FASTTRACK
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Color-Coded Imaging of Breast Cancer Metastatic Niche Formation in Nude Mice

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ABSTRACT

We report here a color-coded imaging model in which metastatic niches in the lung and liver of breast cancer can be identified. The transgenic green fluorescent protein (GFP)-expressing nude mouse was used as the host. The GFP nude mouse expresses GFP in all organs. However, GFP expression is dim in the liver parenchymal cells. Mouse mammary tumor cells (MMT 060562) (MMT), expressing red fluorescent protein (RFP), were injected in the tail vein of GFP nude mice to produce experimental lung metastasis and in the spleen of GFP nude mice to establish a liver metastasis model. Niche formation in the lung and liver metastasis was observed using very high resolution imaging systems. In the lung, GFP host-mouse cells accumulated around as few as a single MMT-RFP cell. In addition, GFP host cells were observed to form circle-shaped niches in the lung even without RFP cancer cells, which was possibly a niche in which future metastasis could be formed. In the liver, as with the lung, GFP host cells could form circle-shaped niches. Liver and lung metastases were removed surgically and cultured in vitro. MMT-RFP cells and GFP host cells resembling cancer-associated fibroblasts (CAFs) were observed interacting, suggesting that CAFs could serve as a metastatic niche. J. Cell. Biochem. 116: 2730–2734, 2015.

KEY WORDS: BREAST CANCER; METASTATIS; NICHE; CANCER-ASSOCIATED FIBROBLASTS; LUNG; LIVER; GFP; RFP; COLOR-CODED IMAGING

Fluorescent proteins of multiple colors were used to develop a color-coded imaging model of the tumor microenvironment (TME). Red fluorescent protein (RFP), cyan fluorescent protein (CFP), and green fluorescent protein (GFP) transgenic nude mice were used as hosts for cancer cells expressing a different color fluorescent proteins [Yang et al., 2003, 2004, 2009; Hoffman and Yang, 2006b; Suetsugu et al., 2012a].

Egeblad et al. [2008] showed that stromal cells had higher motility in the periphery of the tumor than at the center using color-coding imaging. Tumors contain macrophages, fibroblasts, dendritic cells, and lymphocytes in the TME [Egeblad et al., 2008]. Cancer-associated fibroblasts (CAFs) may stimulate proliferation of cancer cells, as well as invasion and angiogenesis [Kalluri and Zeisberg, 2006; Gaggioli et al., 2007; Pietras et al., 2008; Erez et al., 2010]. We have also demonstrated, using color-coded imaging, that tumors acquired stroma over time, including during passage and that the acquired stroma grew along with the tumor [Suetsugu et al., 2012b,c,d]. We previously showed that CAFs are recruited by metastasis which stimulate their growth [Suetsugu et al., 2011, 2012b]. We also reported that stromal cells are necessary for metastasis [Bouvet et al., 2006].

Lyden's group [Kaplan et al., 2005] pioneered the concept of the metastastic niche based on the color-coded models of the TME we previously developed [Yang et al., 2003, 2004, 2009; Hoffman and Yang, 2006b; Suetsugu et al., 2012a].

The present study images the formation of metastatic niches for breast cancer in the lung and liver.

MATERIALS AND METHODS

CELL CULTURE

The mouse mammary tumor cell line MMT 060562 (MMT) was transformed to express RFP [Hoffman and Yang, 2006a,b,c]. RPMI 1,640 with 10% fetal calf serum (FCS) were used for cell culture at 37°C and 5% CO_2.

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GFP TRANSGENIC MICE

C57/B6-GFP mice [Okabe et al., 1997] were originally obtained from the Research Institute for Microbial Diseases (Osaka University, Osaka, Japan). GFP is expressed in these mice driven by the chicken β-actin promoter and cytomegalovirus enhancer. The C57B6-GFP mice were crossed with nude (nu/nu) mice to obtain transgenic GFP nude mice [Yang et al., 2004] in the present study. Mouse studies were conducted under NIH Assurance number A3873-1.

COLOR-CODED BREAST CANCER LUNG METASTASIS MODEL

MMT-RFP cells (2×10^6) were injected in the tail vein of GFP nude mice under anesthesia induced by a ketamine mixture (10 ml ketamine HCl, 7.6 ml xylazine, 2.4 ml acepromazine maleate, and 10 ml H_2O).

COLOR-CODED BREAST CANCER LIVER METASTASIS MODEL

MMT-RFP cells (2×10^6) were injected in the spleen of GFP nude mice during open laparotomy to form experimental liver metastases under ketamine anesthesia (described above).

