

ORIGINAL ARTICLE

The Cyan Fluorescent Protein (CFP) Transgenic Mouse as a Model for Imaging Pancreatic Exocrine Cells

Hop S Tran Cao¹, Hiroaki Kimura^{1,3}, Sharmeela Kaushal², Cynthia S Snyder²,
Jose Reynoso³, Robert M Hoffman^{1,2,3}, Michael Bouvet^{1,2}

¹Department of Surgery and ²Moore's UCSD Cancer Center, University of California;
³AntiCancer, Inc. San Diego, CA, USA

ABSTRACT

Context The use of fluorescent proteins for *in vivo* imaging has opened many new areas of research. Among the important advances in the field have been the development of transgenic mice expressing various fluorescent proteins. **Objective** To report whole-body and organ-specific fluorescence imaging to characterize the transgenic cyan fluorescent protein mouse. **Design** Mice were imaged using two devices. Brightfield images were obtained with the OV100 Small Animal Imaging System (Olympus Corp., Tokyo, Japan). Fluorescence imaging was performed under the cyan fluorescent protein filter using the iBox Small Animal Imaging System (UVP, Upland, CA, USA). **Intervention** All animals were sacrificed immediately before imaging. They were imaged before and throughout multiple steps of a complete necropsy. Harvested organs were also imaged with both devices. Selected organs were then frozen and processed for histology, fluorescence microscopy, and H&E staining. Fluorescence microscopy was performed with an Olympus IMT-2 inverted fluorescence microscope. **Main outcome measure** Determination of fluorescence intensity of different organs. **Results** Surprisingly, we found that there is differential enhancement of fluorescence among organs; most notably, the pancreas stands out from the rest of the gastrointestinal tract, displaying the strongest fluorescence of all organs in the mouse. Fluorescence microscopy demonstrated that the cyan fluorescent protein fluorescence resided in the acinar cells of the pancreas and not the islet cells. **Conclusions** The cyan fluorescent protein mouse should lead to a deeper understanding of pancreatic function and pathology, including cancer.

INTRODUCTION

The use of fluorescent proteins for *in vivo* imaging has opened many new areas of research [1]. Among the important advances in the field have been the development of transgenic mice expressing various fluorescent proteins including green fluorescent protein (GFP) [2], red fluorescent protein (RFP) [3], and cyan fluorescent protein (CFP) [4]. Both the GFP and RFP transgenic mice express their respective fluorescent proteins in almost all organs and cell types [2, 3]. In the CFP mouse, CFP is driven by the beta-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. We investigated this differential fluorescence, and report our results here. 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 tumors, immune cells, stem cells and other cell types of different colors can be added to study their interaction.

MATERIALS AND METHODS

Transgenic Enhanced Cyan Fluorescent Protein (ECFP) Mice

Three transgenic CK6/ECFP mice were acquired from the Jackson Laboratory (Stock No. 003773; Bar Harbor, ME, USA) which are derived from founder lines first developed by the laboratory of Dr. Andras Nagy of the Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada [4]. These mice express CFP under the control of the chicken beta-actin promoter coupled with the cytomegalovirus immediate early enhancer. Three control C57BL/6J non-fluorescent mice were also studied. The mice were bred in the vivarium at AntiCancer, Inc. (San Diego, CA, USA).

Received October 7th, 2008 - Accepted January 5th, 2009

Key words Cyan Fluorescent Protein; Fluorescence; Mice, Transgenic; Pancreas

Abbreviations CFP: cyan fluorescent protein; GFP: green fluorescent protein; RFP: red fluorescent protein

Correspondence Michael Bouvet

Moore's UCSD Cancer Center, 3855 Health Sciences Drive, La Jolla, CA 92093-0987, USA

Phone: +1-858.822.6191; Fax: +1-858.822.6192

E-mail: mbouvet@ucsd.edu

Document URL <http://www.joplink.net/prev/200903/09.html>

Animal Care

Transgenic CK6/ECFP mice and control C57BL/6J non-fluorescent mice were maintained in a barrier facility on high efficiency particulate air (HEPA)-filtered racks. The animals were fed with autoclaved laboratory rodent diet (Teckland LM-485, Western Research Products, Orange, CA, USA). Necropsy and imaging were performed on 6-week-old female mice after they were euthanized with intramuscular injection of 0.05 mL of a solution of 50% ketamine, 38% xylazine, and 12% acepromazine maleate, followed by cervical dislocation.

