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A Novel Orthotopic Mouse Model of Lung Metastasis Using Fluorescent Patient-derived Osteosarcoma Cells

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Abstract. *Background: A mouse model of metastatic osteosarcoma is imperative to identify effective agents for metastatic osteosarcoma, which is a recalcitrant disease. In the present study, we established osteosarcoma patient-derived cells (OS-PDCs) and transfected them with green fluorescent protein (GFP). Materials and Methods: The OS-PDCs were transfected with GFP-lentivirus. GFP-expressing OS-PDCs (2.0×10^5) were then injected into the tibia of nude mice to establish the patient-derived orthotopic cell (PDOC) model (n=3). Six weeks after injection, the primary tumor and each organ were resected and imaged. Results: Primary orthotopic tumors were established in two out of three mice. The GFP-expressing OS-PDCs in the PDOC model were visualized. Multiple GFP-expressing lung metastases were detected in one of the two mice with primary tumor. Conclusion: The present study proves the concept that a GFP-expressing PDOC model can mimic clinical lung-metastatic osteosarcoma. This model can serve as a paradigm to screen for effective drugs for osteosarcoma lung metastasis.*

Osteosarcoma is the most common bone sarcoma in children and adolescents. Surgery and chemotherapy are first-line treatment of osteosarcoma. However, patients with metastasis of osteosarcoma, most frequently lung metastases, have a 5-year survival rate of only 20-30%, which has not improved for decades (1, 2). Therefore, novel approaches are necessary to identify anti-metastatic therapeutics for osteosarcoma.

A patient-derived orthotopic xenograft (PDOX) mouse model was developed by our laboratory for all major cancer types (3-16) and sarcoma (17-22) to identify novel therapeutics, as well for precision individualized therapy. The PDOX model resembles patient tumor behavior compared with subcutaneous mouse models (23). For osteosarcoma, a lung metastatic model is essential.

In the present study, in order to establish such a model, we isolated patient-derived cells (PDCs) from an osteosarcoma PDOX, transfected them with green-fluorescent protein (GFP) to establish GFP-expressing osteosarcoma PDCs and showed that lung metastasis form after the GFP-expressing osteosarcoma PDCs were orthotopically transplanted to establish a patient-derived orthotopic cell (PDOC) mouse model.

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Key Words: Osteosarcoma, metastasis, patient-derived orthotopic xenograft, PDOX, patient-derived cells, PDC, lung metastasis, green fluorescent protein, imaging.

Materials and Methods

Patient-derived tumor. Written informed consent was previously obtained from the patient as part of the UCLA Institutional Review Board-approved protocol (IRB #10-001857). A 16-year-old female patient with high-grade osteosarcoma of the left distal femur underwent neoadjuvant chemotherapy with doxorubicin and limb salvage surgery with distal femoral replacement. One year later, bilateral metachronous lung metastases appeared, which were resected at UCLA (26). A resected tumor was provided to AntiCancer for establishment in nude mice (27).

Experimental protocol. Tumor fragments of the osteosarcoma from PDOX in nude mice were harvested and minced and seeded in medium to establish osteosarcoma-PDCs (OS-PDCs) in primary cell culture. After primary cell culture, a GFP lentivirus was transfected into the OS-PDCs. GFP-expressing OS-PDCs were identified and stably cultured. These cells (2.0×10^5 cells) were injected into the tibia of nude mice to establish a PDOC mouse model (Figure 1).

Mice. Athymic *nu/nu* nude mice (AntiCancer Inc., San Diego, CA, USA), 4-6 weeks old, were used in the present study. Mouse housing, feeding, surgical processes and imaging were conducted as previously described (22, 28-30). The mice were humanely sacrificed as previously described (22, 28-30). All animal studies were conducted with an AntiCancer Institutional Animal Care and Use Committee-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 (22, 31).

Establishment of OS-PDCs. Tumors were minced mechanically and seeded in Dulbecco's modified Eagle's medium (Sigma-Aldrich, St. Louis, MO, USA) with 10% fetal bovine serum (Gibco, Grand Island, NY, USA), 100 U penicillin G, and 100 μg/ml streptomycin (Gibco) at 37˚C in an incubator with 5% CO_2. Twenty-four hours after seeding, medium was changed to remove loose debris and unattached cells. Cell culture was continued using standard protocols.

