Abstract. Background/Aim: Sarcomas of the breast are extremely rare malignant tumors and comprise only 5% of all sarcomas and fewer than 1% of breast cancers. Primary osteosarcoma of the breast is histologically indistinguishable from osteosarcoma of the bone. Effective therapies of this recalcitrant disease have not yet been developed. Materials and Methods: A patient-derived xenograft (PDX) mouse model of primary osteosarcoma of the breast was established by subcutaneous implantation of the surgical specimen, along with surrounding normal tissue. Hematoxylin and eosin (H&E) staining was performed on paraffin-embedded histological sections of the original tumor resected from the patient and from implanted tumors that grew in nude mice. Results: Tumors grew in 46 of 51 mice implanted with the original surgical specimen. The H&E-stained slides of the mouse-grown tumor and the original patient tumor matched, both showing large areas of spindle-shaped cells, characteristic of osteosarcoma. Conclusion: The first PDX mouse model of primary breast osteosarcoma was established which will enable testing of novel therapeutics as well as basic research of osteosarcoma of the breast.

Materials and Methods

Mice. For the establishment of this PDX model of primary osteosarcoma of the breast we used athymic (nu/nu) nude mice (AntiCancer, Inc., San Diego, CA, USA) and followed an AntiCancer, Inc. Institutional Animal Care and Use Committee (IACUC) protocol specifically approved for this study. The present study followed the principles and procedures outlined in the...
Patient-derived tumor. The surgical specimen of a primary osteosarcoma of the breast was obtained under a Kaiser Permanente San Diego Medical Center-approved protocol (IRB#12617).

Establishment of primary osteosarcoma of the breast PDX nude-mouse model. Tumor with surrounding normal tissue was prepared for implantation by mincing the surgical specimen into approximately 2 mm³ fragments (Figure 1A and B). Mice were implanted using the method of Hozumi (5). Mice were anesthetized with isoflurane. A 1 cm skin incision was made at the middle of the back of nude mice. A pocket was created using surgical scissors by dissecting the skin from the dermis. Primary breast osteosarcoma fragments along with surrounding normal tissue were inserted into the pocket (Figure 2A and B). The wound was closed with a 5-0 PDS-II suture (Figure 2C). The implanted tumors were observed for 10 weeks.

Hematoxylin and eosin staining. Fixation, paraffin sectioning, and staining were performed as previously described (7). Hematoxylin and eosin (H&E) staining was performed according to standard protocols (12).

Results

Establishment of primary osteosarcoma of the breast PDX model. Fifty-one mice were subcutaneously implanted with patient-derived primary osteosarcoma of the breast, tumors grew in 46 of them (90.2% establishment) (Table I). Forty-six mice had qualitatively clear growth of tumors within two to four weeks (Figure 3).

Histology of primary osteosarcoma of the breast in the PDX nude-mouse model vs. primary osteosarcoma of the breast

Table I. Establishment frequency of the patient breast osteosarcoma in nude mice.

| Number of mice implanted | Number of mice with tumor growth after 4 weeks |
|--------------------------|-----------------------------------------------|
| 51                       | 46 (90.2%)                                    |

The surgical specimen of the patient breast osteosarcoma was minced into fragments of approximately 2 mm³ (Figure 1). The fragments were inserted into a subcutaneous pocket made in nude mice. Mice were observed for tumor formation starting on day 14 after implantation. By day 28, 90.2% of mice implanted had observable tumor growth.
H&E staining showed that tumors directly from the patient. H&E staining showed that tumors from the PDX mouse model comprised highly-dense cancer cells with a pleomorphic spindle-shape characteristic of osteosarcoma, very similar to the original patient tumor (Figure 4A and B).

**Discussion**

Higher establishment rates for establishing PDX mouse models from patient-derived tumors have been possible using the method of Hozumi, which involves co-implantation of tumor tissue and surrounding normal tissue (5). The present study had a 90.2% establishment rate with tumors growing in 46 of 51 mice implanted. The inclusion of normal tissue surrounding the tumor tissue seems effective in increasing the establishment frequency (5).

The present study was a successful establishment of the first mouse model of an extremely rare primary osteosarcoma of the breast. Establishment of the primary osteosarcoma in nude mice effectively immortalizes the tumor which can be cryopreserved for future implantation in nude or other immuno-compromized mice. For the first time, it is now possible to do scientific studies on osteosarcoma of the breast due to the successful establishment of the first PDX mouse model of the disease. Future experiments will involve drug-sensitivity screening, as currently there is no identified first-line therapy for osteosarcoma of the breast. Orthotopic models will also be established in order to study metastasis of breast osteosarcoma.

**Conflicts of Interest**

The Authors declare that there are no potential conflicts of interest regarding this study.
Authors’ Contributions

N.W., J.Y., and R.M.H. designed and performed experiments and wrote the paper; J.Y., Y.A., and C.H. gave technical support and conceptual advice. J.W. resected the tumor from the patient. Writing, review, and/or revision of the manuscript: N.W., M.B., and R.M.H.

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