IN VIVO IMAGING

The OV100 Small Animal Imaging System (Olympus Corp., Tokyo, Japan) [Yamauchi et al., 2006], the IV100 scanning laser imaging system (Olympus) [Yang et al., 2007], the FV1000 confocal microscope (Olympus) [Uchugonova et al., 2013], and the MVX10 long-working distance fluorescence microscope (Olympus) [Kimura et al., 2010] were used for imaging [Hoffman, 2005; Hoffman and Yang, 2006a,b,c]. Organs were removed for ex vivo imaging.

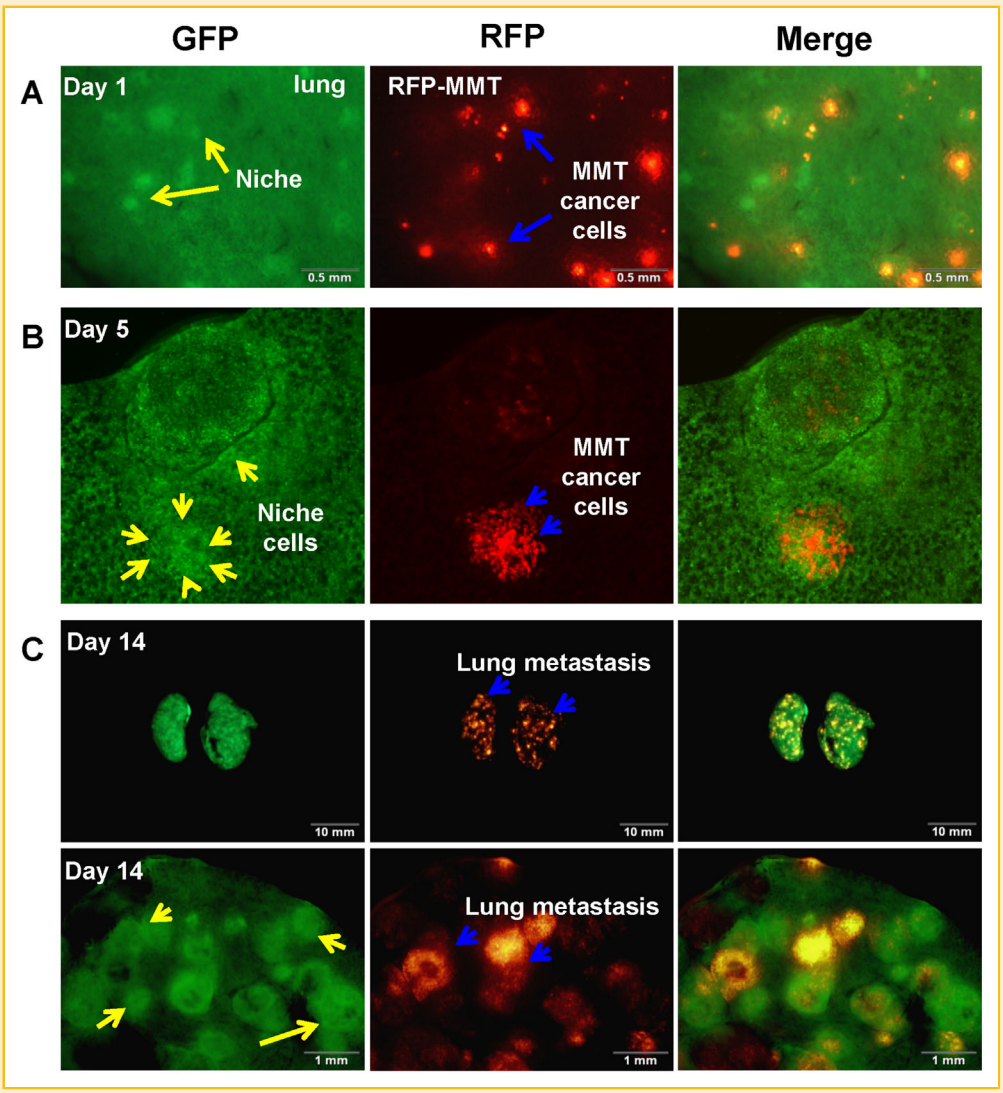

Fig. 1. Imaging niche formation around MMT-RFP cells in the lung Mouse mammary tumor cells (MMT 060562), expressing red fluorescent protein (RFP) (MMT-RFP), were injected in the tail vein of GFP nude mice to establish a lung metastasis model. A: Imaging of MMT-RFP cancer cells in the TME on day 1 after cancer-cell injection. Yellow arrows indicate GFP stromal cells forming a niche for the experimental metastases. Blue arrows indicate MMT cells. (Bar = 0.5 mm). B: Imaging of MMT-RFP cells and GFP stromal cells on day 5 after cancer-cell injection. Yellow arrows indicate the niche. Blue arrows indicate MMT cells. C: Imaging of MMT-RFP cells and stromal cells on day 14 after cancer-cell injection. Upper panels show the entire lungs. Blue arrows indicate lung metastasis. (Bar = 10 mm) Lower panels show high magnification images of the upper panels. Blue arrows indicate lung metastasis. Yellow arrows indicate circle-shaped niches in the lung. (Bar = 1 mm).

