In Vivo Fluorescence Imaging of Angiogenic Vessels in Liver Cancer Animal Model Using the iBox Explorer² Imaging Microscope

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Introduction

Angiogenesis is a vital component of both normal physiological processes and a number of disease states [1]. The angiogenic process is a complex multistep phenomenon involving many growth factors and interactions between different cell types. In angiogenesis, there are the two primary methods of vascular expansion by which nutrient supply to tissues is adjusted to match physiological demand, one being the sprouting of new capillaries from existing blood vessels and another being vasculogenesis, the de novo generation of blood vessels [2]. Accordingly, vasculogenesis is an integral and essential component of embryonic development, and angiogenesis accompanies organ growth and regeneration.

UVP's iBox® Explorer² Imaging Microscope system provides an effective way to image angiogenesis in vivo, aiding researchers in understanding and evaluating different conditions that stimulate or inhibit angiogenesis in preclinical models and human disease. The iBox Explorer² integrates a cooled, high resolution camera capable of detecting low fluorescent signals in angiogenesis cross a wide spectral range within living animals. Here, the iBox Explorer² is used to visualize angiogenesis vessels in the liver cancer animal model

Fig.1. iBox Explorer² Imaging Microscope

Materials and Methods

Vector, Cell Line and Animal

The Hepatocellular Carcinoma Cell Line HepG2 was transfected with GFP-tagged plasmid. Cell culture and selection of HepG2 cells stably expressing GFP were conducted. Six-week-old male nude mice (BALB/c-nu/nu) were acquired (Beijing HFK Bio-Technology Co. Ltd., China) and acclimated for one week. HepG2 cells stably expressing GFP were harvested and kept cold. Cells (1 × 10⁷ cells in 0.1 mL PBS) were subcutaneously injected into the nude mice.

Orthotropic Transplantation Tumor Model

Three weeks after HepG2-GFP inoculation, the nude mice with tumor growths were selected and used for orthotropic transplantation. Tumor tissues were harvested and cut into pieces under aseptic conditions. Tumor pieces were then orthotopically implanted into the liver lobes. The mice were then placed into cages and raised under Specific Pathogen Free (SPF) conditions.

Fluorescent Microscopy

One month after tumor transplantation, mice were anesthetized and images were captured with the iBox Explorer². The system was configured with GFP excitation and emission filter sets, 3.2MP OptiChemi 610 camera, motorized platform with integrated warming plate (set to 37°C), automated BioLite™ MultiSpectral Light Source and VisionWorks®LS Acquisition and Analysis software. The system's integrated magnification optics are variable between 0.17x and 16.5x, providing a wide selection of imaging magnification options for this application.

Results

Imaging of GFP-fluorescence signals in nude mice was achieved with the iBox Explorer² at different levels of magnifications, as discussed below (Fig. 2). The GFP signals were detected in multiple areas of the bodies of the mice, not only in the transplanted position, indicating that the tumor cells had spread within the mice.

Fig.2. In vivo GFP fluorescent imaging of mouse transplanted with HepG2-GFP driven tumor. The image shows the mouse one month after orthotropic transplanted tumor in liver lobes, with survival and migration of transplanted tumor cells.
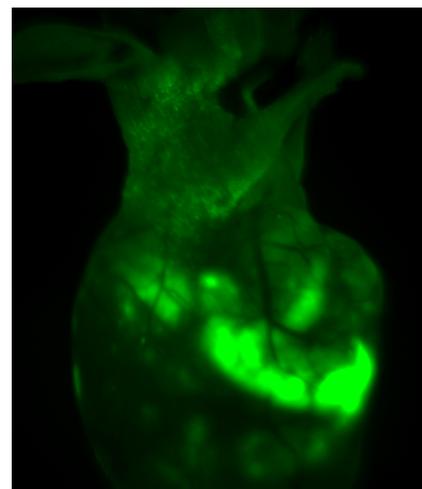
In addition, images of angiogenic vessels around tumor were captured. Figure 3 (A) illustrates the images of the GFP-fluorescent tumor signals at 0.5x magnification, which corresponds to a field of view of 30mm^2.

