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Labeling the Stroma of a Patient-Derived Orthotopic Xenograft (PDOX) Mouse Model of Undifferentiated Pleomorphic Soft-Tissue Sarcoma With Red Fluorescent Protein for Rapid Non-Invasive Imaging for Drug Screening

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ABSTRACT

Our laboratory pioneered patient-derived orthotopic xenograft (PDOX) mouse models using surgical orthotopic implantation (SOI). PDOX models are patient-like, in contrast to the ectopic subcutaneous-transplant cancer models. In the present study, we demonstrate that an undifferentiated pleomorphic soft-tissue sarcoma (UPS-STS) PDOX model acquired bright RFP-expressing stroma through one passage in red fluorescent protein (RFP) transgenic mice, which upon passage to non-colored nude mice was non-invasively imageable. A PDOX nude mouse model of UPS-STS was established in the biceps femoris of nude mice. After the tumors grew to a diameter of 10 mm, the tumors were subsequently passaged to RFP transgenic mice, and after tumor growth were then passaged to non-transgenic nude mice. Tumors were divided into small fragments and transplanted in the biceps femoris at each passage. The OV100 Small Animal Fluorescence Imaging System and FV1000 laser scanning confocal microscope were used to image RFP fluorescence in the UPS-STS PDOX models. UPS-STS PDOX tumors, previously grown in RFP transgenic nude mice for only one passage, had very bright fluorescence and after passage to non-transgenic nude mice maintained the bright fluorescence and were non-invasively imageable. FV1000 confocal imaging revealed diffusely distributed bright RFP stromal cells in the PDOX tumor, both in RFP transgenic mice and after passage to non-transgenic mice. These results demonstrate a powerful method to make the PDOX UPS-STS model brightly fluorescent for non-invasive imaging, as well as for confocal microscopy of individual stromal cells associated with the tumor. The RFP-labeled UPS PDOX has the potential to rapidly screen for novel effective agents for individual patients, including stroma-targeting drugs, whereby the stromal cells are a visual target. J. Cell. Biochem. 118: 361–365, 2017.

KEY WORDS: SARCOMA; SOFT TISSUE; UNDIFFERENTIATED; PLEOMORPHIC; PDOX; RED FLUORESCENT PROTEIN; TRANSGENIC NUDE MOUSE; STROMA; IMAGING; NONINVASIVE; CONFOCAL

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Orthotopic implantation of intact tumor tissue in appropriate mouse models can result in metastasis resembling the clinical pattern, unlike subcutaneous transplantation [Hoffman, 2015]. We have established patient-derived orthotopic xenograft (PDOX) nude-mouse models of the following patient cancers: colon [Fu et al., 1991]; pancreas [Fu et al., 1992; Hiroshima et al., 2014a,b,c,d, 2015a, b; Yano et al., 2015]; lung [Wang et al., 1992]; ovarian [Fu and Hoffman, 1993]; breast [Fu et al., 1993]; stomach [Furukawa et al., 1993]; mesothelioma [Astoul et al., 1996]; soft tissue sarcoma [Hiroshima et al., 2015c,d; Murakami et al., 2016a]; follicular dendritic-cell sarcoma [Kiyuna et al., 2016]; and Ewing's sarcoma [Murakami et al., 2016b].

We previously developed an imageable PDOX model for pancreatic cancer [Suetsugu et al., 2012a]. Pancreatic cancer PDOX models were serially passaged to transgenic green fluorescent protein (GFP), red fluorescent protein (RFP), and cyan fluorescent protein (CFP), respectively. The PDOX acquired and maintained fluorescent stroma for each mouse. The PDOX, which acquired GFP-expressing stroma, subsequently metastasized to the liver and formed peritoneal metastases which both maintained the stroma from the primary tumor [Suetsugu et al., 2012b]. PDOX that acquired GFP and RFP stroma, were then orthotopically passaged to non-transgenic nude mice which enabled noninvasive longitudinal imaging as the tumor progressed in non-transgenic nude mice [Suetsugu et al., 2012c].

Previously, an undifferentiated pleiomorphic soft-tissue sarcoma (UPS) was established by our laboratory in the biceps muscle of nude mice and was eradicated with tumor-targeting *Salmonella typhimurium* A1-R followed by doxorubicin (DOX) [Murakami et al., 2016a].

The present report demonstrates a non-invasive imageable UPS PDOX model, established with brightly labeled RFP-expressing sarcoma through only a single passage through an RFP transgenic nude mouse.

