ARTICLE
Journal of Cellular Biochemistry 107:328–334 (2009)

Journal of Cellular Biochemistry

Development of the Transgenic Cyan Fluorescent Protein (CFP)-Expressing Nude Mouse for "Technicolor" Cancer Imaging

Hop S. Tran Cao,[1] Jose Reynoso,[2] Meng Yang,[2] Hiroaki Kimura,[1,2] Sharmeela Kaushal,[1] Cynthia S. Snyder,[1] Robert M. Hoffman,[1,2*] and Michael Bouvet[1]

[1]*Department of Surgery, University of California, San Diego, California*

[2]*AntiCancer, Inc., San Diego, California*

ABSTRACT

A major goal for in vivo biology is to develop models which can express multiple colors of fluorescent proteins in order to image many processes simultaneously in real time. Towards this goal, the cyan fluorescent protein (CFP) nude mouse was developed by crossing non-transgenic nude mice with the transgenic CK/ECFP mouse in which the β-actin promoter drives expression of CFP in almost all tissues. In crosses between nu/nu CFP male mice and nu/+ CFP female mice, approximately 50% of the embryos fluoresced blue. In the CFP nude mice, the pancreas and reproductive organs displayed the strongest fluorescent signals of all internal organs which vary in intensity. Orthotopic implantation of XPA-1 human pancreatic cancer cells expressing red fluorescent protein (RFP); or green fluorescent protein (GFP) in the nucleus and RFP in the cytoplasm, was performed in female nude CFP mice. Color-coded fluorescence imaging of these human pancreatic cancer cells implanted into the bright blue fluorescent pancreas of the CFP nude mouse afforded novel insight into the interaction of the pancreatic tumor and the normal pancreas, in particular the strong desmoplastic reaction of the tumor. The naturally enhanced blue fluorescence of the pancreas in the CFP mouse serves as an ideal background for color-coded imaging of the interaction of implanted cancer cells and the host. The CFP nude mouse will provide unique understanding of the critical interplay between the cancer cells and their microenvironment. J. Cell. Biochem. 107: 328–334, 2009.

KEY WORDS: MULTICOLOR IN VIVO IMAGING; PANCREATIC CANCER; TUMOR MICROENVIRONMENT; BLUE FLUORESCENCE

The use of fluorescent proteins for imaging is revolutionizing in vivo biology [Hoffman, 2005, 2008]. Green fluorescent protein (GFP) has been shown to be able to be genetically linked with almost any protein thereby providing a permanent and heritable label in live cells to study protein function and location. Many different colors of fluorescent proteins have now been produced in the laboratory or found in nature [Matz et al., 1999; Shaner et al., 2004]. With multiple colors, many processes can be visualized simultaneously in cells. Thus, live cells can be multiply labeled for imaging processes that heretofore could be seen only on fixed and stained cells.

Our laboratory pioneered in vivo imaging with fluorescent proteins [Chishima et al., 1997], including noninvasive whole-body imaging [Yang et al., 2000]. Whole-body imaging with fluorescent proteins depends in large part on the brightness of the protein. Whole-body imaging with fluorescent proteins can track tumor growth and metastasis, gene expression, angiogenesis, and bacterial infection quantitatively [Hoffman, 2005]. Noninvasive in vivo imaging can be performed even at subcellular resolution [Hoffman and Yang, 2006; Yang et al., 2007], depending on the position of the cells in the animal. Interference by skin autofluorescence is kept to a minimum with the use of proper excitation filters. Simple equipment, such as an LED flashlight with a narrow-band excitation filter in combination with a bandpass emission filter, can be used to whole-body image mice implanted with cells expressing fluorescent proteins [Yang et al., 2005].

Whole-body imaging is more effective when the fluorescent protein emits at longer wavelengths that are absorbed less by tissues and by physiological molecules, such as hemoglobin. Longer wavelength light is also less scattered [Hoffman, 2008].

Grant sponsor: National Cancer Institute; Grant numbers: CA109949, CA132971; Grant sponsor: American Cancer Society; Grant number: RSG-05-037-01-CCE; Grant sponsor: T32 training grant; Grant number: CA121938; Grant sponsor: National Cancer Institute; Grant number: CA103563.

*Correspondence to: Dr. Robert M. Hoffman, 7917 Ostrow Street, San Diego, CA 92111. E-mail: all@anticancer.com

Received 10 February 2009; Accepted 11 February 2009 • DOI 10.1002/jcb.22128 • 2009 Wiley-Liss, Inc.
Published online 20 March 2009 in Wiley InterScience (www.interscience.wiley.com).