Animal Necropsy

Six-week-old female mice were sacrificed in the manner reported above before performing a complete necropsy. Hair on their back was removed with Nair lotion (Church & Dwight Co., Inc., Princeton, NJ, USA). Necropsy was performed via a midline laparotomy incision that was extended along the sternal border to enter the thoracic cavity. The gastrointestinal tract was isolated and removed en bloc from the abdominal cavity, from the esophagus to the rectum. Subsequently, the spleen, pancreas, and stomach were separated from the small and large bowel. Next, all major organs were harvested individually.

Animal Imaging

Mice were imaged using two devices. Brightfield images were obtained with the OV100 Small Animal Imaging System (Olympus Corp., Tokyo, Japan) containing an MT-20 light source (Olympus Biosystems, Planegg, Germany) and DP70 CCD camera (Olympus Corp., Tokyo, Japan). Fluorescence imaging was performed under the CFP filter using the iBox Small Animal Imaging System (UVP, Upland, CA, USA) equipped with a BioChemi HR 500 CCD camera (UVP, Upland, CA, USA) and a BioLite automated multispectral light source (UVP, Upland, CA, USA) delivered to the dark imaging chamber via fiber optics. All animals were sacrificed immediately

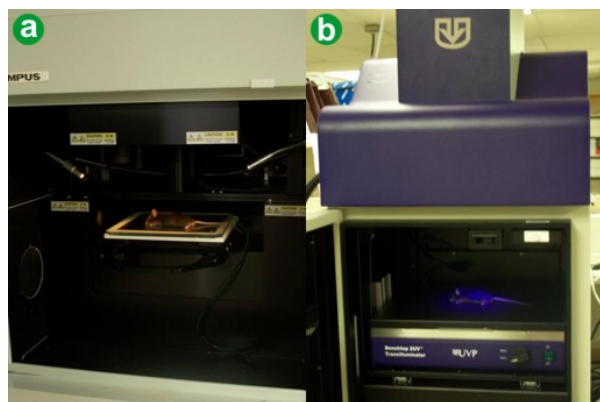


Figure 1. Imaging devices. **a.** Brightfield images of the CK6/ECFP mice and control C57BL/6J mice were obtained using the OV100 Small Animal Imaging System (Olympus Corp., Tokyo, Japan). **b.** Fluorescence imaging of the animals was performed with the iBox Small Animal Imaging System (UVP, Upland, CA, USA).

before imaging. They were imaged before and throughout multiple steps of a complete necropsy. Harvested organs were also imaged with both devices. Selected organs were then frozen and processed for histology, fluorescence microscopy, and H&E staining. Fluorescence microscopy was performed with an IMT-2 (Olympus Corp., Tokyo, Japan) inverted fluorescence microscope.

Data Processing

All images were analyzed with Image-J (National Institutes of Health, Bethesda, MD, USA), and the data obtained were tabulated and graphed using Microsoft Excel (Microsoft, Redmond, WA, USA).

ETHICS

All animal studies were conducted in accordance with the principles and procedures outlined in the NIH Guide for the Care and Use of Animals.

STATISTICS

Fluorescence intensity was expressed as arbitrary units and was reported as mean \pm SD. Fluorescence intensity was compared between the pancreas and the other organs by means of the paired Student-t test. The SPSS (version 13.0 for Windows; SPSS Inc., Chicago, IL, USA) was used for the statistical analysis and the significance level was chosen at a 0.05 two-tailed P-value.

RESULTS

The CK6/ECFP mice express the cyan fluorescent protein, which is discernable at the lower end of the visible light wavelength spectrum (370-475 nm), with the excitation peak and emission peak at 433 nm and 475 nm, respectively. All images were obtained in pairs, with a brightfield image obtained in the Olympus OV100 Small Animal Imaging System and a fluorescent image obtained in the UVP iBox under the CFP filter (Figure 1). Three CK6/ECFP mice were sacrificed for this analysis.

For the purpose of comparison, three control non-CFP mice were also sacrificed, necropsied, and imaged in the exact same manner and sequence. As expected, none of the control mice displayed any fluorescence.

The following results pertain to the CK6/ECFP mice only: **a)** whole body imaging revealed that the skin of the mouse displayed blue fluorescence, but the hair did not (Figure 2ab); **b)** however, upon initiation of the necropsy, to our surprise, we observed a marked difference in the level of fluorescence between organs in the CFP mouse (Figure 2cd); **c)** immediately, it was apparent that the peritoneum and the pancreas very strongly expressed the cyan fluorescent protein, along with the thigh muscles; **d)** the pancreas, in fact, stood out in stark contrast to the fairly dark background made up by the rest of the GI tract.