Transfection of OS-PDCs with GFP lentivirus. GFP lentivirus (1.0×10^8 transducing units/ml; Cellomics Technology, LLC, Helethope, MD, USA) were prepared for transfection into OS-PDCs. OS-PDCs (1.0×10^4 cells), GFP lentivirus and polybrene (8 μg/ml) were mixed in medium in 24-well plates. Multiplicities of infection (MOIs) of GFP lentivirus at 1, 5, 10 and 20, were determined by dividing the number of viral particles added (ml added x transducing units/ml) by the number of cells. Lentivirus at the above MOIs were added to the OS-PDCs. Six hours after incubation, the GFP lentivirus and polybrene mixture medium was removed and fresh culture medium was added. Three days after incubation, GFP-expressing OS-PDCs were visualized with fluorescence imaging. Puromycin (1-10 μg/ml) was added in culture medium to select stable GFP-expressing OS-PDCs.

Establishment of the OS-PDOC mouse model and detection of metastasis. A suspension of GFP-expressing OS-PDCs (2.0×10^5 cells) was injected into the tibia of nude mice to establish the PDOC mouse model (n=3). All surgical procedures were performed under anesthesia with a ketamine mixture solution as previously described (16, 32). Six weeks after injection, the primary tumor and each organ were resected and imaged to identify GFP-expressing OS-PDCs.

Fluorescence imaging. An FV1000 confocal microscope with XLUMPLFLx20x (0.95 numerical aperture) water immersion objective (Olympus, Tokyo, Japan) was used for imaging of the GFP-expressing OS-PDCs in PDOC. GFP was excited at 488 nm with an Argon laser (24, 33). An OV100 small animal imaging system (Olympus) and FluorVivo (INDEC BioSystem, Los Altos, CA, USA) were used to detect GFP in the tumor and other organs (22, 34).

Results

Transfection of OS-PDCs with a GFP lentivirus. The OS-PDCs were transfected with a GFP lentivirus at MOIs of 1, 5, 10 and 20. GFP was not detected at MOIs of 1 and 5; however, GFP was detectable at MOIs of 10 and 20. GFP-expressing OS-PDCs were selected with puromycin and passaged to establish GFP-expressing OS-PDCs (Figure 2).

Establishment of the OS-GFP PDOC mouse model. GFP-expressing OS-PDCs (2.0×10^5 cells) were injected into the tibia of nude mice (n=3). Orthotopic tumors were established in two out of three mice 6 weeks after injection. GFP-expressing primary tumors in the tibia were detected non-invasively using the FluorVivo imaging system (Figure 3). The OV100 visualized GFP in the resected tumor (Figure 4).

Lung metastases. Six weeks after orthotopic implantation of GFP-expressing OS-PDCs, bilateral lungs and other organs were resected from the osteosarcoma PDOC mouse model. In one out of two tumor-growing PDOC mouse models, multiple GFP-expressing lung metastases were imaged using FluorVivo (Figure 5). There were no metastases visualized in other organs.

Discussion

In the present study, OS-PDCs were established from a patient-derived osteosarcoma. GFP lentivirus was transfected into OS-PDCs to establish GFP-expressing OS-PDCs, which were used to establish an osteosarcoma PDOC model. GFP-expressing lung metastases were identified in the PDOC model, matching the clinical course of the patient tumor.

Lung metastases are a significant problem of morbidity and mortality in patients with osteosarcoma and other cancer types (1, 35-37). In patients with osteosarcoma, the lung is the most frequent site of metastasis, in approximately 80-90% of patients with metastasis, greatly reducing survival (38, 39).

Future studies will perform real-time fluorescent imaging of metastatic dynamics in PDOC models using fluorescent-protein-expressing PDCs in order to identify novel efficacy therapeutics against lung metastases from osteosarcoma and other cancer.