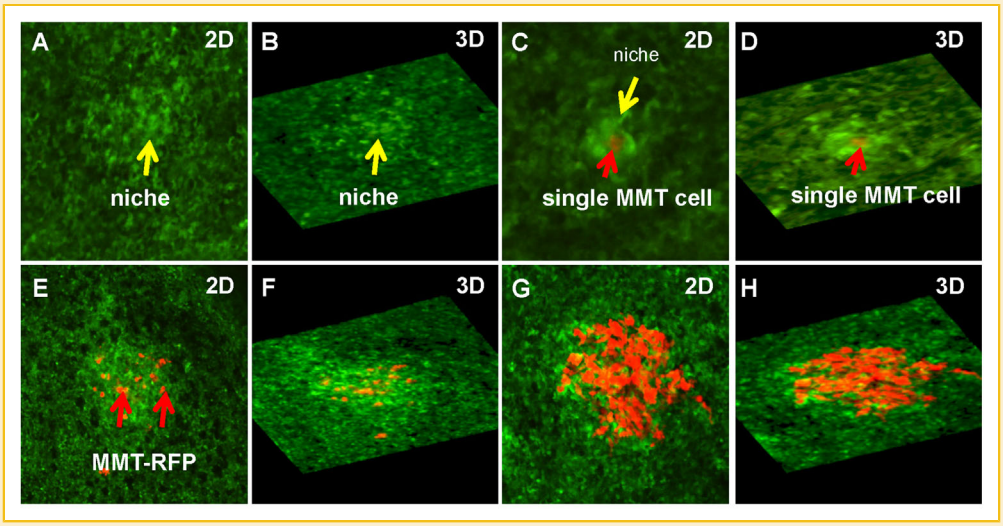

Fig. 2. Confocal 2D and 3D imaging of niche formation around MMT-RFP cells in the lung. Images were taken with a FV1000 scanning laser confocal microscope. Yellow arrows indicate metastatic niches. Red arrows indicates MMT-RFP cells. GFP host-mouse cells accumulated around a single MMT-RFP cell (C, D) and also seemed to form a niche before arrival of metastatic cancer cells (A, B). Eventually, many MMT-RFP cells accumulated in the niche formed by GFP-host cells (E–H).

RESULTS AND DISCUSSION

IMAGING NICHE FORMATION AROUND BREAST CANCER LUNG METASTASIS

MMT-RFP cells were injected in the tail vein of transgenic GFP nude mice. At day-1, GFP host cells appeared to form niches in the lungs around the injected MMT-RFP cancer cells. By day-5, the niches became more extensive as the experimental breast cancer colonies grew.

On day 14 after cancer-cell injection, extensive GFP fluorescent host cell formation was observed around the experimental lung metastasis. High-magnification imaging visualized GFP fluorescent host cells surrounding the metastatic colonies. The lung metastasis became enriched with GFP-expressing host cells (Figs. 1 and 2). GFP host cells were observed to form circle-shaped niches in the lung with one or even without MMT-RFP cells, which was possibly a niche in which future metastasis could be formed (Fig. 2). Eventually, very large numbers of MMT-RFP cells accumulated in the niche (Figs. 1 and 2).

