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Detection of Metastasis in a Patient-derived Orthotopic Xenograft (PDOX) Model of Undifferentiated Pleomorphic Sarcoma with Red Fluorescent Protein

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Abstract. *Background/Aim: Undifferentiated pleomorphic sarcoma (UPS) is a common soft tissue sarcoma and highly recalcitrant. We have previously developed patient-derived orthotopic xenograft (PDOX) mouse models of UPS and other major sarcoma types. Unlike PDOX models of other cancer types, it has been difficult to demonstrate metastasis in the sarcoma PDOX models. Materials and Methods: To visualize metastasis at high resolution in the UPS PDOX model, established tumor fragments were implanted in transgenic nude mice expressing red fluorescent protein (RFP) for one passage. The tumors acquired RFP-expressing stroma from transgenic host. UPS tumor with RFP stromal cells were harvested and implanted orthotopically in non-transgenic nude mice. After six weeks of UPS tumor growth in the PDOX*

model, the primary tumor was imaged non-invasively and lung, liver, and spleen were resected and imaged ex-vivo in order to visualize the presence of RFP, with a FluorVivo[®] imaging system and FVI1000[®] confocal laser microscope, respectively. Results: The primary tumor was imaged non-invasively. Confocal microscopy visualized the presence of RFP in the lung and liver indicating metastases in these organs. This is the first report of metastasis in a sarcoma PDOX model. Conclusion: This study should prove very useful to screen for anti-metastatic drugs for the PDOX donor patients and to understand the metastatic process in sarcoma.

Undifferentiated pleomorphic soft-tissue sarcoma (UPS) is a common soft tissue sarcoma and is highly recalcitrant with a metastatic rate of 31-35%. The common metastatic sites are lung (90%), bone (8%), and liver (1%) (1, 2). UPS patients are treated with surgical resection and radiotherapy. Because UPS is resistant to chemotherapy, standard chemotherapy protocols have not been developed. Therefore, development of effective therapy for UPS patients is needed.

Traditional imaging procedures such as optical imaging, intravital videomicroscopy, histopathological examination or immunohistochemistry (3-5) are either not sensitive or lacking spatial resolution to image early stage tumor growth or metastasis in mouse models of patient tumors. Therefore, developing stable, sensitive, non-invasive and ex-vivo methods are required to visualize early stages of tumor growth or metastasis.

In order to study metastasis of patient tumors in a mouse model, a patient-derived orthotopic xenograft (PDOX) nude mouse model was developed for all major cancers by our laboratory (6-17). The use of fluorescent proteins for *in vivo*

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imaging was also established by our laboratory (18, 19). We previously developed an imaginable PDOX model for pancreatic cancer, gastric leiomyosarcoma and undifferentiated pleomorphic soft-tissue sarcoma by passage of the PDOX tumor in transgenic red-fluorescent protein (RFP)-expressing nude mice, whereby the PDOX tumor stably acquired RFP-expressing stroma (16, 20, 21).

In the present report, using this method for labeling the UPS tumor, we demonstrate for the first-time that metastasis can be visualized in a sarcoma PDOX model.

Materials and Methods

Mice. Athymic *nu/nu* nude mice and transgenic RFP-expressing athymic *nu/nu* mice (22) (AntiCancer Inc., San Diego, CA, USA), 4-6 weeks old, were used in this study. All animal studies were conducted 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. Mouse housing, feeding, surgical processes and imaging were conducted as previously described (23-29). The mice were humanely sacrificed as previously described (22-26).

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 at the 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 (IRB #10-001857)-approved protocol. We previously reported the establishment of a PDOX model of this UPS (16, 21, 22-26).

Establishment of a fluorescent UPS PDOX model by surgical orthotopic implantation (SOI) in transgenic RFP-expressing nude mice. UPS tumors growing in nude mice were harvested, cut into 5-mm fragments and then implanted subcutaneously in transgenic RFP-expressing nude mice. After the UPS tumor grew subcutaneously in transgenic RFP-expressing nude mice, the tumors were harvested and cut into small fragments and transplanted orthotopically in non-transgenic nude mice as follows: a 5-mm skin incision was made on the right high thigh into the biceps femoris, which was split to make space for the UPS tissue fragment. A single tumor fragment was implanted orthotopically into the space to establish the PDOX model in the non-transgenic nude mice (16). The wound was closed with a 6-0 nylon suture (Ethilon, Ethicon, Inc., NJ, USA). In the present study, 5 UPS PDOX models were established (Figure 1A).