Proceeding with the necropsy, we first removed the entire gastrointestinal tract, including the spleen and pancreas, en bloc, from the esophagus to the rectum

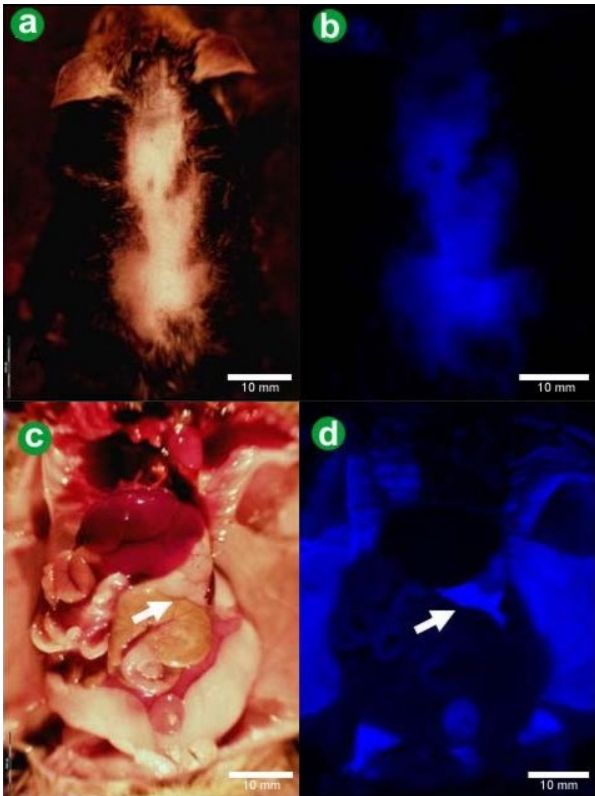


Figure 2. Brightfield and fluorescent images of the mouse before and during necropsy. The CK6/ECFP mouse was imaged with both brightfield and fluorescence filters throughout the study. The mouse's hair was removed, and whole body imaging shows that the skin, including that on the ears, expressed cyan fluorescence, while the hair did not (a. and b.). Necropsy of the mouse with exposure of the abdomen and thoracic cavities immediately allowed visualization of differential fluorescence among the organs, with the brightest signals coming from the pancreas (arrow), thigh muscles, peritoneum, and pleural covering (c. and d.).

(Figure 3). The GI tract was then imaged. As can be seen in Figure 3, the pancreas was obviously much more fluorescent than the rest of the GI tract. The GI tract was then separated into the spleen, stomach, pancreas, and bowel.

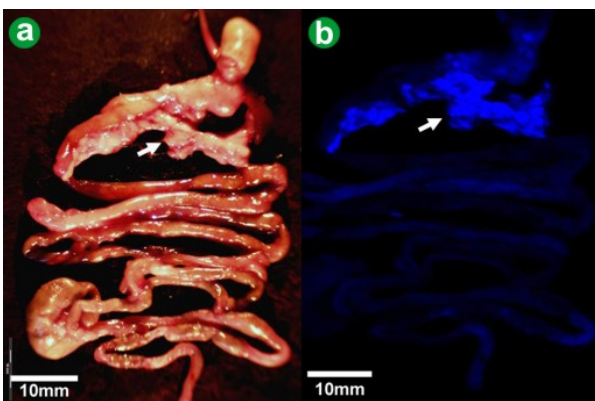


Figure 3. Gastrointestinal tract imaging. a. The gastrointestinal tract was isolated from the body, and imaged alone under brightfield. b. There is a sharp contrast in the level of fluorescence between the pancreas (arrow) and the rest of the GI tract when imaged under the cyan fluorescent protein filter.

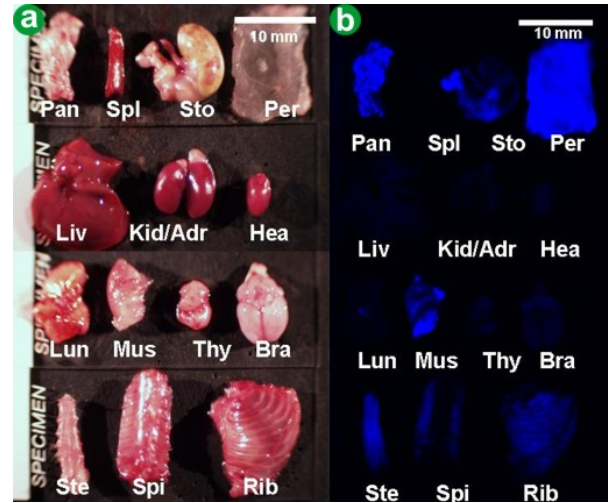


Figure 4. Single organ imaging. a. Complete necropsy of the mouse comprised harvesting of the organs. b. Differential expression of fluorescence is observed.

