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Visualizing Portal Vein Metastatic Trafficking to the Liver with Green Fluorescent Protein-expressing Tumor Cells

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Abstract. *Cell migration or trafficking is an integral aspect of cancer metastasis and is a target for development of novel antimetastatic therapy. Tumor cell trafficking has been a poorly understood phenomenon due to the inability to visualize the process. In this study, we visualized the trafficking of metastatic cells targeting the liver via the portal vein using green fluorescent protein (GFP)-expressing cancer cells. Within 72 h after transplantation of tumor cells, on the ascending colon in nude mice, metastasis was visualized ex vivo on a single-cell basis around the portal vein by GFP imaging. At this early time-point, a few cells were visualized trafficking to the liver via the portal vein. By post-implantation day-5, the caudate lobe of the liver was involved with trafficking metastatic cells. Metastasis around the portal vein increased more rapidly than those in other areas of the liver. By day-7 post-implantation, the right lateral lobe of the liver was involved with trafficking metastatic cells. By days-9 and -11, metastasis increased rapidly around the portal vein and then spread to other areas of the liver. These experiments demonstrate the critical role of the portal vein in metastasis to the liver.*

Early events in metastasis are poorly understood, because single cancer cells or micrometastases in tissues could not be visualized. Previously, cancer cells were transfected with the *Escherichia coli* β-galactosidase (lacZ) gene, which enables detection of micrometastases in tissue sections (1-3). However, lacZ does not allow direct visualization of cancer cells in live animals, which would enable understanding of real-time events. An approach to visualizing cancer cells *in vivo* is green fluorescent protein (GFP), which has been used to image cancer cells in live tissues *ex vivo* (4-6), in intact animals using time-lapse confocal laser scanning microscopy (CLSM) (7), intravital videomicroscopy (IVVM) (8, 9), and by whole-body imaging (10, 11).

In a recent study, IVVM was used to study the behavior of GFP-expressing rat colon cancer cells in livers of live rats after portal vein administration (12). It was shown that initial arrest of colon cancer cells in sinusoids of the liver was due to size restriction. Tumors cells divided intravascularly during the first 4 days (12). Adhesion of cancer cells to endothelial cells was not observed, and interactions were observed only between cancer cells and hepatocytes (12).

In the current study, we visualized the trafficking of GFP-expressing metastatic cells from the colon to the liver *via* the portal vein, after tumor transplantation on the colon.

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Key Words: Portal vein, metastatic trafficking, liver metastasis, green fluorescent protein.

Materials and Methods

Animals. Male athymic CD-1 nude mice, between 5 and 6 weeks of age, were used in this study. The animals were bred and maintained in a HEPA-filtered environment with cages, food and bedding sterilized by autoclaving. The breeding pairs were obtained from Charles River Laboratories (Wilmington, MA, USA). The animal diets were obtained from Harlan Teklad (Madison, WI, USA). 5.0% (w/v) Ampicillin (Sigma, St Louis, MO, USA) was added to the autoclaved drinking water.

Tumor implantation. A tumor fragment (1 mm^3) from the liver-metastatic, GFP-expressing AC3488 tumor (13) was implanted on the colon. After proper exposure of the colon following a lower midline abdominal incision, the serosa of the colon was removed and a 1 mm^3 tumor fragment was implanted. An 8-0 surgical suture was used to penetrate the small tumor piece and suture it on the wall of the intestine, then the intestine was returned to the abdominal cavity. The incision in the abdominal wall was closed with a 6-0 surgical suture in one layer. The animals were kept under isoflurane anesthesia during surgery. All procedures of the operation described above were performed with a 7x magnification microscope (Olympus). Animals were kept in a barrier facility under HEPA filtration.