The present study is the first to use GFP-expressing PDCs in an orthotopic model of metastasis, thereby establishing a proof of concept. This model can serve as a paradigm to screen for drugs effective for lung metastasis of osteosarcoma.

Conflicts of Interest

This study did not receive any grant from funding agencies.

Authors' Contributions

HO, YT, and RMH wrote this article. YT, and RMH contributed to the final version of the article. HO, KM, TH, NS, JHP, ZZ, and SR conceived and planned the experiments. FK and KN supervised this study.

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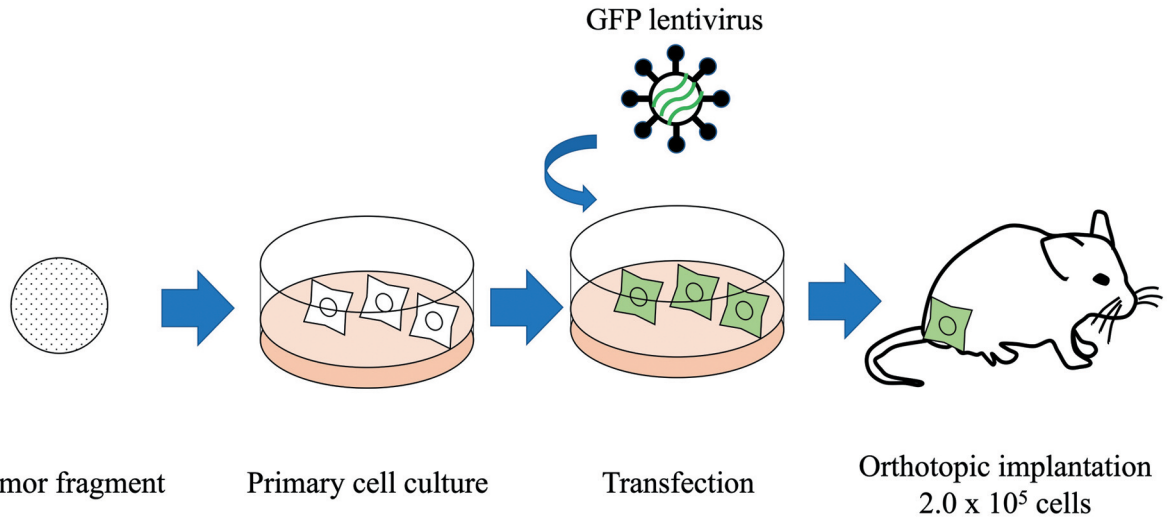

Figure 1. *Experimental schema. Patient-derived tumor fragments grown subcutaneously in nude mice were minced and seeded in medium to produce osteosarcoma patient-derived cells (OS-PDCs). A green fluorescent protein (GFP) lentivirus was used to transfect OS-PDCs. GFP-expressing OS-PDCs (2.0×10^5 cells) were then injected into the tibia of nude mice to establish an orthotopic mouse model of GFP-expressing lung-metastatic patient-derived osteosarcoma.*

