (1). Improved mouse models of metastatic pancreatic cancer are needed. We developed orthotopic models of cancer, including the patient-derived orthotopic xenograft (PDOX) model of cancer, comprising pancreatic and other cancers, more than 30 years ago (2). We have also evaluated therapies with the pancreatic-cancer PDOX model (3-5). In order to develop a model for high-throughput *in vivo* screening of novel therapeutics, a readily-imageable mouse model of pancreatic cancer is necessary. Our laboratory pioneered *in vivo* imaging with fluorescent proteins (6-14).

In the present report, we demonstrate an orthotopic model of pancreatic cancer expressing green fluorescent protein (GFP) imaged with a very-narrow-band-width laser apparatus, the UVP Biospectrum Advanced (Analytik Jena US LLC, Upland, CA, USA). This precise model can non-invasively visualize the growth of primary and metastatic pancreatic cancer with a very strong signal and no background from skin autofluorescence, and with high concordance to imaging *via* laparotomy.

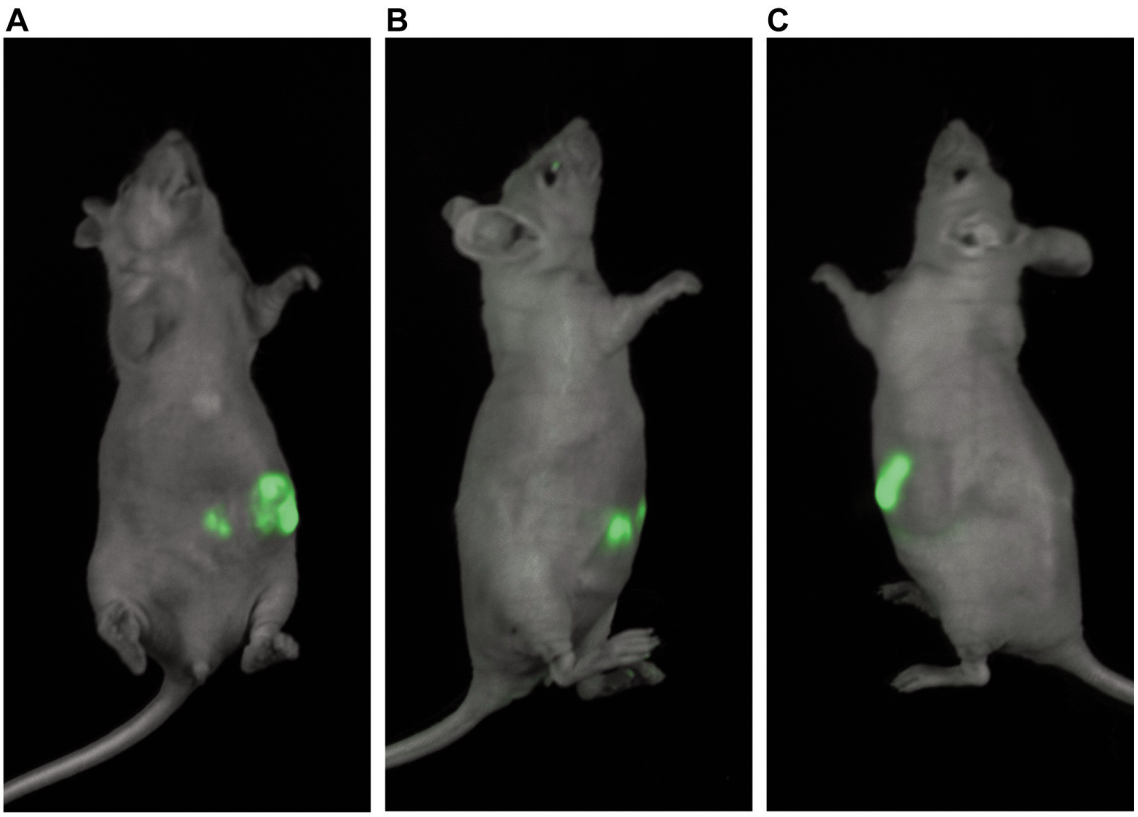

Figure 1. *Non-invasive fluorescence images of the primary pancreatic cancer growing in the pancreas and expressing GFP. A) Front side. B) Right side. C) Left side.*

Materials and Methods

Cell line. Panc-1, a human pancreatic cancer cell line, was stably transduced to express green fluorescent protein (GFP) as previously described and used for *in vivo* experiments (9). Panc-1 cells were cultured in DMEM with 10% fetal bovine serum.