Bra: brain; Hea: heart; Kid/Adr: kidneys/adrenals; Liv: liver; Lun: lungs; Mus: muscle; Pan: pancreas; Per: peritoneum; Rib: ribcage; Spi: spine; Spl: spleen; Ste: sternum; Sto: stomach; Thy: thymus.

Next, we performed a complete necropsy, whereby we harvested the following additional organs for imaging: kidneys and adrenals, liver, heart, lungs, thymus, brain, peritoneum, thigh muscle, ribcage, sternum, and spine (Figure 4). Imaging with the CFP filter revealed differential fluorescence among the organs, with the peritoneum, pancreas, and muscle displaying much stronger signals than the rest of the organs, including the liver and lungs. Interestingly, the ribcage, spine, and sternum also displayed some degree of enhanced fluorescence. Likewise, fluorescence of the stomach was most notable along its greater curvature towards the duodenum.

Analysis of the images with the Image-J software allowed quantification of the fluorescence intensity, which was then converted into a bar graph (Figure 5) where a clear difference is discernable. The pancreas displays much greater fluorescence than the other organs of the GI tract although not reaching statistical significance due to the low number of animals and the

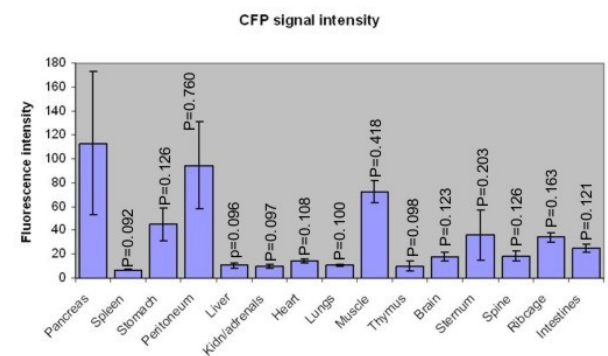


Figure 5. Cyan fluorescent protein fluorescence intensity by organ. Fluorescence intensity was quantified using Image-J software. The pancreas expressed the cyan fluorescent protein most strongly, particularly in comparison to the rest of the GI tract and the lungs. (Data are expressed in arbitrary units; mean±SD in 3 animals).

high variability observed among the different mice; its fluorescence intensity, on average, was 113.0 ± 60.3 arbitrary units. By comparison, the fluorescence intensity for the spleen was 7.1 ± 0.7 ($P=0.092$), the liver 10.8 ± 2.2 ($P=0.096$), the lungs 10.9 ± 0.8 ($P=0.100$), the stomach 45.1 ± 14.1 ($P=0.126$), and the intestines 25.5 ± 2.2 ($P=0.121$).

The next step in our analysis was to obtain microscopic fluorescence imaging and H&E staining of some select tissues. Using an inverted fluorescence microscope, we imaged the pancreas, along with the peritoneum and the muscle as well as the liver and the spleen (Figure 6). The findings from macro fluorescence imaging were confirmed at the microscopic level. Subsequently, we performed an H&E stain of the pancreatic tissue (Figure 7). The staining demonstrated that the acinar cells of the exocrine pancreas expressed blue fluorescence under fluorescence microscopy, while islet cells did not.

DISCUSSION

Fluorescent proteins offer the advantages of color-coding cells and proteins, permitting identification of the different cellular components of tissues as well as proteins, and organelles within cells. With fluorescent proteins, even single molecules can be imaged in cells [5]. Up to now, fluorescence imaging has been limited by a fairly narrow range of color choices, relying mainly on the GFP and RFP, which can, for example, be combined in a single cell to develop dual colored cells, with each protein coloring either the cytoplasm or the nucleus [6] or be used to color code cell-cell interaction [7, 8]. Although CFP has been available, its use has been limited due to its short wavelength. CFP