Visualization of metastasis in the fluorescent UPS PDOX model. Six weeks after the UPS tumor was implanted orthotopically in non-transgenic nude mice, the tumor, lung, liver and spleen were resected. Fresh tissues were first cut thin with scissors and placed on slides to confirm the presence of RFP under a fluorescence microscope.

The tissues described above were embedded using optimal cutting temperature (OTC) compound (Tissue-Tek; Sakura Finetek Europe BV, Zoeterwude, the Netherlands) in order to make frozen sections

Table I. *Metastases in the UPS PDOX model.*

Mouse	Tumor	Lung	Liver
1	+	+	+
2	+	+	-
3	+	-	+
4	+	-	-
5	-	-	-

and preserved in liquid nitrogen. The frozen tissue was sectioned at 7 µm with a Cryomicrotome (Leica CM1850, Wetzler, Germany).

Imaging. The FluorVivo® (INDEC Bio System) and FV1000® (Olympus) confocal laser microscope were used to observe RFP. The FluorVivo® was used to non-invasively image the primary tumor. Frozen tissue sections were observed with the FV1000® confocal laser microscope. RFP was excited by a laser emitting at 559 nm. A 4x objective lens and 20x objective lens were used in this experiment.

Results and Discussion

Six weeks after the UPS tumor with RFP stromal cells was implanted orthotopically in non-transgenic nude mice, the primary tumor was non-invasively imaged (Figure 1B). Then the primary tumor, lung, liver and spleen were resected.

Confocal microscopy demonstrated the presence of RFP in the lung and liver in one mouse, in another mouse in the lung and in another mouse in the liver, indicating the metastasis in these organs (Figure 2, Table I).

We were the first to report that stromal cells were necessary for metastasis (27). We subsequently reported that stromal cells from the primary tumor traveled to the metastasis site in a PDOX model of pancreatic cancer (20). Recently, our results that stromal cells are essential for cancer cell homing and colonization to other organs have been confirmed (28-30).

We have also shown that RFP host stromal cells from the primary tumor were observed in the metastasis to the lung in an orthotopic model of human osteosarcoma cell line (31).

The present study is the first to observe metastasis in a sarcoma PDOX model. Being able to visualize metastasis in a sarcoma PDOX model will be of value to screen for drugs to prevent or eradicate metastasis, which should be helpful to the donor patient. The metastatic sarcoma PDOX model will also be valuable to fully understand this disease throughout its entire course.

Conflicts of Interest

The Authors declare that there are no potential conflicts of interest in regard to this study. AntiCancer Inc. uses PDOX models for contract research. HO, TK, YT, KM, KK, TH, MM, ZZ, SR, MBa, SW and RMH are or were unsalaried associates of Anticancer Inc.