MATERIALS AND METHODS

MICE

Athymic *nu/nu* nude mice and transgenic RFP expressing athymic *nu/nu* mice (AntiCancer Inc., San Diego, CA), 4–6 weeks old, were used in this study [Yang et al., 2009]. All surgical procedures and imaging were performed with an AntiCancer Institutional Animal Care and Use Committee (IACUC)-protocol specifically approved for this study, and in accordance with the principles and procedures outlined in the National Institute of Health Guide for the Care and Use of Animals under Assurance Number A3873-1. In order to minimize any suffering of the animals, the use of anesthesia and analgesics were used for all surgical experiments. Animals were anesthetized by subcutaneous injection of a 0.02 ml solution of 20 mg/kg ketamine, 15.2 mg/kg xylazine, and 0.48 mg/kg acepromazine maleate. The response of animals during surgery was monitored to ensure adequate depth of anesthesia. The animals were observed on a daily basis humanely sacrificed by CO_2 inhalation, if they met the following humane endpoint criteria: severe tumor burden (more than 20 mm in diameter), prostration, significant body weight loss, difficulty breathing, rotational motion, and body temperature drop. Animals were housed in a barrier facility on a high efficiency particulate arrestance (HEPA)-filtered rack under standard conditions of 12-h light/dark cycles. The animals were fed an autoclaved laboratory rodent diet.

PATIENT-DERIVED TUMOR

The patient was diagnosed with UPS of the left thigh and had the tumor resected. The disease recurred locally a few months later and the patient was treated with radiotherapy, and subsequent re-resection by F.C.E., Division of Surgical Oncology, University of California, Los Angeles (UCLA). Written informed consent was obtained from the patient as part of a UCLA Institutional Review Board-approved protocol.

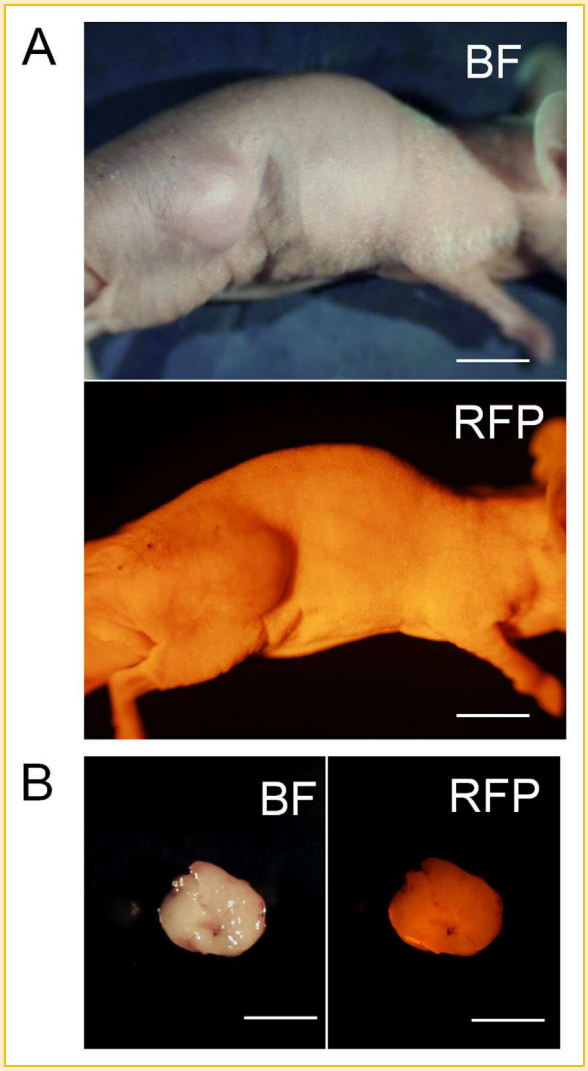

Fig. 1. Establishment of a fluorescent undifferentiated pleomorphic soft-tissue sarcoma (UPS) PDOX model. (A) A growing PDOX tumor in the right biceps femoris of an RFP-expressing nude mouse. (B) Images of the resected tumor derived from the RFP-expressing nude mouse. Scale bars: 10 mm (A and B). RFP, red fluorescent protein; BF, brightfield.