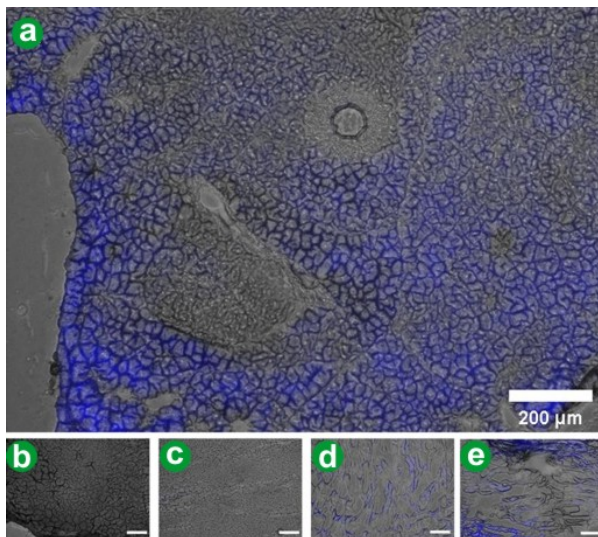


Figure 6. Fluorescence microscopy of selected tissue. Selected organs were processed for fluorescence microscopy with an IMT-2 (Olympus Corp., Tokyo, Japan) inverted fluorescence microscope. The microscopic images were obtained in parallel with whole organ images, clearly demonstrating cyan fluorescence in the pancreas (a.). By contrast, microscopic images of the liver (b.) and spleen (c.) failed to pick up any blue signal. The muscle (d.) and peritoneum (e.) displayed cyan fluorescence.

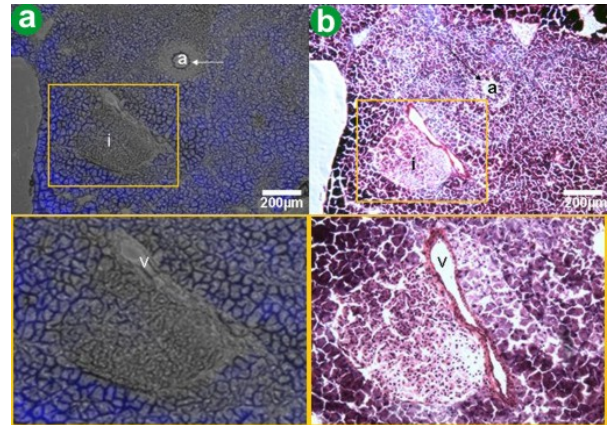


Figure 7. H&E staining of the pancreas. H&E staining of the pancreas was performed to identify which components were responsible for the cyan fluorescence. **a.** The image shows the microscopic fluorescence of pancreatic tissue. The adjoining picture is a blow-up of the inset box and focuses on an area of non-fluorescence. **b.** The H&E stain of this exact same area of the tissue demonstrates that the cyan fluorescence is due to the pancreas' exocrine parenchyma (acinar cells), and not from islet cells (i), seen in the blow-up box of the image. The vein (v), collapsed in this picture, and an artery (a) do not display cyan fluorescence either. Although not shown in this figure, the pancreatic duct cells did not display cyan fluorescence.

has been mostly directed to Förster resonance energy transfer (FRET) [9]. The UVP iBox imaging system now allows us to conveniently use this protein because it possesses a narrow range CFP filter that does not spill into the GFP or RFP wavelengths.

Hadjantonakis *et al.* first developed the ECFP (enhanced cyan fluorescent protein) transgenic mouse using the ECFP gene which is a spectrally-distinct color variant of wild type *Aequorea victoria* GFP [4]. These transgenic mice were bred to generate homozygotes and reported to express fluorescence in all cell types, except erythrocytes and adipocytes. As reported here, we observed that there is a markedly differential expression of the CFP among the organs of the CK6/ECFP mouse. Of particular interest, the pancreas was by far the most fluorescent of all intra-abdominal organs. The basis of the enhanced fluorescence of the pancreas is as yet unclear and should be the subject of further study. It is possible that the activity of the beta-actin promoter is highest in the pancreas. Nevertheless, our serendipitous finding should allow for the development of color-coded imaging studies of pancreatic function and pathology by breeding our model with currently available color-coded imaging models. For instance, Hara *et al.* [10] previously developed a model of dual-colored pancreas, where the beta-cells can be genetically engineered to express a fluorescent protein that allows their differentiation from the rest of the pancreas. The application of this technology to the CFP mouse would yield an ideal tool to specifically study differences in function and responses to pathology between the endocrine and exocrine components of the pancreas. Likewise, our laboratory has previously published models for color-coded imaging of the tumor

microenvironment and tumor-host interactions [7, 8, 11, 12]. In this context, the CFP mouse would add yet another color to our armamentarium for color-coding tumor-host interactions in the case of pancreatic cancer.

Acknowledgements 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.).

Conflict of interest The authors have no potential conflicts of interest

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