A number of attempts have been made to image tumor–host interaction. To study tumor angiogenesis, Fukumura et al. [1998] and Brown et al. [2001] have used transgenic mice that express GFP under the control of human vascular endothelial cell growth factor (VEGF) promoter. After implantation of solid tumors, highly fluorescent fibroblasts were observed surrounding and infiltrating the tumor mass. When spontaneous mammary tumors developed in these mice, GFP was visualized in fibroblasts surrounding the neoplastic nodules but not in the tumor cells themselves. Thus, the VEGF promoter of nontransformed cells is strongly activated by the tumor microenvironment, which in turn stimulates tumor angiogenesis. However, these models did not enable simultaneous imaging of tumor and host cells.

Okabe et al. [1997] produced transgenic mice with GFP under the control of a chicken β-actin promoter and cytomegalovirus enhancer. All of the tissues from these transgenic mice, with the exception of erythrocytes and hair, fluoresce green. Tumor cells transplanted in the GFP transgenic mouse were made visible by transforming them with red fluorescent protein (RFP) [Yang et al., 2003]. To gain further insight into the tumor–host interaction in the living state, including tumor angiogenesis and immunology, we visualized RFP-expressing tumors transplanted in GFP-expressing transgenic mice using dual-color fluorescence imaging and microscopy [Yang et al., 2003].

We developed the transgenic GFP nude mouse with ubiquitous GFP expression [Yang et al., 2004]. The GFP nude mouse was obtained by crossing nontransgenic nude mice with the transgenic C57/B6 GFP mouse. In crosses between nu/nu GFP male mice and nu/+ GFP female mice, the embryos fluoresced green. Approximately 50% of the offspring of these mice were GFP nude mice. Newborn mice and adult mice fluoresced very bright green and could be detected with a simple blue–light-emitting diode flashlight with a central peak of 470 nm and a bypass emission filter. In the adult mice, the organs all brightly expressed GFP, including the heart, lungs, spleen, pancreas, esophagus, stomach, and duodenum. The following systems were dissected out and shown to have brilliant GFP fluorescence: the entire digestive system from tongue to anus; the male and female reproductive systems; brain and spinal cord; and the circulatory system, including the heart and major arteries and veins. The skinned skeleton highly expressed GFP. Pancreatic islets showed GFP fluorescence. The spleen cells were also GFP positive. RFP-expressing human cancer cell lines, including PC-3-RFP prostate cancer, HCT-116-RFP colon cancer, MDA-MB-435-RFP breast cancer, and HT1080-RFP fibrosarcoma were transplanted to the transgenic GFP nude mice. All of these human tumors grew extensively in the transgenic GFP nude mouse. Dual-color fluorescence imaging enabled visualization of human cancer cell/host cell interaction.

However, most donor sources of stromal cell types, appropriate for study in the tumor microenvironment (TME), are from mice expressing GFP. Therefore, a nude mouse expressing RFP would be an appropriate host for transplantation of GFP-expressing stromal cells. The RFP nude mouse was obtained by crossing non-transgenic nude mice with the transgenic C57/B6 mouse in which the beta-actin promoter drives RFP (DsRed2) expression in essentially all tissues [Vintersten et al., 2004]. In crosses between nu/nu RFP male mice and nu/+ RFP female mice, the embryos fluoresced red. Approximately 50% of the offspring of these mice were RFP nude mice. In the RFP nude mouse, the organs all brightly expressed RFP, including the heart, lungs, spleen, pancreas, esophagus, stomach, duodenum; the male and female reproductive systems; brain and spinal cord; and the circulatory system, including the heart, and major arteries and veins. The skinned skeleton highly expressed RFP. The bone marrow and spleen cells were also RFP positive. GFP-expressing human cancer cell lines, including HCT-116-GFP colon cancer and MDA-MB-435-GFP breast cancer, were orthotopically transplanted to the transgenic RFP nude mice. These human tumors grew extensively in the transgenic RFP nude mouse and were visible by noninvasive whole-body imaging. Dual-color fluorescence imaging enabled visualization of the interaction of the human cancer cells and the host stromal cells.

Additional colored mice would allow even more processes to be imaged simultaneously. In the cyan fluorescent (CFP) mouse developed by Nagy's laboratory [Hadjantonakis et al., 2002], CFP is driven by the β-actin promoter similar to the GFP and RFP transgenic mice. In the CFP mouse, however, we have observed that the pancreas displays markedly enhanced fluorescence compared to the other organs and to the rest of the GI tract in particular. This interesting finding provides a unique model wherein the organ of interest in pancreatic research is naturally highlighted in blue, establishing an ideal background on which cancer cells, immune cells, stem cells, and other cell types of different colors can be added to study their interaction. There is differential expression of blue fluorescence among the various organs. However, the pancreas stands out from the rest of the GI tract, displaying the strongest fluorescence of all organs in the mouse. Fluorescence microscopy demonstrated that the CFP fluorescence resided in the acinar cells of the pancreas and not the islet cells [Tran Cao et al., 2009].