ESTABLISHMENT OF A FLUORESCENT PDOX MODEL OF UPS BY SURGICAL ORTHOTOPIC IMPLANTATION (SOI)

A fresh sample of UPS was obtained immediately after patient surgery. The tumor was transported immediately to the laboratory at AntiCancer, Inc. on wet ice. The tumor was cut into fragments and implanted subcutaneously in nude mice. Two weeks later, the subcutaneously implanted tumors grew to more than 10 mm in diameter. The subcutaneously grown tumors were then harvested and cut into small fragments. A 5-mm skin incision was made on the high thigh into the biceps femoris, under ketamine anesthesia, which was split, and a single tumor fragment was implanted orthotopically in this space to establish PDOX model in RFP transgenic nude mice. The wound was closed with a 6-0 nylon suture (Ethilon, Ethicon, Inc., NJ). When the PDOX tumors in the RFP transgenic mice grew to more than 10 mm in diameter, the tumor was harvested and passaged to non-transgenic nude mice with the same method described above.

IMAGING OF THE FLUORESCENT UPS PDOX MODEL

Fluorescence imaging of the macroscopic tumor was performed with the OV100 Small Animal Imaging System (Olympus, Tokyo, Japan) [Yamauchi et al., 2006]. When PDOX tumors reached 10 mm in diameter, the tumors were resected and cut into 1.5 mm-thick slices. RFP-expressing tumor stroma in the specimens was observed with the FV1000 confocal laser microscope (Olympus) [Uchugonova et al., 2011].

RESULTS AND DISCUSSION

ESTABLISHMENT OF A FLUORESCENT UPS PDOX MODEL

The PDOX tumor grew orthotopically in the right biceps femoris of an RFP expressing nude mouse. The PDOX tumor in the RFP-expressing nude mouse became brightly fluorescent (Fig. 1A). The resected PDOX tumor from the RFP transgenic nude mouse was also brightly fluorescent (Fig. 1B). The RFP expressing PDOX tumor was passaged orthotopically to the right biceps femoris of non-fluorescent nude mice to establish a non-invasively imageable PDOX model (Fig. 2). The bright RFP-expressing UPS PDOX was readily visible without opening the skin (Fig. 2A-left). The UPS PDOX tumor in the non-transgenic nude mouse was then exposed by a skin flap. Tumor RFP expression was very bright when imaged through the skin-flap (Fig. 2A-right). The resected RFP-expressing PDOX was also very bright (Fig. 2B).

CONFOCAL IMAGING OF THE STROMA IN THE UPS PDOX MODEL

FV1000 confocal laser microscope imaging showed RFP-expressing tumor stroma of PDOX tumors derived from RFP-expressing nude mice and after passage to a non-transgenic nude mouse. The PDOX model tumors had bright and diffusely distributed RFP expressing stroma (Fig. 3). Fibroblast-like cells and lymphocyte-like cells from the PDOX tumors were observed (Fig. 3).

UPS-STS PDOX tumors, previously grown in RFP transgenic nude mice for only one passage, had very bright fluorescence and after

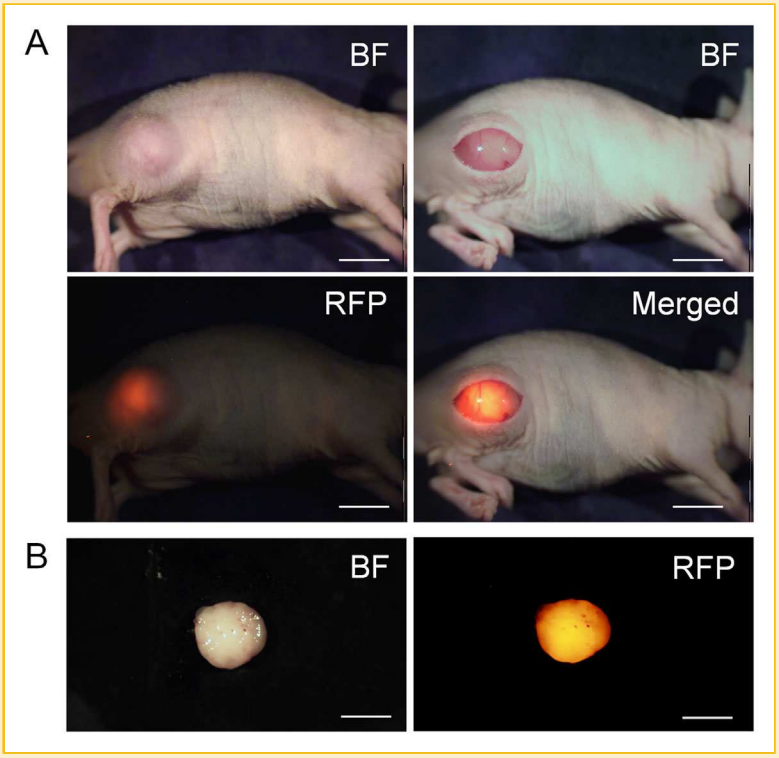

Fig. 2. (A) Non-invasive imaging of a RFP-expressing UPS PDOX tumor in a non-transgenic nude mouse, previously grown in an RFP-transgenic mouse (left). Imaging of the RFP-expressing UPS PDOX after a skin-flap was raised (right). (B) Images of the resected PDOX tumor derived from a non-transgenic nude mouse model. Tumor RFP expression was still strongly detectable. Imaging with the OV100. Scale bars: 10 mm (A and B). RFP, red fluorescent protein; BF, brightfield.