Mice. To conduct this study, 4-6-week-old nu/nu mice were used (AntiCancer Inc., San Diego, CA, USA). Mice were bred and maintained in a barrier facility with HEPA filtration. The AntiCancer Institutional Animal Care and Use Committee approved all mouse investigations. All investigations were conducted in accordance with the ARRIVE 2.0 criteria (15).

Surgical orthotopic implantation (SOI). After culturing in flasks, Panc-1-GFP cells were injected subcutaneously into the flanks of nude mice and allowed to engraft and proliferate for 3-4 weeks to form subcutaneous tumors. Subsequently, 30 mm^3 tumor fragments from subcutaneous tumors were sutured to the tail of the pancreas using 7-0 PDS-II surgical sutures (Ethicon, Inc., Somerville, NJ, USA). The incision was closed in one layer using 5-0 PDS-II surgical sutures (Ethicon, Inc.) (2).

Imaging. A UVP Biospectrum Advanced imaging instrument (Analytik Jena US LLC, Upland, CA, USA) was used in the present study with excitation at 487 nm and peak emission at 513 nm.

Results

Non-invasive images, obtained with the Biospectrum Advanced, of the pancreatic tumor showed a very brilliant fluorescent, well-defined tumor in the peritoneal cavity, measuring approximately 17.21 mm × 15.48 mm with no background from skin autofluorescence (Figure 1A). Images obtained from the right and left sides of the abdomen showed that the tumor was within the peritoneal cavity (Figure 1B and C). Opening the skin and peritoneum of the abdomen and imaged, showed that the tumor was growing within the peritoneal cavity with apparent peritoneal metastasis (Figure 2).

Discussion

The present report described an important advance in the development of imaging mouse models of pancreatic cancer that precisely visualizes the main features of clinical pancreatic cancer. The model can be rapidly non-invasively imaged in less than one second with a very high signal and no background from skin autofluorescence. The primary pancreatic cancer, as well as metastases, were

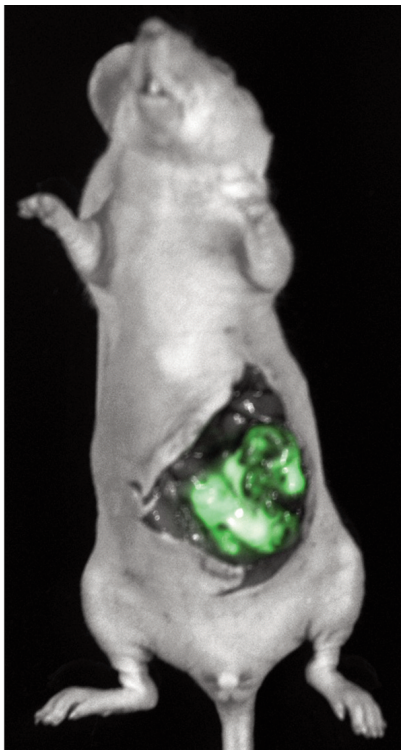

Figure 2. *Fluorescence images acquired from opening the skin and peritoneum of the abdomen showing the primary tumor growing on the pancreas and expressing GFP.*

imaged non-invasively with high concordance with open imaging. This model will enable the rapid screening of drugs that can target primary and metastatic metastatic pancreatic cancer to improve the outcome of this recalcitrant disease.

The present study demonstrates the very high power of imaging with GFP using instruments that comprise very-narrow-band-width laser excitation. Our laboratory pioneered in vivo imaging with fluorescent proteins starting in the last century (6-14).

The present study should set the field of small-animal imaging on its proper course as it has been severely set back and confused by misinformation that GFP is inappropriate for *in vivo* imaging due to skin autofluorescence (16-18), which as the present study shows is readily overcome by very-narrow-band fluorescence excitation.

Conflicts of Interest

AW, NC, ST and SG are employees of Analytik Jena. YA, YT, NM, KO, SM and RMH are non-salaried associates of AntiCancer Inc. AntiCancer Inc. uses mouse models of cancer for contract research.

Authors' Contributions

YK, AW, NC, ST, SG, YA, NM, KO, and SM performed experiments. YK and RMH wrote the article. TT critically reviewed the article.

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