To further visualize the angiogenic vessels around tumors, higher magnification images were captured. Figures 3(B) to 3(D) correspond to the areas with the same names marked in Fig. 3(A). Vessels were then visualized at 1.66x and 2.5x magnifications, which correspond to fields of view of 9mm^2 and 6mm^2, respectively. Angiogenic vessels are marked in yellow lines.
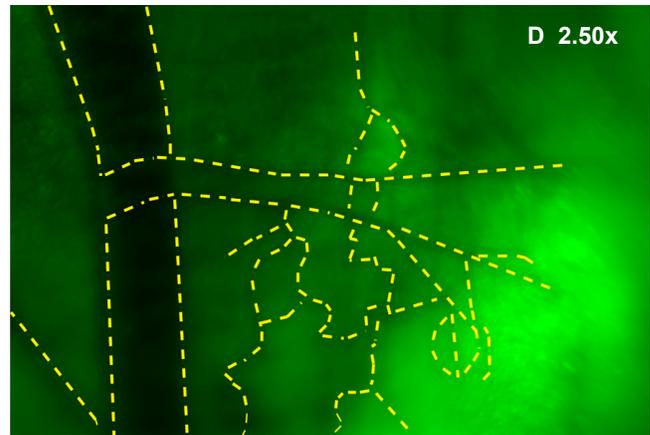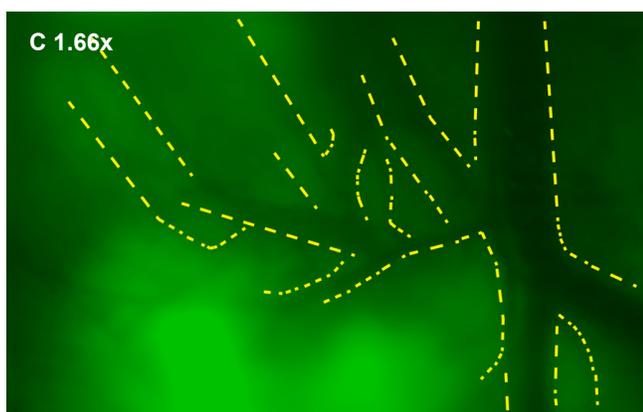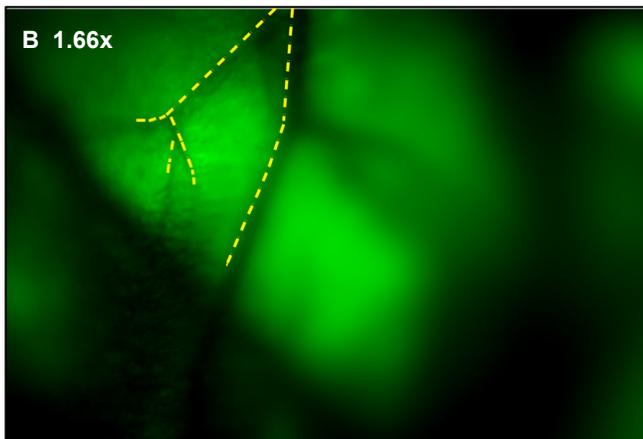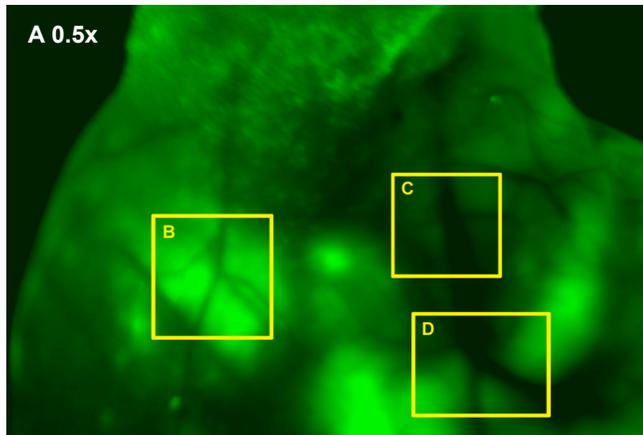

Fig. 3 (A) GFP-Fluorescent signals were imaged with iBox Explorer at 0.5x magnification corresponds to a field of view of 30mm^2 window. Areas with angiogenic vessels around tumor were marked. Figs. 3 (B, C, D) correspondent areas with angiogenic vessels were imaged at 1.66x or 2.50x magnifications, which correspond to fields of view of 9 mm2 or 6 mm2 respectively. Angiogenic vessels were marked with yellow dashes.

Conclusion

In this experiment, we captured strong fluorescence signals in mouse one month after tumor transplantation. The tumor cell was spread from the liver lobes to other tissues inside the mouse body. To analyze the angiogenic responses, angiogenic vessels around the tumor tissues were visualized with higher quality image, the numbers and lengths of the vessels were measured and recorded for further study. The experiment verified that the HepG2-GFP liver cancer mouse model is a reliable, reproducible system for angiogenesis study.

The iBox Explorer^2 Imaging Microscope is a valuable tool for preclinical in vivo angiogenesis imaging. The system enables high quality imaging technique to monitor the changes in vessels. This technique could be used for qualitative and quantitative assessment of various angiogenesis studies and monitoring antiangiogenic therapies, such as to understand the regulating pathways or to analyze angiogenic responses to specific perturbations of the angiogenc process. The system makes vessel imaging in mouse models easier, faster and more accurate.

References

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