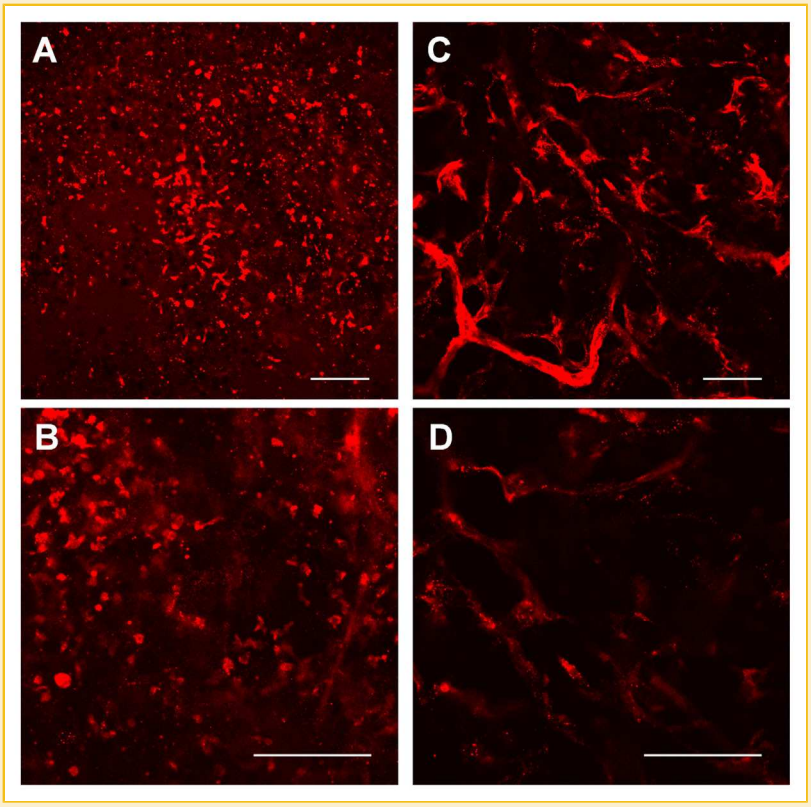

Fig. 3. Confocal fluorescence microscopy of individual stromal cells of the UPS PDOX. FV1000 confocal laser microscopy imaged RFP-expressing stroma of PDOX tumors grown in the RFP-expressing nude mouse (A and B) and after passage and growth in non-transgenic nude mice (C and D). Bright and diffusely-distributed RFP expressing stroma could be seen both in the UPS PDOX grown in the RFP transgenic nude mouse and after passage and growth in a non-transgenic nude mouse. Scale bars: 100 μm.

passage to non-transgenic nude mice were non-invasively imageable due to maintenance of the RFP-expressing stroma within the PDOX. FV1000 confocal imaging demonstrated diffusely distributed bright RFP stromal cells in the PDOX tumor, both in RFP transgenic nude mice and after passage to non-transgenic nude mice. These results demonstrate a powerful method to make the PDOX UPS-STS model brightly fluorescent for non-invasive imaging, as well as for confocal microscopy of individual stromal cells associated with the tumor.

CONCLUSIONS

Implantation of human tumors in nude mice changed the paradigm of cancer research [Rygaard and Povlsen, 1969] and orthotopic implantation of tumor in nude mice [Sordat and Wang, 1984; Fidler, 1990; Fu et al., 1991; Hoffman, 2015] enabled patient-like models to be established for individualized patient therapy. In the present study, we established an imageable PDOX model of UPS-STS brightly labeled with RFP-expressing stroma in non-transgenic nude mice passaged from RFP transgenic nude mice. Only one passage in an RFP nude mouse was necessary to brightly and stably label the stroma of the UPS PDOX. The RFP-labeled UPS PDOX has the potential to rapidly screen for novel effective agents for individual patients, including stroma-targeting drugs, whereby the stromal cells are a visual target.

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