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Journal

Journal of Cellular Biochemistry, 118(10)

ISSN

0730-2312

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Publication Date

2017-10-01

DOI

10.1002/jcb.25991

Peer reviewed

HHS Public Access

Author manuscript

J Cell Biochem. Author manuscript; available in PMC 2018 October 01.

Published in final edited form as:

J Cell Biochem. 2017 October ; 118(10): 3367–3371. doi:10.1002/jcb.25991.

Analysis of stroma labeling during multiple passage of a sarcoma imageable patient-derived orthotopic xenograft (iPDOX) in red fluorescent protein transgenic nude mice

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Abstract

A patient-derived orthotopic xenograft (PDOX) model of undifferentiated pleomorphic sarcoma (UPS) was previously established that acquired red fluorescent protein (RFP)-expressing stroma by growth in an RFP transgenic nude mouse. In the present study, an imageable PDOX model (iPDOX) of UPS was established by orthotopic implantation in the biceps femoris of transgenic RFP nude mice. After the tumors grew to a diameter of 10 mm, they were harvested and the brightest portion of the tumors were subsequently orthotopically transplanted to both RFP and non-colored nude mice. The UPS PDOX tumor was again transplanted to RFP transgenic and non-colored nude mice and finally a 3rd passage was made in the same manner. Five UPS tumors from each passage in both RFP and non-colored mouse models were harvested. The FV1000 confocal microscope was used to visualize and quantitate the RFP area of the resected tumors. The average percent fluorescent area in the first passage of RFP mice was 34 ± 22%; in the second passage, 34 ± 20%; and 36 ± 11% in the third passage of RFP transgenic nude mice. The average tumor RFP area in the first passage from RFP mice to non-colored mice was 20±7%; in the second passage, 28±11%; in the third passage was 27±13%. The present results demonstrate the extensive and stable acquisition of stroma by the UPS-tumor growing orthotopically in transgenic RFP nude mice (iPDOX). This model can be used for screening for effective drugs for individual patients and drug discovery.

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Keywords

Soft tissue sarcoma; patient-derived orthotopic xenograft (PDOX); red fluorescent protein (RFP); transgenic nude mouse; passaging; stroma labeling; imaging

Introduction

Kiyuna, T., Murakami, T., Tome, Y., Igarashi, K., Kawaguchi, K., Russell, T., Eckhardt, M.A., Crompton, J., Singh, A., Bernthal, N., Bukata, S., Federman, N., Kanaya, F., Eilber, F.C., and Hoffman, R.M. Labeling the stroma of a patient-derived orthotopic xenograft (PDOX) mouse models of undifferentiated pleomorphic soft-tissue sarcoma with red fluorescent protein for rapid non-invasive drug screening. J. Cell. Biochem. **118**, 361–365, 2017.

Appropriate mouse models of cancer that accurately represent patient cancer behavior, most importantly, metastasis [Hoffman, 2015]. Therefore, our laboratory has established patient-derived orthotopic xenograft (PDOX) nude-mouse models of the major cancers: colon [Fu et al., 1991; Metildi et al., 2014; Hiroshima et al., 2014a]; pancreas [Fu et al., 1992; Hiroshima et al., 2014b,c,d,e, 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,2015d; Murakami et al., 2016a, b; Kiyuna et al., 2017]; follicular dendritic-cell sarcoma [Kiyuna et al., 2016]; Ewing's sarcoma [Murakami et al., 2016a, b] and melanoma [Yamamoto et al., 2016; Kawaguchi et al., 2016a, b].

We previously developed an imageable PDOX model for pancreatic cancer [Suetsugu et al., 2012a] by passaging the tumor in fluorescent-protein-expressing mice. The pancreatic PDOX acquired and maintained fluorescent stroma for each mouse [Suetsugu et al., 2012b]. The labeled PDOX tumors were then orthotopically passaged to non-transgenic non-colored nude mice where they could be non-invasively imaged [Suetsugu et al., 2012c].