We wished to study tumor–host interaction of human pancreatic cancer and other types of human cancer in the CFP mouse. Therefore, we developed and characterized the transgenic CFP nude mouse. Human pancreatic cancer cells expressing RFP; or GFP in the nucleus and RFP in the cytoplasm [Yamamoto et al., 2004], were orthotopically implanted in the CFP nude mouse. Their growth was visualized over time and the tumor and tumor microenvironment were subsequently visualized with spectacular color-coded imaging and reported here.

MATERIALS AND METHODS

TRANSGENIC CYAN FLUORESCENT PROTEIN NUDE MICE

Wild-type transgenic CK6/ECFP mice were obtained from the Jackson Laboratory (Bar Harbor, ME). These mice express cyan fluorescent protein under the control of the chicken β-actin promoter coupled with the cytomegalovirus (CMV) immediate early enhancer [Hadjantonakis et al., 2002]. Six-week-old transgenic CFP female mice were crossed with 6- to 8-week-old BALB/c nu/nu or NCR nu/nu male mice (Harlan, Indianapolis, IN). Male F1 CFP nude mice were crossed with female F1 immunocompetent CFP mice. When male F2 nu/nu CFP mice were crossed with female F1 or

F2 nu/+ CFP mice, approximately 50% of their offspring were transgenic CFP nude mice. This schema can reliably and consistently produce nude CFP mice.

ANIMAL CARE

Transgenic CK6/ECFP mice, athymic nu/nu nude mice, and transgenic nu/nu CFP nude mice were maintained in a barrier facility on high efficiency particulate air (HEPA)-filtered racks. The animals were fed with autoclaved laboratory rodent diet (Teklad LM-485; Western Research Products, Orange, CA). Breeding was carried out per the schema described above. Anesthesia for surgical procedures and imaging was achieved by intramuscular injection of 0.02 ml of a solution of 50% ketamine, 38% xylazine, and 12% acepromazine maleate. Mice were euthanized by injecting 0.05 ml of the same solution, followed by cervical dislocation. All animal studies were conducted in accordance with principles and procedures outlined in the NIH Guide for the Care and Use of Animals.

CULTURE OF RFP AND DUAL-COLOR GFP-RFP XPA-1 CANCER CELLS

Human pancreatic cancer XPA-1 cells were a gift from Dr. Anirban Maitra at Johns Hopkins University. They were previously engineered in our laboratory to express RFP (XPA-1-RFP) or to express GFP in the nucleus and RFP in the cytoplasm (XPA-1-GFP-RFP) [Yamamoto et al., 2004]. Cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) (HyClone Laboratories, Inc., Logan, UT) and sodium bicarbonate (Cellgro, Mediatech, Inc., Manassas, VA). Additionally, all media were supplemented with 2 mM L-glutamine, penicillin-streptomycin, sodium pyruvate, and MEM non-essential amino acids, all of which were obtained from Gibco-BRL, Life Technologies, Inc. (Grand Island, NY). Cultures were incubated at 37°C with 5% CO_2.

ORTHOTOPIC PANCREATIC CANCER MODEL

Six-week-old female nude CFP mice were anesthetized in the manner described above. A 6- to 10-mm transverse incision was made on the left flank of the mouse through the skin and peritoneum, and the tail of the pancreas was then exposed through this incision. RFP-expressing XPA-1 (1×10^6) cells in 20 μl total volume were injected into the pancreatic tail, which was subsequently returned into the abdomen. The incision was closed in two layers using 6.0 Ethibond non-absorbable sutures (Ethicon, Inc., Somerville, NJ). The model was also made using XPA-1-GFP-RFP cells using the methods described above.

IMAGING

All animals were imaged using the UVP iBox Small Animal Imaging System (UVP, Upland, CA), equipped with a BioChemi HR 500 CCD camera and a BioLite automated multispectral light source (UVP) delivered to the dark imaging chamber via fiber optics. Non-tumor-bearing nude CFP mice were sacrificed before imaging, and a complete necropsy was performed, with all major organs being imaged with the same device. Orthotopic pancreatic tumor-bearing mice were anesthetized before each imaging session, and tumor growth was noninvasively imaged over the course of 6 weeks after implantation. At 6 weeks post-implantation, these mice were sacrificed and intravital imaging was performed. The pancreas-tumor block was harvested, frozen, and processed for