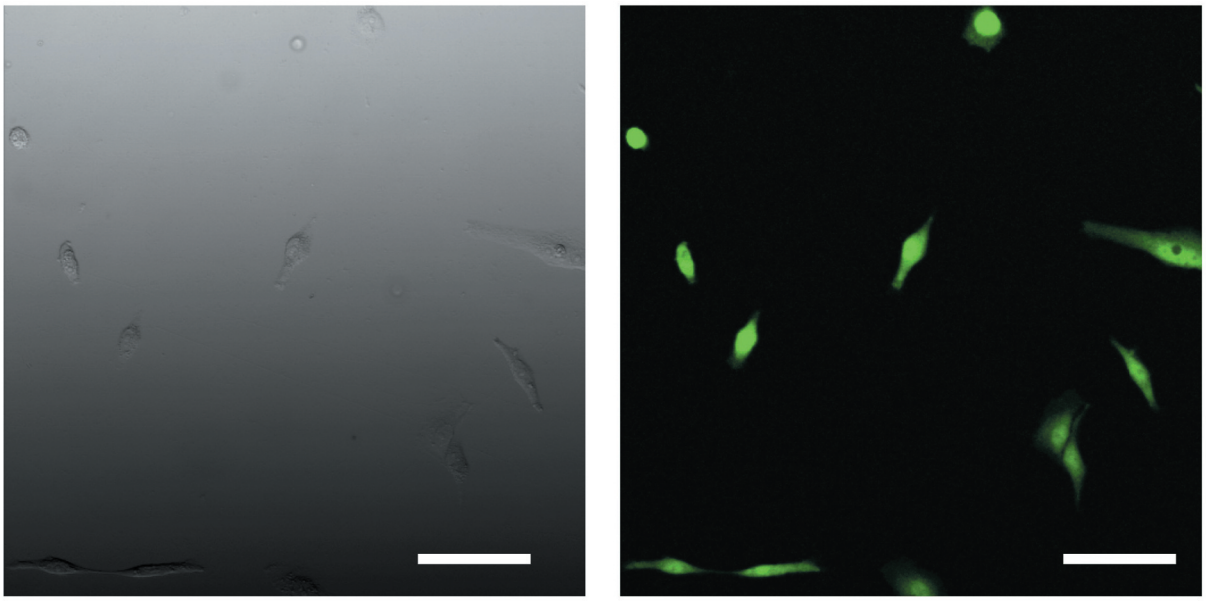

Figure 2. *Green fluorescent protein (GFP) transfection of osteosarcoma patient-derived cells (OS-PDCs). GFP lentivirus was transfected into OS-PDCs. After selection with puromycin, GFP-expressing OS-PDCs were detected using a FV1000 confocal microscope. Left: Bright field. Right: GFP filter. Scale bars are 100 μm.*

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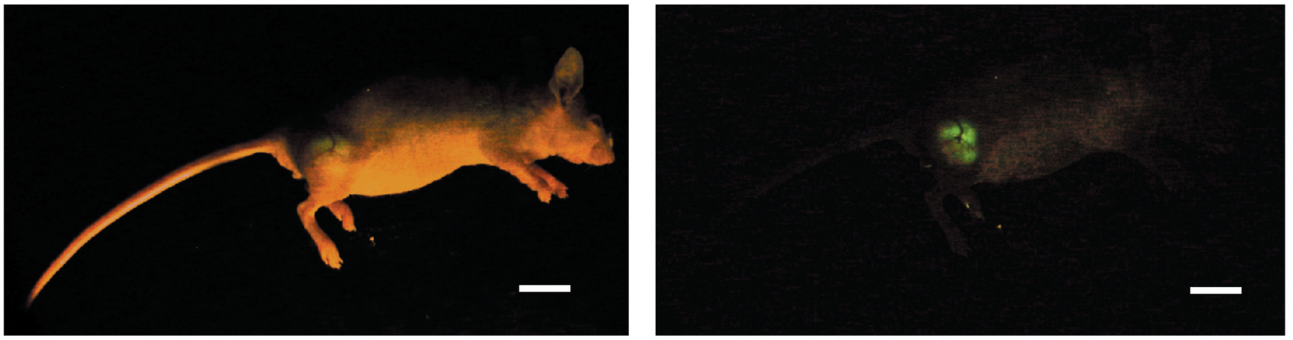

Figure 3. *Orthotopic mouse model of green fluorescent protein (GFP)-expressing lung-metastatic patient- derived osteosarcoma. GFP-expressing osteosarcoma patient-derived cells (2.0×10^5 cells) were injected into the tibia of nude mice. Six weeks after injection, GFP tumors in the right knee were detected using the FluorVivo imaging system. Left: Bright field with GFP filter. Right: GFP filter. Scale bars are 10 mm.*