We previously established an undifferentiated pleomorphic sarcoma (UPS) PDOX model [Murakami et al., 2016] and subsequently showed it acquired bright red fluorescent protein (RFP)-expressing stroma through one passage in RFP transgenic mice. Upon passage to non-colored nude mice, the RFP-expressing UPS PDOX was non-invasively imageable [Kiyuna et al., 2017].

The present report describes the extent of acquisition of RFP stroma in the UPS iPDOX after multiple serial passages in RFP mice.

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. Animals were housed in a barrier facility on a high efficiency particulate arrestance (HEPA)-filtered rack under

standard conditions of 12-hour light/dark cycles. The animals were fed an autoclaved laboratory rodent diet. 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 and humanely sacrificed by CO_2 inhalation and 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.

Patient-derived tumor

The patient was previously diagnosed with UPS of the thigh received tumor resection. The disease recurred locally a few months later and was surgical resected by F.C.E., Division of Surgical Oncology, University of California, Los Angeles (UCLA). Written informed consent was obtained from the patient as a part of UCLA Institutional Review Board approved protocol [Murakami et al., 2016a].

Establishment of a fluorescent PDOX model of UPS by surgical orthotopic implantation (SOI)

The UPS was previously established in nude mice orthotopically in the biceps femoris [Murakami et al., 2016a]. Subsequently we established an imageable PDOX (iPDOX) of UPS in RFP transgenic nude mice [Kiyuna et al., 2017]. In the present study, the iPDOX tumors in the RFP transgenic mouse grew to 10 mm in diameter, the tumors were harvested and the brightest portion of the tumors were subsequently serially transplanted orthotopically to both RFP and non-colored nude mice for a total of 3 times (Fig. 1).

Frozen section imaging of the fluorescent UPS PDOX model

The OV100 Small Animal Fluorescence Imaging System (Olympus, Tokyo, Japan) [Yamauchi et al., 2006] was used to visualize RFP-expression in the UPS stroma by whole-body imaging. Five UPS tumors at each passage of both RFP and non-colored nude mice were harvested at each passage. The center portion of each tumor was selected for frozen sectioning. The frozen blocks of tumors were sliced at 10 μm thickness. Three different images of field per one slide were observed with the FV1000 confocal laser microscope (Olympus) [Uchugonova et al., 2011]. The percentage of RFP-expressing tumor stroma area was analyzed with Image J v1.440 (National Institutes of Health).

Statistical analysis

Statistical analysis was performed with JMP pro version 12. The percentage of RFP-expressing tumor stroma area was expressed as mean ± SD. The two-tailed Student’s t-test

was used to compare continuous variables between 3 groups. A P value of < 0.05 was considered statistically significant.

Results and Discussion

Whole body imaging of the UPS iPDOX model during multiple passages

The tumor grew orthotopically in the right biceps femoris of RFP-expressing nude mice (Fig. 2). The iPDOX tumors had sufficient shows RFP expression to be imaged through the skin in the first passage in RFP and non-colored nude mice (Fig. 2A). The tumors harvested from RFP mice and after subsequent passage in non-colored nude mice had acquired sufficient bright RFP stroma to be very brightly imaged.

Confocal imaging of the RFP stroma in the UPS iPDOX model during multiple passages

Images obtained with the FV1000 confocal laser microscope showed RFP-expressing stroma in the UPS tumors harvested from each passage of RFP and non-colored nude mice (Fig 3). The images were obtained from frozen sections of the central tumor area containing bright tumor stroma (Fig 3). Tumors from each passage of RFP and non-colored mice contained bright red fluorescent stroma.

Quantitation of RFP-expressing tumor stroma area at each passage of the UPS iPDOX

The average tumor RFP area in the first passage of RFP mice was 34 ± 22%; in the second passage was 34 ± 20%; and in the third passage was 36 ± 11%. The average tumor RFP area in the first passage from RFP mice to non-colored mice was 20±7%; in the second passage was 28±11%; and in the third passage was 27±13%. The average tumor RFP area after all passages in RFP mice and to non-colored nude mice did not significantly differ.