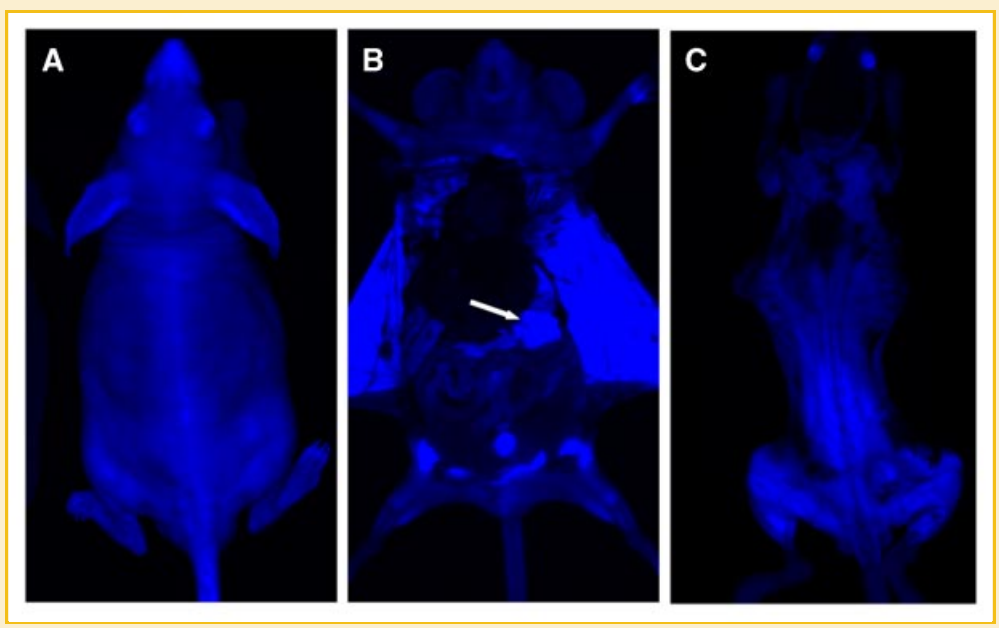

Fig. 1. Imaging the transgenic CFP nude mouse. All the images in this figure were obtained with the CFP filter of the UVP iBox Small Animal Imaging System with exposure time of 1 s. A: Whole-body imaging of the live mouse reveals strong blue fluorescence throughout. B: After euthanasia, the abdominal and thoracic cavities were exposed. The peritoneum displayed a strong blue signal. The enhanced fluorescence of the pancreas (arrow) made it stand out from the rest of the internal organs. C: The musculoskeletal system of the mouse also expresses CFP strongly.

fluorescence imaging and H&E staining. Fluorescence microscopy was performed with an Olympus IMT-2 inverted fluorescence microscope (Olympus Corp., Tokyo, Japan) and with a DeltaVision Deconvolution Microscope System (Applied Precision, Inc., Issaquah, WA) mounted on a Nikon TE-200 microscope (Nikon Corp., Tokyo, Japan).

IMAGE PROCESSING

All images were analyzed using Image-J (National Institute of Health, Bethesda, MD), and processed for contrast and brightness using Photoshop Element-4 (Adobe Systems, Inc., San Jose, CA).

RESULTS

CHARACTERIZATION OF THE TRANSGENIC CYAN FLUORESCENT PROTEIN (CFP) NUDE MOUSE

We have successfully developed the CFP nude mouse by crossing non-fluorescent nude mice with the transgenic CK6/ECFP mouse (Fig. 1A). The CFP nude mouse exhibits the same fluorescence pattern that we previously described in the wild-type CK6/ECFP mouse [Tran Cao et al., 2009]. Namely, it expresses blue fluorescence in nearly all its tissues but at much different intensities. Amongst internal organs, the pancreas displays the strongest CFP blue fluorescent signal (Fig. 1B).

In addition to intravital imaging, all organs were harvested and individually imaged under the CFP filter of the UVP iBox. The musculoskeletal system (Fig. 1C) displayed strong blue fluorescence. Internal organs expressed varying levels of CFP expression. The lungs, cardiovascular system, kidneys, and adrenals glands (Fig. 2A), as well as the neurological system (Fig. 2B), were very weakly fluorescent. Likewise, the liver (Fig. 2C) required a prolonged exposure time (5 s) to display a fairly low level of blue fluorescence. In contrast, both female and male reproductive organs brightly fluoresced blue (Fig. 2D,E, respectively) after a 1-s exposure. Within the gastrointestinal tract, the brilliant fluorescence of the pancreas stood out in sharp contrast to the relatively weak fluorescent signal of the rest of the tract (Fig. 3A,B). When the pancreas was isolated (Fig. 3C) and examined under higher magnification (Fig. 3D), the glandular nature of the organ became evident.