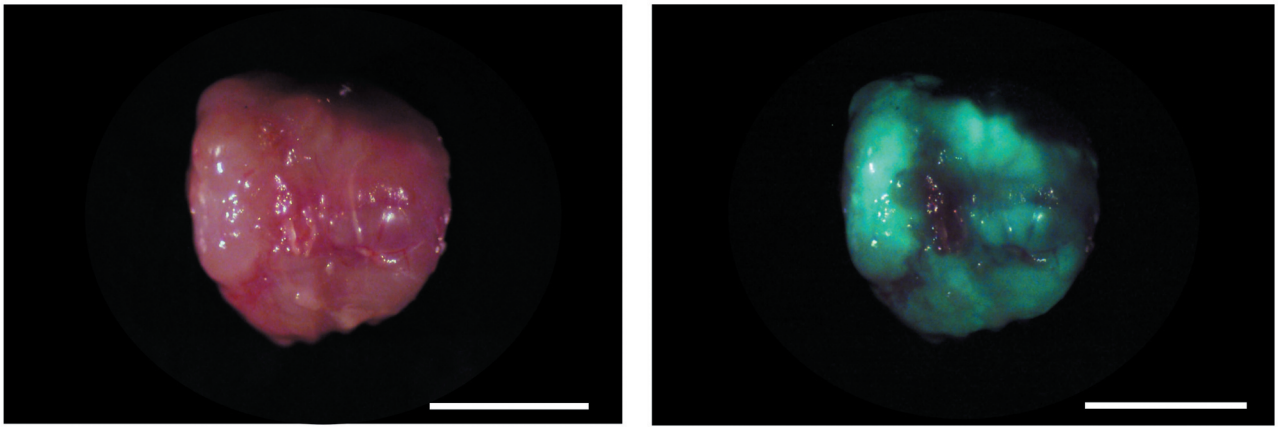

Figure 4. *Resected primary tumor resulting from orthotopic injection of green fluorescent protein (GFP)-expressing osteosarcoma patient-derived cells. Tumor was resected to confirm GFP expression using the OV100 imaging system. Left: Bright field. Right: Merge. Scale bars are 10 mm.*

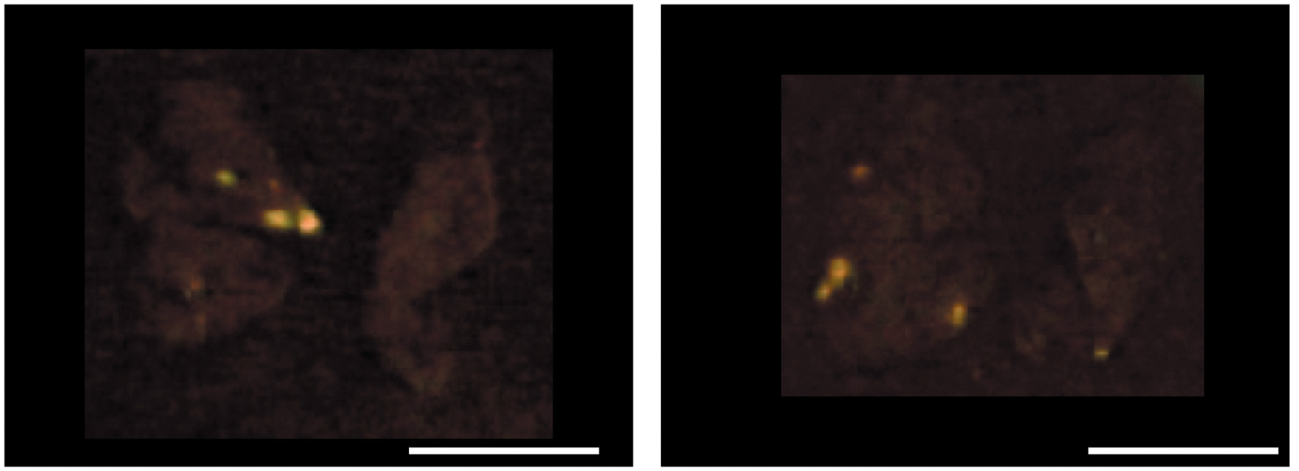

Figure 5. *Lung metastasis from orthotopic injection of green fluorescent protein (GFP)-expressing osteosarcoma patient-derived cells in nude mice. Bilateral lungs were resected to confirm GFP-expressing metastases. Multiple lung metastases were detected with the FluorVivo imaging system. Left and Right GFP filter. Both sides of the lung were imaged. Scale bars are 10 mm.*

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