Imaging of green fluorescent protein-expressing tumor cells. A Leica stereo fluorescence microscope model LZ2 equipped with a mercury lamp power supply was used to image the excised livers of

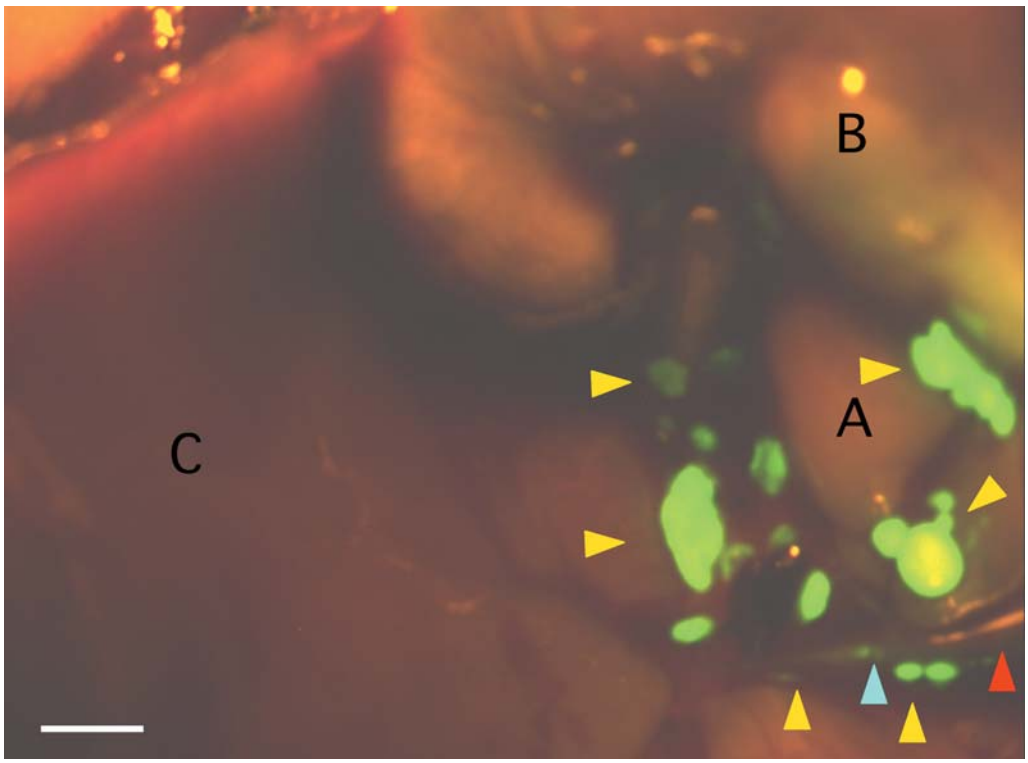

Figure 1. *At day-5 post-SOI, the caudal lobe of the liver was involved with trafficking metastatic cells. Red arrow: Portal vein. Yellow arrows: Liver metastasis. Blue arrow: Single metastatic cell was observed at the site where the portal vein enters the liver. (A) Caudal lobe of liver. (B) Stomach. (C) Left lateral lobe of liver. Metastasis around the portal vein increased more rapidly than those in other areas of the liver. (Bar = 0.7 mm)*