The present results demonstrate the extensive and stable acquisition of stroma by the UPS-tumor growing orthotopically in transgenic RFP nude mice (iPDOX). This is shown by the bright stroma in the UPS iPDOX after a single passage in RFP mice, which is not diluted after passage in non-colored nude mice. Serial passage of UPS iPDOX did not increase the extent of acquired RFP-expressing stroma which was probably maximal in the first passage in RFP nude mice.

Acknowledgments

This study was supported in part by National Cancer Institute grant CA213649.

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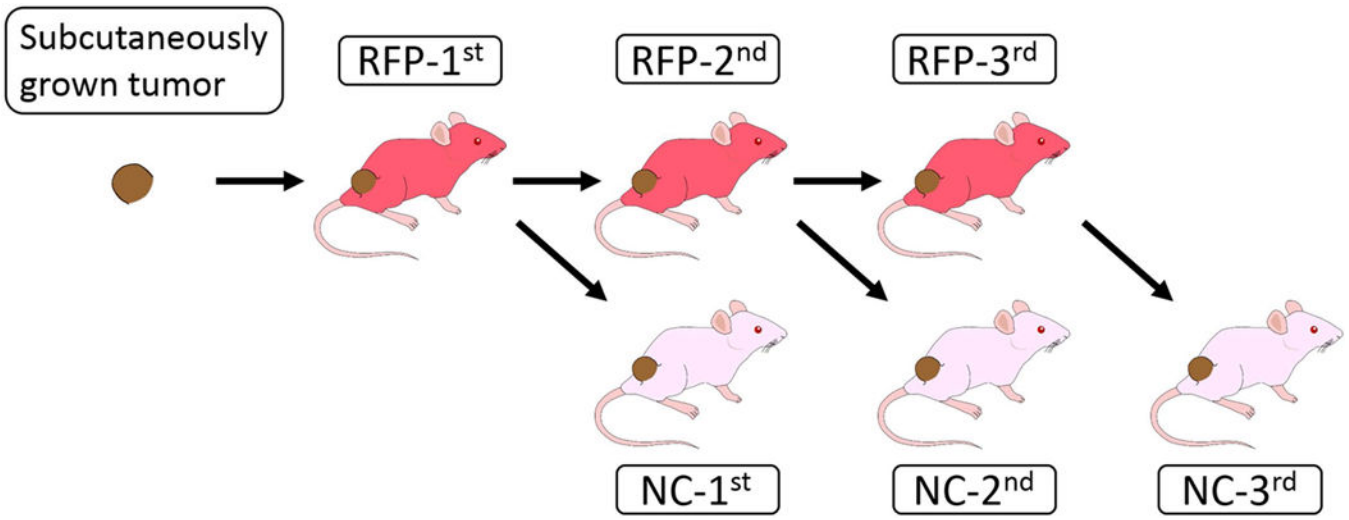

Figure 1. Experimental design

N = 5 tumor harvested at each passage. NC = non-colored.