ORTHOTOPIC RFP OR DUAL-COLORED GFP-RFP PANCREATIC CANCER MODEL IN THE CFP NUDE MOUSE

RFP or dual-colored, expressing GFP in the nucleus and RFP in the cytoplasm, XPA-1 pancreatic cancer cells were orthotopically implanted into separate mice. The mice were imaged weekly under three different filters (CFP, RFP, and GFP) in the iBox, and tumor growth was monitored. The mice were capable of serving as hosts, with no rejection of the xenograft. The growth rate of the tumors mirrored that of tumors in non-transgenic nude mice. The tumor

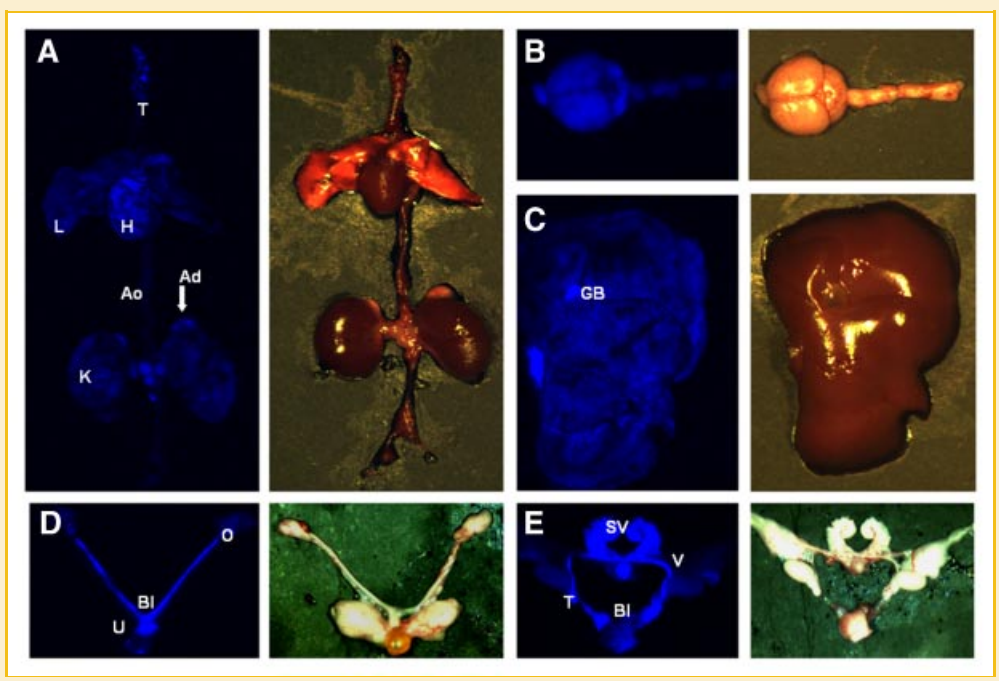

Fig. 2. Imaging the organs of the CFP nude mouse. After necropsy, individual organ systems were removed and imaged under both the CFP filter and brightfield. Due to the low signal in some organs, the exposure time was changed for optimal visualization. Each image has one panel for fluorescence and one panel for brightfield. A: The cardiovascular (heart—H and aorta—Ao) and pulmonary (lungs—L and trachea—T) systems were resected en bloc with the kidneys (K) and adrenal glands (Ad) and imaged at an exposure time of 5 s. B: The neurological system also required 5 s exposure for adequate visualization of blue fluorescence. C: The liver was imaged for 5 s as well; the gallbladder (GB) was brightly fluorescent. D: The female reproductive organs fluoresced strongly, mostly in the uterus (U), and less so in the ovaries (O); exposure time: 1 s. E: The male reproductive organs, likewise, were brightly fluorescent at 1 s; the seminal vesicles (SV) emitted the strongest signals, more so than the vas deferens (V) and the testes (T). The urinary bladder (Bl) is also strongly fluorescent in both images.

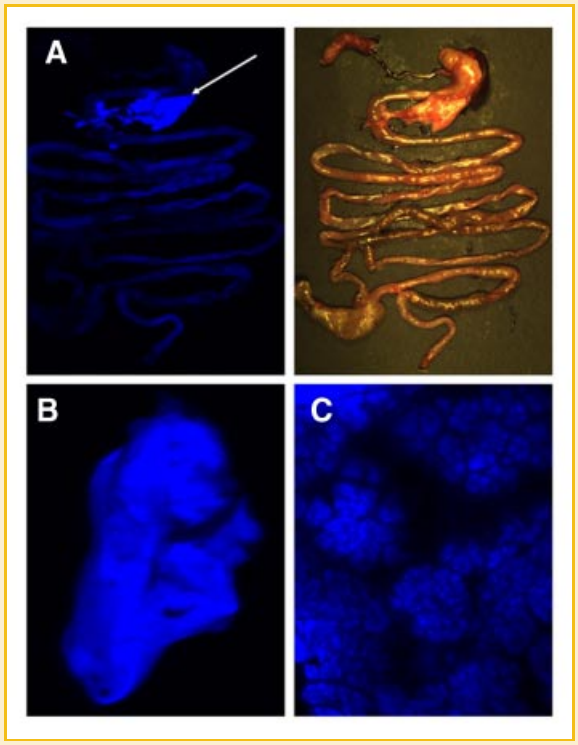

Fig. 3. Imaging the pancreas and gastrointestinal tract. The GI tract was dissected en bloc, from the tongue to the rectum. A: The fluorescence of the pancreas (arrow) was much stronger than that of the rest of the GI tract. Exposure time of 1.5 s was used. A brightfield image is also shown. B: The pancreas was isolated from the rest of the GI tract and imaged individually. C: Examination under the microscope at 10× revealed clearly the glandular nature of the pancreas.