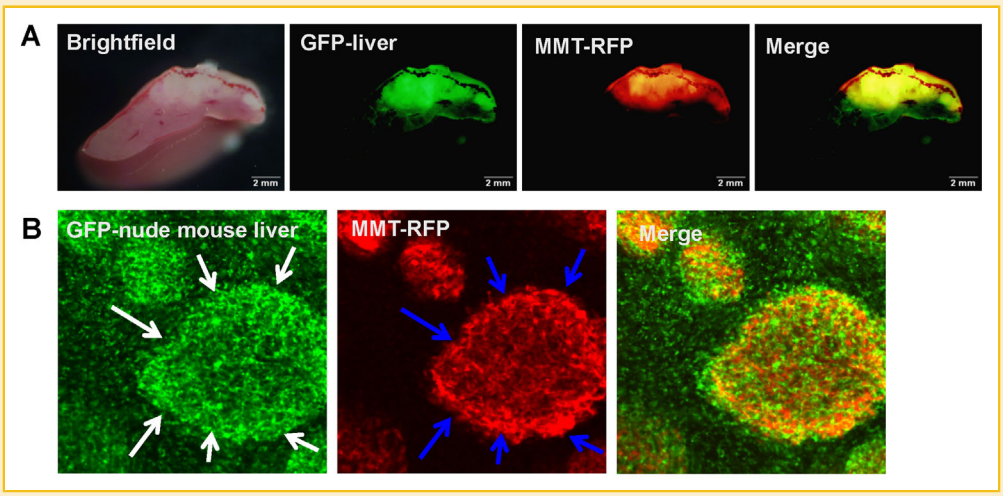

Fig. 3. Imaging MMT-RFP metastatic niche formation in the liver. MMT-RFP cells were injected in the spleen of GFP nude mice to establish a liver metastasis model. A: Images of MMT-RFP cells and GFP host cells in the liver on day 14 after cancer-cell injection. B: High-magnification images of liver metastasis. Images were taken with an IV100 microscope. White arrows indicate liver metastatic niches. Blue arrows indicate MMT-RFP cells.

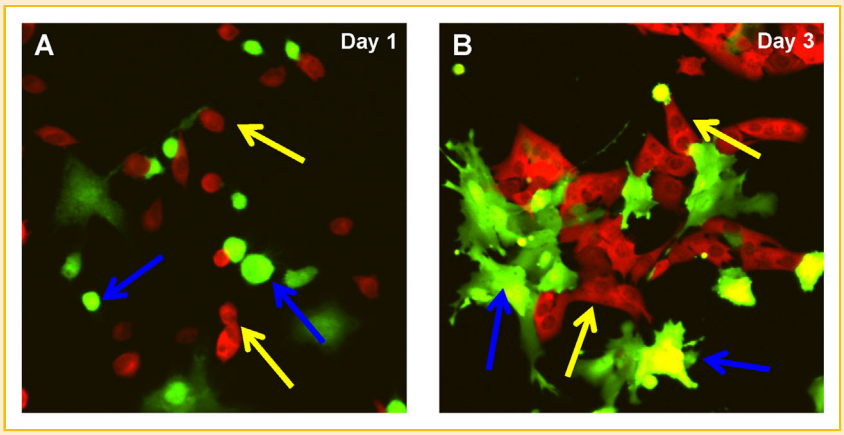

Fig. 4. Imaging of GFP host and metastatic MMT-RFP cells interactions. Liver metastases were removed surgically and cultured in vitro. On day1, round GFP cells and MMT-RFP cells were observed in culture. On day 3, stretched GFP fibroblast-like cells and MMT-RFP cells were observed interacting. Yellow arrows indicate MMT-RFP cells. Blue arrows indicate host GFP fibroblast-like cells.

IMAGING NICHE FORMATION AROUND BREAST CANCER LIVER METASTASIS

The spleen of GFP nude mice was used for injection of MMT-RFP cells. By day 14, the experimental liver metastasis were enriched with GFP-expressing host cells. The GFP-expressing hosts cells were recruited by the round liver metastasis and appeared to form a niche for the metastatic colonies to grow (Fig. 3). The metastases were highly enriched with GFP cells compared to the rest of the liver.

IMAGING THE INTIMATE INTERACTION OF BREAST CANCER AND HOST CELLS

Liver metastases were removed surgically and cultured in vitro. On day 1, round GFP cells and MMT-RFP cancer cells were observed interacting in culture. On day 3, stretched GFP fibroblasts-like and MMT-RFP cancer cells were observed (Fig. 4).

DISCUSSION

The GFP nude mouse is an especially useful model for imaging the TME in the liver since the liver parenchymal cells only dimly express GFP. Therefore, GFP host stromal cells recruited by metastases are readily visualized. The present report demonstrates that MMT metastatic colonies in the lung and liver are coordinately formed by host and cancer cells. The host cells appear to form a niche for the metastatic cancer cells to proliferate. We previously observed that host cells are necessary for metastasis to occur [Bouvet et al., 2006]. The intimate relationship we have observed between the host niche cells and the cancer cells in the metastatic colonies suggest host cell-cancer cell interactions promote metastatic colony growth. Culturing the metastatic colonies and confocal microscopy showed that host cells within the colony are fibroblast-like. Future experiments will determine the role of cancer-associated fibroblasts in metastatic niche formation.

The real-time color-coded tumor-stroma imaging model described in this report will be very useful for identifying host cells which form niches to enhance metastatic growth. The present report suggests cancer-associated fibroblasts participate in niche formation.

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AUTHOR CONTRIBUTIONS

AS and RMH conceived and designed the experiments. AS performed the experiments. AS, MM, YH, MS, SS, HM, MB, and RMH analyzed the data. RMH contributed reagents/materials/analysis tools. AS and RMH wrote the article.

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SUPPORTING INFORMATION

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