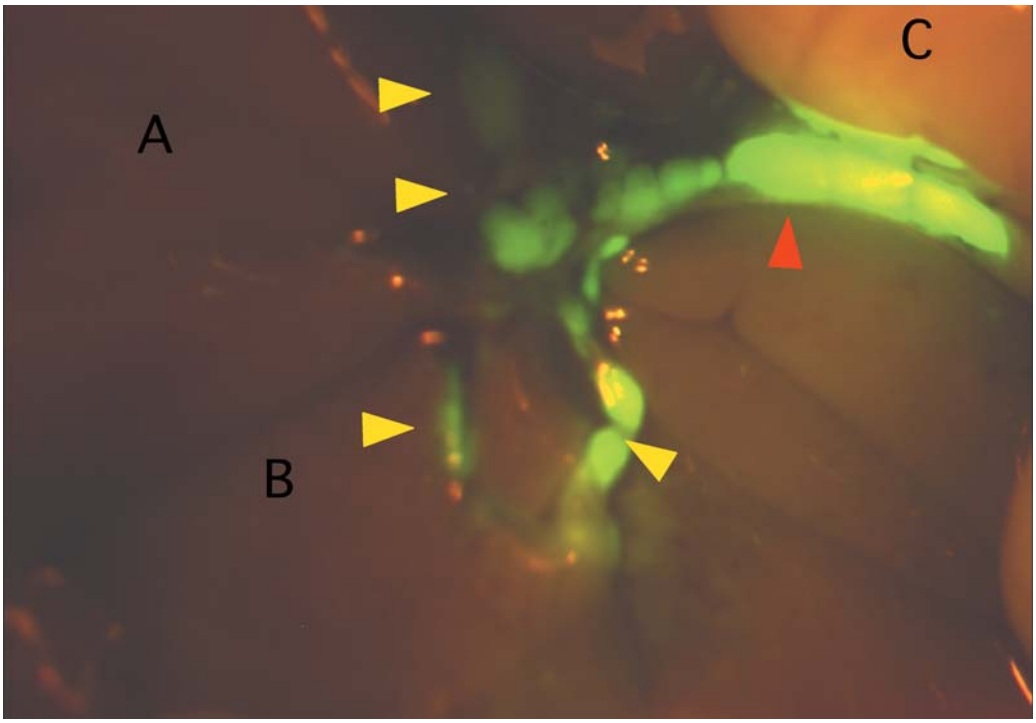

Figure 2. *At day-7 post-SOI, the trafficking metastatic cells had reached the left and right lateral lobe of the liver. Red arrow: Portal vein. Yellow arrow: Liver metastasis. (A) Left lateral lobe of liver. (B) Right lateral lobe of liver. (C) Stomach. (Original magnification x8).*