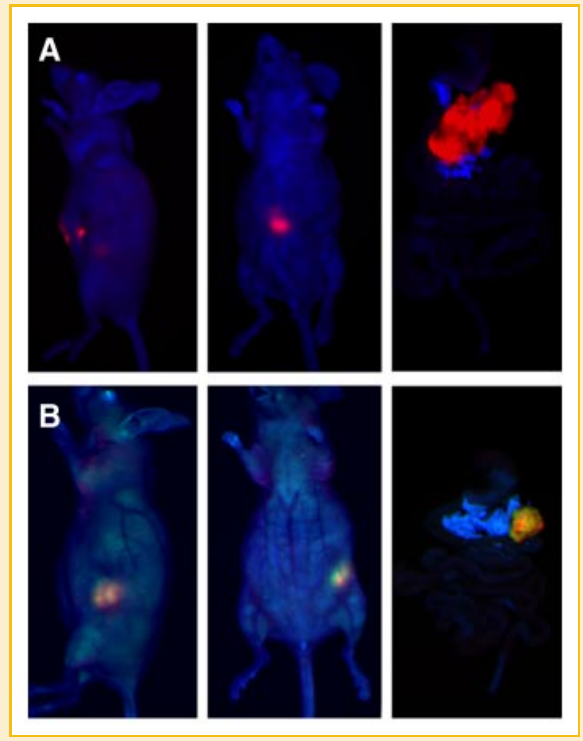

Fig. 4. Imaging the orthotopic human pancreatic cancer CFP nude mouse model with XPA-1-RFP cells. Orthotopic injection of human pancreatic cancer cells was performed in CFP nude mice; these images were obtained at week 6 post-implantation. Exposure time for CFP signals was 1 s. A: In this series, whole-body dual-color imaging permits identification of the tumor in the whole animal. This brightly red fluorescent tumor had grown extensively into the blue pancreas. This could be seen after isolation of the GI tract, when only small slivers of blue pancreas could be seen around the red tumor. B: The dual-colored XPA-1-GFP-RFP tumor was grown from human pancreatic cancer cells engineered to express orthotopic GFP in nucleus and RFP in the cytoplasm. Whole-body tri-color imaging permitted identification of the tumor within the mouse. After isolation of the GI tract, one could see a dual-colored tumor sitting on a blue-fluorescent pancreas.

first became discernable under noninvasive whole-body imaging between one and 2 weeks post-implantation. When the mice were sacrificed at 6 weeks, images were obtained of the whole body and after exposure of the abdominal and thoracic cavities (Fig. 4). In both the RFP and dual-color tumor models, the bright blue fluorescence of the intact mouse body and native pancreas provided a sharp background for the bright red or red-green fluorescent tumors which were visible both noninvasively and intravitally on the pancreas.

HOST–TUMOR INTERACTION IN THE TUMOR MICRO-ENVIRONMENT

Fluorescence microscopy permitted visualization of the interaction between the tumor and the host's pancreas. This was especially impressive in the case of the dual-colored XPA-1-GFP-RFP cancer cells, where the cancer cell nuclei fluoresce green and the cytoplasm red. The use of a deconvolution microscope allowed the identification of a "leading edge" of the tumor into the normal pancreas, where relatively few tumor cells can be seen (Fig. 5A,B). Instead, it appears to be a region of heavy desmoplastic activity with some infiltrating XPA-1-GFP-RFP tumor cells. One such cell can be seen dividing near the edge of the normal pancreas (Fig. 5C). When the tissue slide was stained for blood vessels with primary CD31 antibody and Alexa 488-secondary antibody conjugate, the greatest signal corresponded to this interaction zone (Fig. 5D), a finding confirmed by H&E staining (Fig. 5E).

DISCUSSION

Our laboratory has previously applied color-coded fluorescence imaging in an effort to elucidate the critical interplay between stromal and cancer cells in the tumor microenvironment, first in the transgenic wild-type GFP mouse [Yang et al., 2003], then the transgenic GFP nude mouse [Yang et al., 2004], and recently the RFP nude mouse [Yang et al., 2009]. We have studied the interaction between pancreatic cancer cells and splenocytes in the tumor microenvironment using this technology [Bouvet et al., 2006]. Fluorescent-protein-based imaging offers the advantage of color-coding the cells, permitting identification of the different components of the tumor microenvironment, including stromal elements, immune cells, and the cancer cells themselves. In addition,