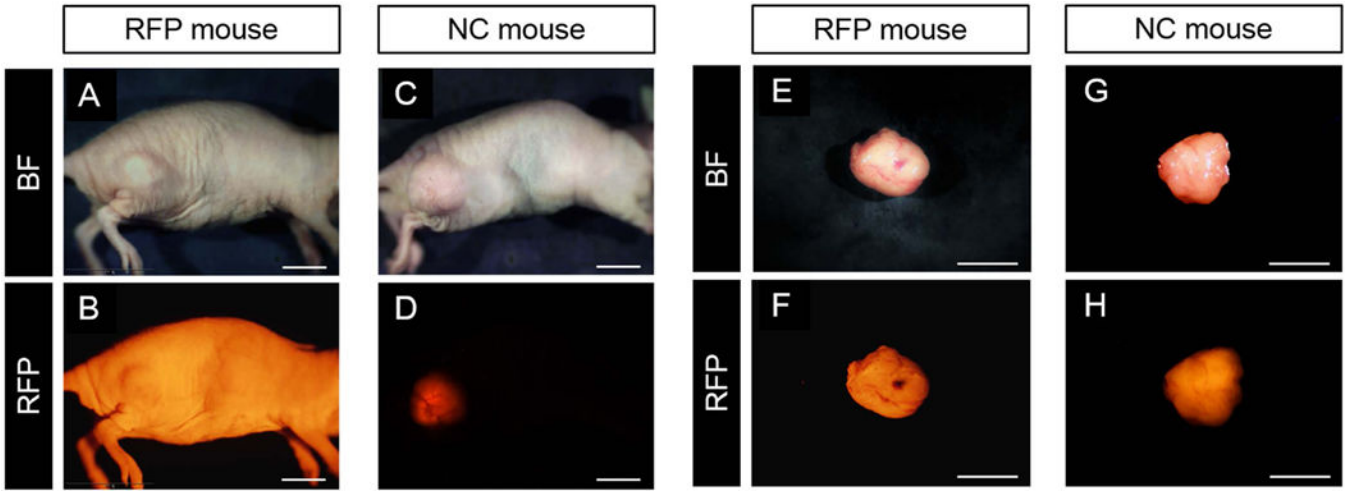

Figure 2. Imaging of UPS iPDOX tumor in first passage in an RFP transgenic mouse and after first passage to a non-colored nude mouse

Left panels: Noninvasive image of the UPS iPDOX in transgenic RFP and non-colored nude mice. Right panel: Excised tumors from RFP and non-colored nude mice. The UPS tumor was passaged from an RFP to a non-colored nude mouse.

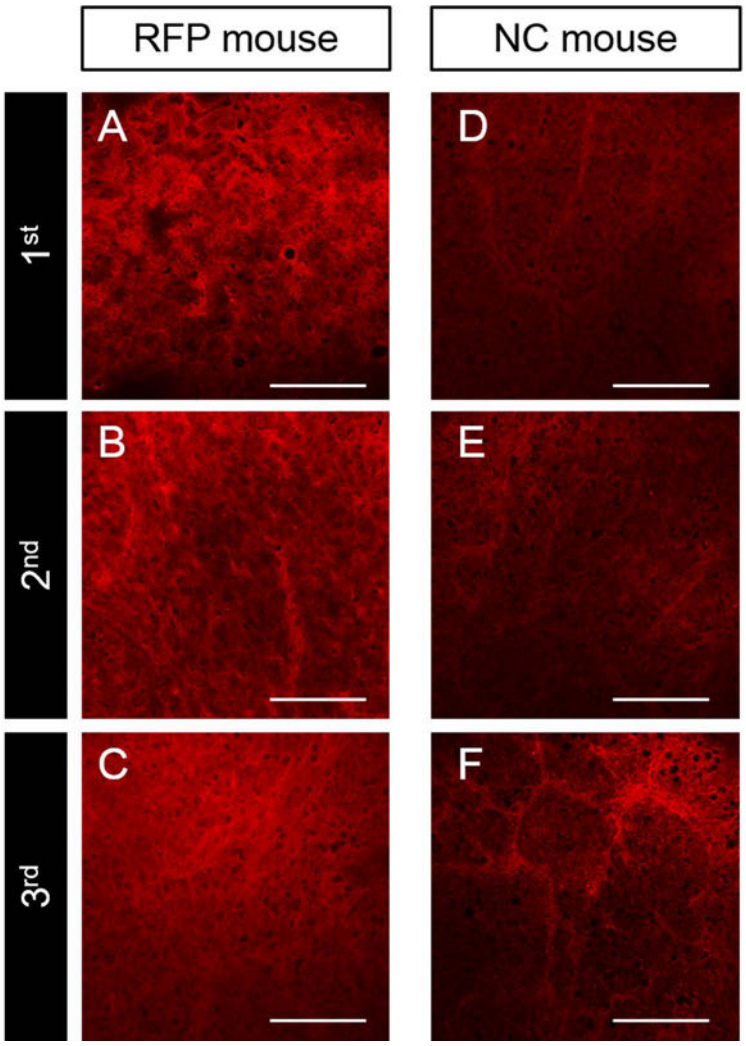

Figure 3.

