Bioluminescence and Fluorescence Based 3-D Imaging of Tumor in a Spontaneous Pancreatic Tumor Model

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Abstract
We have generated a spontaneous pancreatic tumor model in transgenic mouse where T-Antigen and luciferase expression is driven by the elastase 1 promoter in the acinar cells of the pancreas (EL1-luc/EL1-SV40-T-Antigen transgenic mouse). Development of pancreatic tumor lesions in this model can be monitored non-invasively through imaging and quantifying light emission which is proportional to the total luciferase expression, which, in turn, is proportional to the tumor burden. Due to the recent development of 3-D reconstruction algorithm for bioluminescence imaging, we were able not only to monitor luciferase expression in the pancreas, but also to obtain spatial resolution of the signal in a 3-D dimension through optical imaging. One of the critical features of the imaging system is its capability to perform 3-D bioluminescence imaging reconstruction using the Diffused Luminescent Imaging Tomography (DLIT) software. The 3-D bioluminescence imaging is performed through collecting two dimensional images with different optical filters in the range of 560-660 nm. Reconstruction with the DLIT software not only allows quantification of the signal, but also generates a spatial resolution of the signal in a 3-D dimensional space.

Introduction
Bioluminescence and fluorescence are currently used in pre-clinical research and drug development in multiple therapeutic areas, such as oncology, neurodegenerative diseases, and inflammation-mediated diseases. In order to facilitate effective uses of fluorescent labels, Caliper Life Sciences (Formerly Xenogen Corporation) developed a new imaging system, IVIS Spectrum. This new system is equipped with a set of 18 narrow band emission filters and 10 narrow band excitation filters. It also allows fluorescence imaging with transillumination, where the excitation light is delinated from below of the object observed, at 180 degrees angle to the camera installed at the top of the imaging space. The IVIS Spectrum retains all the bioluminescence imaging capabilities of its predecessor, the IVIS 200, thus enabling dual imaging on one single instrument.

Materials and Methods
EL1-Luc/EL1-TAg mice: EL1-Luc/EL1-TAg mouse model was established at Xenogen Corp. as previously reported (Scott L. AACR 97th Annual Meeting 2006). Conjugation of Xenogen Fluor®750 with Herceptin antibody: Commerically available Herceptin antibody (Genentech Inc.) was conjugated to the Xenogen Fluor®750 dye (Caliper Life Sciences, Inc.) by succinimidyl ester amine labeling according to manufactures' protocol. The Herceptin-dye conjugates were purified using a desalting column that comes with the Xenogen Fluor®750 Kiti.

Bioluminescence Imaging: In vivo imaging of luciferase activity from the spontaneous pancreatic tumor model was performed using the IVIS Spectrum imaging system. Mice were anesthetized with isoflurane and injected intraperitoneally with 150 mg/kg of luciferin. Ten minutes after the luciferin injection mice were imaged for 1.5 seconds on three sides, ventral, lateral, and right flank. Photons emitted from a specific region were quantified using Living Image 3.0 software and total ROI was calculated. 3-D bioluminescent imaging tomography was performed with Living Image 3.0 software using sequential images taken with filters ranging from 580 to 660 nm.

Fluorescence imaging: EL1-Luc/EL1-TAg transgenic mice were injected with 50 μg of Xenogen Fluor® 750 – Herceptin probe and imaged with the IVIS Spectrum at various time points with a combination of a 745 nm excitation filter and a 630 nm emission filter. Fluorescence Imaging Tomography (FIT) was performed with Living Image 3.0 software. Photon density map and source depth were calculated based on the measured signal strength on the surface of the animal, taking into account the spectral properties of luciferase signal when penetrating the animal tissue (absorption and dispersion). Reconstruction of the images using DLIT software created an image that allows evaluation of the bioluminescent signal in a 3-D dimension. The strength of the light source and its spatial location are shown on the 3-D images.

Results
Figure 1. 3D reconstruction of bioluminescence imaging results in mapping of bioluminescent hot spots in the pancreatic tissue. Excitation light originating underneath the stage trans-illuminates the subject at precise x-y-locations, enabling transillumination imaging. Transillumination-fluorescence imaging enables more sensitive detection and accurate quantification of deep sources and reduces the effects of autofluorescence.

Figure 2. EL1-Luc/EL1-TAg mouse as a spontaneous pancreatic adenocarcinoma model. A 220 base pair fragment of the rat elastase 1 promoter was used to confer acinar cell specific expression of both SV40 T-Antigen and firefly luciferase reporter in the transgenic mouse. Both transgenes were co-injected into the embryo of the IVIS mouse.

Figure 3. Monitoring of pancreatic tumor development in EL1-Luc/EL1-TAg mice. A) Imaging of the transgenic mice showed specific bioluminescence signal in the pancreas. The total amount of the signal was quantified using the IVIS Spectrum Imaging System. B) In vivo images and image of pancreas were taken using a set of 5 filters of 580 nm to 660 nm and open filter.

Summary
We have established a spontaneous pancreatic tumor mouse model where tumor development can be monitored through bioluminescence imaging. Diffused Luminescent Imaging Tomography (DLIT) analysis of bioluminescence imaging showed the signal in a 3-D dimension space and allowed tumor depth assessment. When the mice were injected with the Xenogen Fluor®750-Herceptin probe, we were able to observe weak fluorescence labeling of the pancreatic tumor. Accumulation of the probe in the liver and the bladder was also noted. We demonstrated the fluorescence imaging tomography (FIT) feature of the IVIS Spectrum through analysis of the fluorescent images with the Living Image 3.0 software. Reconstruction of the signals from the bladder was successful and provided information on the depth of the source of fluorescence in 3 dimensional space. Application of this novel method of 3-D reconstruction of the bioluminescent signal could improve visualization and quantification of deep sources and reduce the effects of autofluorescence.