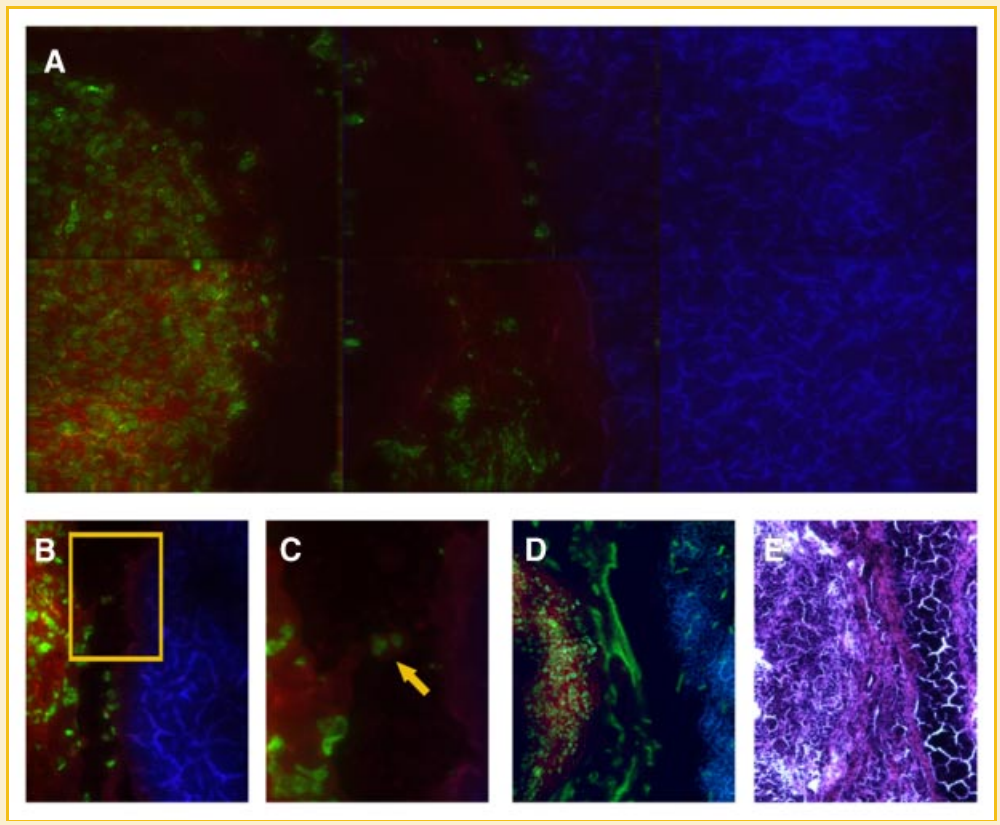

Fig. 5. Color-coded fluorescence microscopy of the pancreatic tumor microenvironment in the CFP nude mouse. The dual-colored pancreatic tumor was removed en bloc and frozen in OTC, and subsequently imaged under fluorescence microscopy. A: A high-resolution deconvolved image of the tumor-host interaction area is shown. The blue fluorescent pancreas in invaded by the dual-colored XPA-1-GFP-RFP cells, with an intervening area of dense fibrous tissue infiltrated by only few tumor cells. B,C: A dividing tumor cell (arrow) is identified at the edge of this interaction zone, near the pancreas. D: CD31 staining for blood vessels confirms the increased vascularity of this interaction zone. E: H&E staining of this area is attached for comparison.

imaging at the single-cell level is afforded by this technology as well.

The limitations of fluorescence imaging, up to very recently, have centered around a narrow choice of colors, relying primarily on green and red fluorescent proteins. Although Nagy's laboratory first developed the CFP mouse in 2002 [Hadjantonakis et al., 2002], the use of the cyan fluorescent protein had been limited by the low wavelength of its signal, leading to a high degree of interference from background autofluorescence. The iBox imaging system is a novel device that can detect the fluorescence of the cyan fluorescent protein through its narrow range CFP filter, thus minimizing background interference. Using this device, we recently found that the pancreas of the wild-type CK6/ECFP mouse exhibited substantially stronger fluorescence than the rest of the organs in the GI tract [Tran Cao et al., 2009].

The transgenic CFP nude mouse, having retained the characteristic enhanced blue fluorescence of the pancreas, serves as an excellent host for the orthotopic pancreatic cancer model. This model allows clear and distinct visualization of both single- and dual-colored cancer cells that express RFP; or GFP in the nucleus and RFP in the cytoplasm, both in the intact mouse as well as intravitally. In the GFP and RFP transgenic nude mice, the host tissue displayed either red or green fluorescence that was at times difficult to differentiate from the dual-colored tumor cells. In the CFP nude mouse, no such ambiguity exists on the blue background of the native pancreas. The CFP nude mouse should add to our understanding of pancreatic and other cancers with the addition of stromal and immune cells of various combinations of color to more clearly define the critical interplay between the cancer cells and their microenvironment.