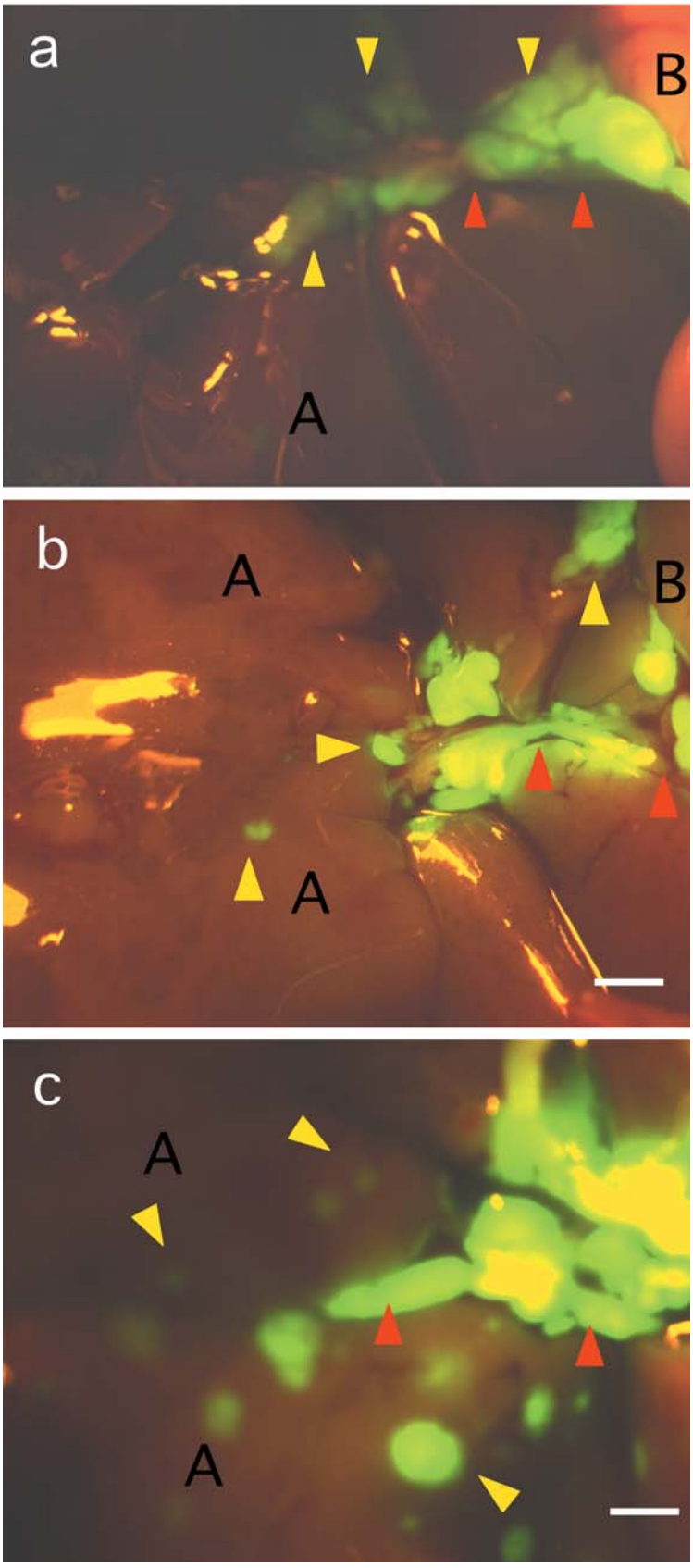

Figure 3. *At day-9 (a), day-11 (b) and day-15 (c) post-SOI, massive invasion of metastatic cells was visualized around the portal vein, which increased more rapidly than those in other areas of the liver. Red arrows: Portal vein. Yellow arrows: Liver metastasis. (A) Left lateral lobe of liver. (B) Stomach. (Original magnification x8). (Bar = 0.6 mm)*

the mice with metastasizing GFP-expressing tumor cells. Selective excitation of GFP was produced through a D425/60 band-pass filter and 470 DCXR dichroic mirror. Emitted fluorescence was collected through a long-pass filter GG475 (Chroma Technology, Brattleboro, VT, USA) on a Hamamatsu C5810 3-chip cooled color CCD camera (Hamamatsu Photonics Systems, Bridgewater, NJ, USA). Images were processed for contrast and brightness and analyzed with the help of Image Pro Plus 3.1 software (Media Cybernetics, Silver Spring, MD, USA). High resolution images were captured directly on the computer or continuously through video output on a high-resolution Sony VCR.

Results and Discussion

Seventy-two hours post-implantation of AC3488, two mice were sacrificed and the abdominal cavity was opened. GFP-expressing trafficking metastatic cells in the liver around the portal vein were imaged. At this time, metastasis was visualized on a single-cell basis around the portal vein by GFP-expression. One metastatic cell had already invaded the caudal lobe of the liver.

By day-5 post-implantation, a few malignant cells had already invaded the caudal lobe of the liver *via* the portal vein. Single GFP-expressing trafficking metastatic cells were observed at the site where the portal vein enters the liver (Figure 1).

At day-7 post-implantation, large numbers of metastatic cells invaded the left and right lateral lobes of the liver *via* the portal vein. At this stage, islands of metastases were found on the surface of the liver (Figure 2).

By days-9, -11, and -15 post-implantation, massive numbers of metastatic cells were visualized at the above sites. Metastasis increased more rapidly around the portal vein than in other areas of the liver, and metastatic cells arriving from the portal vein subsequently spread to other areas of the liver (Figure 3).

Clinically, the liver is the organ most often involved with metastasis. In this study, we visualized trafficking of metastatic cells in the liver *via* the portal vein using GFP-expressing cancer cells which preferentially metastasize to the liver.

The major advantage of GFP-expressing tumor cells is that even a single cell can be visualized in the liver. The brightness and high resolution of GFP enabled us to follow the trail of single malignant cells metastasizing to distal sites in the liver tissue, starting early after tumor implantation through late-stage tumor progression and metastasis. The use of GFP allowed the visualization of the origin and trafficking of liver metastasis at the single-cell level and demonstrated the critical role of the portal vein.

Future studies will visualize cancer cell movement from the portal vein to the liver parenchyma in living animals. These studies will be useful to screen novel agents that target the early metastatic process.

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