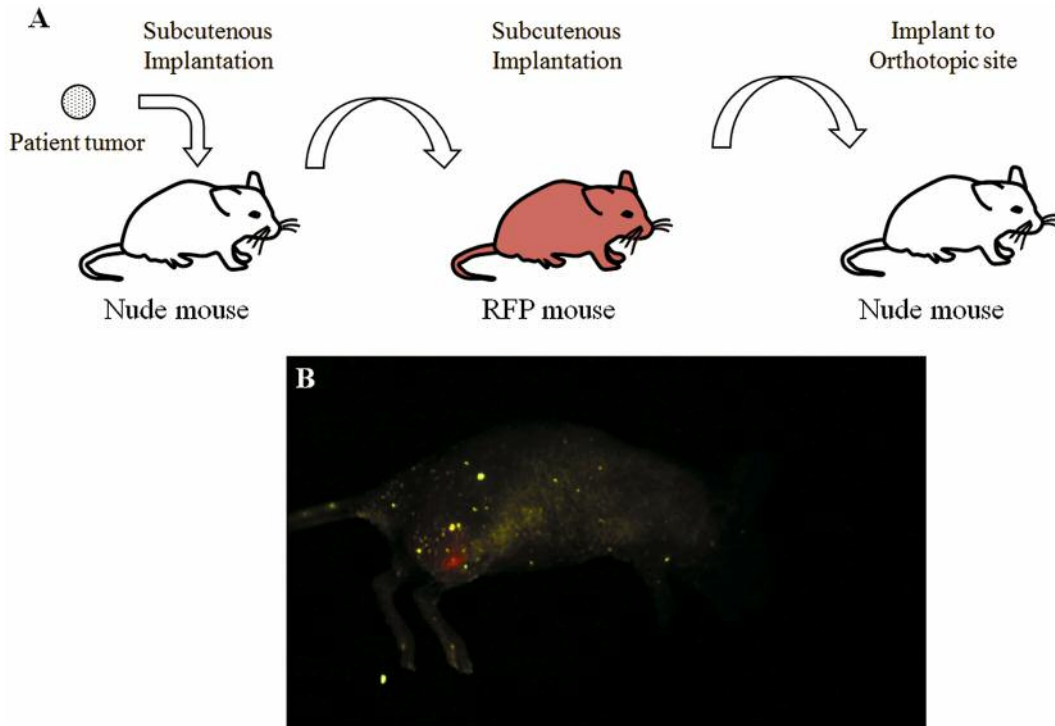


Figure 1. Non-invasive imaging of a fluorescent tumor derived from a primary tumor of a patient with undifferentiated pleomorphic sarcoma, growing orthotopically in nude mice. (A) Experiment schema for developing the imageable metastatic undifferentiated pleomorphic sarcoma (UPS) PDOX model. The patient tumor was initially implanted in the subcutis of a non-transgenic nude mouse. After tumor growth, the tumor was then implanted in the subcutis of an RFP transgenic nude mouse. After tumor growth in RFP transgenic mice, tumor were finally implanted in an orthotopic site in the biceps femoris of non-transgenic nude mice. (B) Non-invasive imaging of the UPS PDOX primary tumor. The mouse was placed in the left lateral decubitus position. RFP expression of the primary tumor can be non-invasively visualized in the right high thigh.

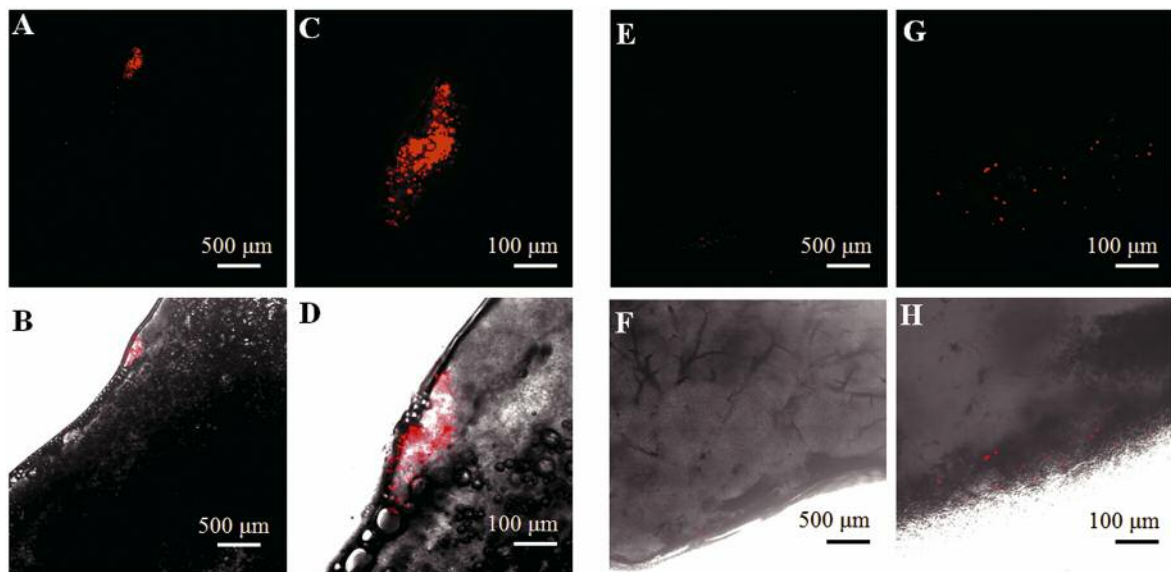


Figure 2. FV1000[®] confocal laser microscopy of metastases in frozen sections. (A-D) Lung, (E-H) liver. (A, E) RFP expression at low magnification (Bar: 500 μm). (B, F) Merge at low magnification (Bar: 500 μm). (C, G) RFP expression at high magnification (Bar: 100 μm). (D, H) Merge at high magnification (Bar: 100 μm).

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