Frozen section images of PDOX tumors with RFP-expressing stroma visualized with the FV1000 confocal laser microscope from 3 successive passages as shown in Figure 1.

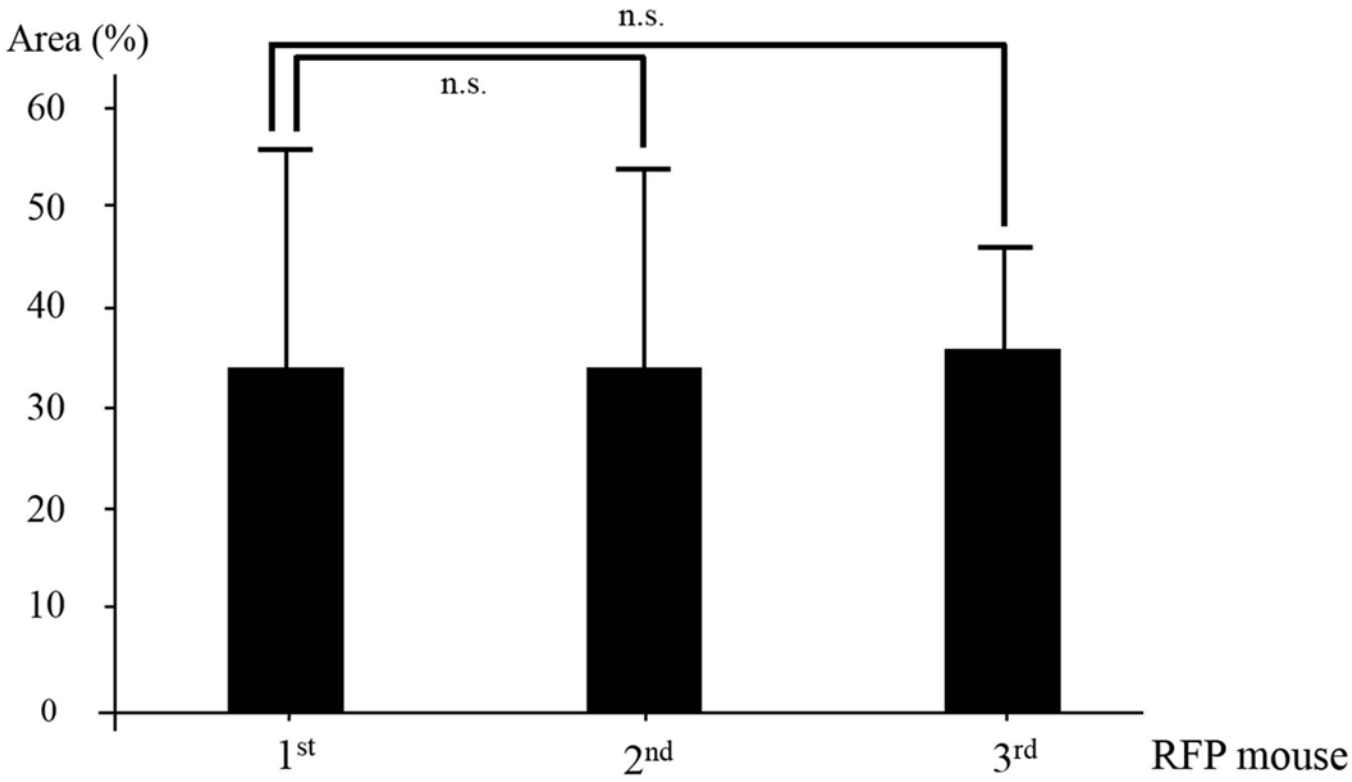

Figure 4. Bar graphs indicate percentage fluorescent area in UPS iPDOX growing in RFP transgenic nude mice at 1st, 2nd, and 3rd passage