ACKNOWLEDGMENTS

Work supported in part by grants from the National Cancer Institute CA109949 and CA132971 and American Cancer Society RSG-05-037-01-CCE (to M.B.), T32 training grant CA121938 (to H.S.T.C.), and National Cancer Institute grant CA103563 (to AntiCancer, Inc.).

REFERENCES

Bouvet M, Tsuji K, Yang M, Jiang P, Moossa AR, Hoffman RM. 2006. In vivo color-coded imaging of the interaction of colon cancer cells and splenocytes in the formation of liver metastases. Cancer Res 66:11293–11297.

Brown EB, Campbell RB, Tsuzuki Y, Xu L, Carmeliet P, Fukumura D, Jain RK. 2001. In vivo measurement of gene expression, angiogenesis and physiological function in tumors using multiphoton laser scanning microscopy. Nature Med 7:864–868.

Chishima T, Miyagi Y, Wang X, Yamaoka H, Shimada H, Moossa AR, Hoffman RM. 1997. Cancer invasion and micrometastasis visualized in live tissue by green fluorescent protein expression. Cancer Res 57:2042–2047.

Fukumura D, Xavier R, Sugiura T, Chen Y, Park EC, Lu N, Selig M, Nielsen G, Taksir T, Jain RK, Seed B. 1998. Tumor induction of VEGF promoter activity in stromal cells. Cell 94:715–725.

Hadjantonakis AK, Macmaster S, Nagy A. 2002. Embryonic stem cells and mice expressing different GFP variants for multiple non-invasive reporter usage within a single animal. BMC Biotechnol 2:11.

Hoffman RM. 2005. The multiple uses of fluorescent proteins to visualize cancer *in vivo*. Nature Rev Cancer 5:796–806.

Hoffman RM. 2008. A better fluorescent protein for whole-body imaging. Trends Biotechnol 26:1–4.

Hoffman RM, Yang M. 2006. Subcellular imaging in the live mouse. Nature Protoc 1:775–782.

Matz MV, Fradkov AF, Labas YA, Savitsky AP, Zaraisky AG, Markelov ML, Lukyanov SA. 1999. Fluorescent proteins from nonbioluminescent Anthozoa species. Nat Biotechnol 17:969–973.

Okabe M, Ikawa M, Kominami K, Nakanishi T, Nishimune Y. 1997. 'Green mice' as a source of ubiquitous green cells. FEBS Lett 407:313–319.

Shaner NC, Campbell RE, Steinbach PA, Giepmans BN, Palmer AE, Tsien RY. 2004. Improved monomeric red, orange and yellow fluorescent proteins derived from *Discosoma* sp. red fluorescent protein. Nat Biotechnol 22: 1567–1572.

Tran Cao HS, Hoffman RM, Bouvet M. 2009. The cyan fluorescent protein (CFP) transgenic mouse as a model for imaging pancreatic exocrine cells. J Pancreas 10:152–156.

Vintersten K, Monetti C, Gertsenstein M, Zhang P, Laszlo L, Biechele S, Nagy A. 2004. Mouse in red: Red fluorescent protein expression in mouse ES cells, embryos, and adult animals. Genesis 40:241–246.

Yamamoto N, Jiang P, Yang M, Xu M, Yamauchi K, Tsuchiya H, Tomita K, Wahl GM, Moossa AR, Hoffman RM. 2004. Cellular dynamics visualized in live cells *in vitro* and *in vivo* by differential dual-color nuclear-cytoplasmic fluorescent-protein expression. Cancer Res 64:4251–4256.

Yang M, Baranov E, Jiang P, Sun F-X, Li X-M, Li L, Hasegawa S, Bouvet M, Al-Tuwaijri M, Chishima T, Shimada H, Moossa AR, Penman S, Hoffman RM. 2000. Whole-body optical imaging of green fluorescent protein-expressing tumors and metastases. Proc Natl Acad Sci USA 97:1206–1211.

Yang M, Li L, Jiang P, Moossa AR, Penman S, Hoffman RM. 2003. Dual-color fluorescence imaging distinguishes tumor cells from induced host angiogenic vessels and stromal cells. Proc Natl Acad Sci USA 100:14259–14262.

Yang M, Reynoso J, Jiang P, Li L, Moossa AR, Hoffman RM. 2004. Transgenic nude mouse with ubiquitous green fluorescent protein expression as a host for human tumors. Cancer Res 64:8651–8656.

Yang M, Luiken G, Baranov E, Hoffman RM. 2005. Facile whole-body imaging of internal fluorescent tumors in mice with an LED flashlight. BioTechniques 39:170–172.

Yang M, Jiang P, Hoffman RM. 2007. Whole-body subcellular multicolor imaging of tumor–host interaction and drug response in real-time. Cancer Res 67:5195–5200.

Yang M, Reynoso J, Bouvet M, Hoffman RM. 2009. A transgenic red fluorescent protein-expressing nude mouse for color-coded imaging of the tumor microenvironment. J Cell Biochem 106:279–284.