Error bars: +1 SD. n.s.: not significant. RFP: red fluorescent protein

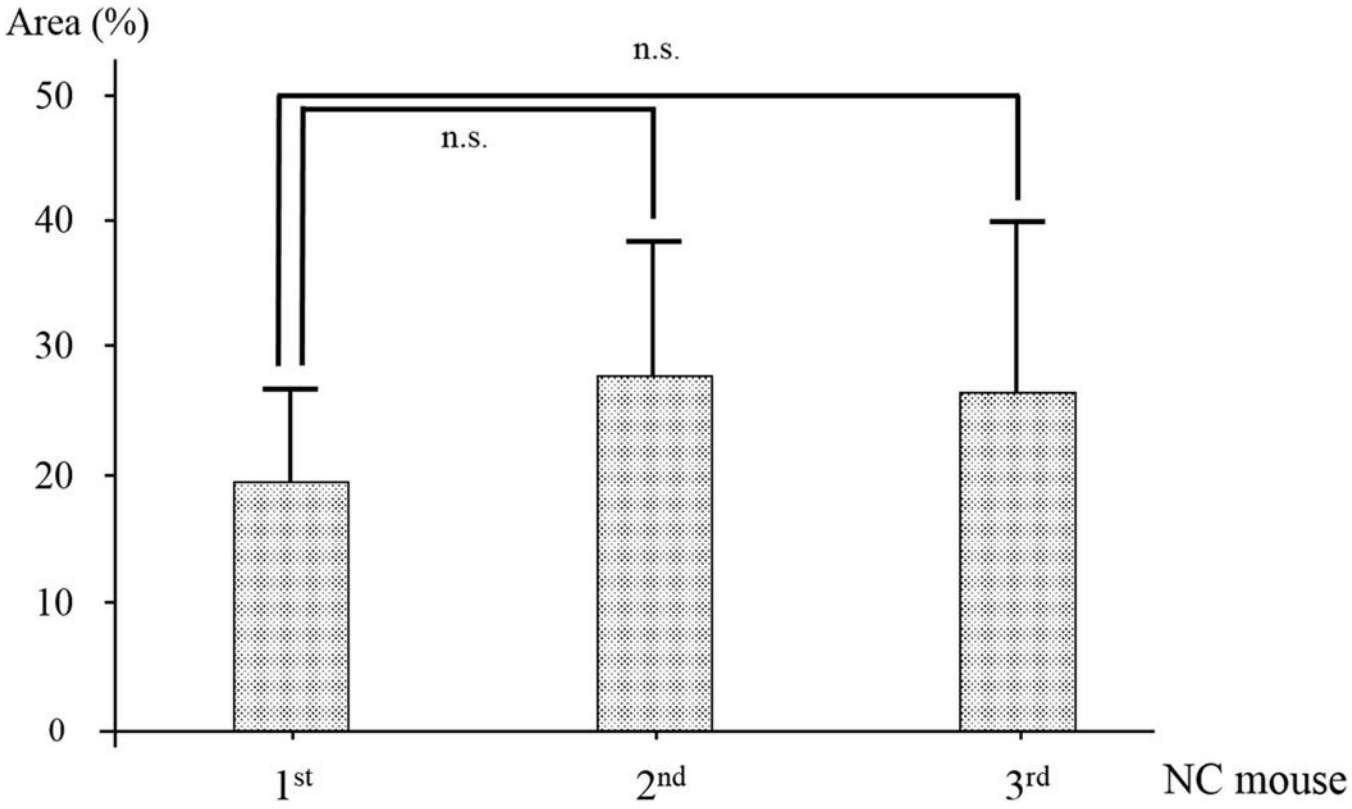

Figure 5. Bar graph indicate percentage fluorescent area in UPS iPDOX growing in non-colored nude mice at 1st, 2nd, and 3rd passage

Error bars: +1 SD. n.s.: not significant. NC: non-colored.