J Toxicol Pathol 2021; 34: 89–93

Original Article

Establishment of a patient-derived xenograft mouse model of pleomorphic leiomyosarcoma

Yasuhiro Shimada[1], Tomoharu Naito[1], Takuo Hayashi[1]*, Tsuyoshi Saito[1], Yoshiyuki Suehara[2], Chihaya Kakinuma[1], Yuji Nozaki[3], Hisayoshi Takagi[3], and Takashi Yao[1]

[1] Department of Human Pathology, School of Medicine, Juntendo University, 1-1-19 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
[2] Department of Orthopaedic Surgery, School of Medicine, Juntendo University, 1-1-19 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
[3] Biotechnical Center, Japan SLC, Inc., 3-5-1 Aoihigashi, Naka-ku, Hamamatsu, Shizuoka 433-8114, Japan

Abstract: Soft tissue sarcomas are difficult to treat using chemotherapy owing to a current deficiency in candidate drugs for specific targets. Screening candidate compounds and analyzing therapeutic targets in sarcomas is insufficient, given the lack of an appropriate human sarcoma animal model to accurately evaluate their efficacy, as well as the lack of an adequate technical protocol for efficient transplantation and engraftment of sarcoma specimens in patient-derived xenograft (PDX) models. Accordingly, in this study, we sought to identify the optimal type of sarcoma and develop a protocol for generating a PDX model. We characterized a PDX mouse model using histopathological and immunohistochemical analyses to determine whether it would show pathological characteristics similar to those of human sarcomas. We achieved engraftment of one of the 10 transplanted sarcoma specimens, the xenografted tumor of which exhibited massive proliferation. Histologically, the engrafted sarcoma foci resembled a primary tumor of pleomorphic leiomyosarcoma and maintained their histological structure in all passages. Moreover, immunohistochemical analysis revealed the expression of specific markers of differentiation to smooth muscle, which is consistent with the features of leiomyosarcoma. We thus demonstrated that our pleomorphic leiomyosarcoma PDX mouse model mimics at least one aspect of human sarcomas, and we believe that this model will facilitate the development of novel therapies for sarcomas. (DOI: 10.1293/tox.2020-0061; J Toxicol Pathol 2021; 34: 89–93)

Key words: patient-derived xenograft (PDX), pleomorphic leiomyosarcoma (PLMS), mouse model

Introduction

Cell-based assay systems are the primary tools used for drug discovery owing to their simplicity, which facilitates assessment of the effects of drugs on cells cultured *in vitro*. Although *in vivo* tumor models established using tumor cell lines obtained from immunodeficient mice (nude and SCID) have been used to mimic human cancer pathology[1], in many cases, the cell characteristics and tumor formation differ from those of the original tumor, owing to the different microenvironments, including the immune system and cancer stroma in host animals[2–4]. Therefore, translational studies that use patient-derived tissues are required for the development of more effective cancer drugs. Some patient-derived xenograft (PDX) models have been established on the basis of this consideration[5]. These models are increasingly being used as tools for drug development in pre-clinical settings and have been shown to recapitulate the histology and behavior of the cancers from which they are derived[6]. Furthermore, studies conducted to aid clinical decision-making have shown high concordance between individual PDX and patient responses to therapy[7]. Although these findings are encouraging, the role of this approach in sarcoma-derived models and in the context of genomic drug-matching strategies remains undefined. This has thus created an opportunity to evaluate the utility of PDX models as clinical predictors to direct the use of chemo- and targeted therapies in combination with comprehensive genomic and epigenetic analyses for patients with sarcomas. Moreover, the National Cancer Institute recommends the screening of anti-cancer drugs using PDX models, given that unlike cell lines, the tissue structure and gene expression patterns of PDX models more closely resemble those of patients[8]. Thus, PDX models are predicted to emerge as the mainstream approach for drug discovery, and represent a valuable support tool for investigator-initiated clinical trials.

However, owing to the low efficiency of engraftment on host tissue and slow growth rate[9, 10], a PDX model suitable for all tumor types, particularly sarcomas, is currently unavailable. In this study, we aimed to establish a PDX model for certain types of sarcoma, using SCID mice, for drug

Received: 3 September 2020, Accepted: 29 October 2020
Published online in J-STAGE: 12 December 2020
*Corresponding author: T Hayashi (e-mail: tkhyz@juntendo.ac.jp)

discovery. We describe the development of a novel PDX model of pleomorphic leiomyosarcoma (PLMS), a morphological variant of leiomyosarcoma with aggressive clinical behavior[11], which shows histological and immunohistological features similar to those of the original PLMS in mice. Our case model may aid in elucidating the biological characteristics of PLMS and in developing novel candidate drugs for the treatment of this type of sarcoma.

Materials and Methods

Preparation of patient-derived tumor samples

Tumor tissue samples were obtained with informed consent from a 71-year-old man who underwent surgery at the Juntendo University Hospital. The tumor specimens originating from his thigh were preserved in minimum essential medium (Cat. #51200038; GIBCO, Grand Island, NY) supplemented with antibiotics (penicillin) under refrigerated conditions. The specimens were subsequently washed twice in Dulbecco's modified Eagle's medium without fetal bovine serum and minced at 1 mm^3 per sample block on ice using a razor before transplantation into the mice. This study was approved by the ethics committee of Juntendo University (approval code: 2017040).

Animals

C.B-17/IcrHsd-*Prkdc*^{scid} mice (Japan SLC, Shizuoka, Japan) were obtained at 6 weeks of age and maintained under specific pathogen-free conditions. The mice were used for the experiments at 7 weeks of age. All experiments and procedures for the care and treatment of the animals used in the present study were performed in accordance with the requirements of the Juntendo University Animal Care and Experimentation Committee (Experimental Protocol: No. 2017040; Juntendo University, Tokyo, Japan) and the SLC Animal Care and Experimentation Committee (Experimental Protocols: No. BT18098 and No. 18099).

Preparation of a PDX mouse model

Twenty mice were subcutaneously transplanted with tumor tissues on day 0. Tumor specimens were subcutaneously injected into the right flank region of each animal using a transplant needle (φ2.5 × L85 mm, Cat. #KN-391-25; Natsume Seisakusho, Tokyo, Japan). The tumor diameter and body weight were measured twice per week. To calculate the tumor volume, both long and short diameters (mm) were measured using a Solar Digital Caliper (Cat. #1-3255-02; AZ-One, Osaka, Japan). The formula used to calculate tumor volume was as follows: tumor volume (mm^3) = long diameter (mm) × short diameter (mm) × short diameter (mm) × 0.5. The volumes of tumor were measured until reaching 2,000 mm^3, in line with experimental ethics. Animals with moribundity [marked reduction in body weight, hypothermia, significant drop in temperature, and significant exhaustion (crouching position)] were euthanized immediately by bloodletting under isoflurane anesthesia (induction: 4.0%; maintenance: 1.0–3.0%) without any other pain[12]. Proliferated tumor nodules were collected at the time of autopsy.

Histological examination

All removed tissues were fixed in 10% neutral buffered formalin. Tissue slices were routinely processed for paraffin embedding and sectioned at 3 μm thickness. The sections were stained with hematoxylin and eosin and observed under a light microscope.

Immunohistochemistry (IHC)

The PDX specimens were fixed with 10% formaldehyde, embedded in paraffin blocks, cut into 4-μm-thick sections, and mounted on glass slides. Staining of the sections was performed following standard methods described previously[13]. The sections were then incubated overnight at 4°C and thereafter at room temperature for 30 min with mouse monoclonal anti-Caldesmon (clone: h-CD; Dakopatts, Glostrup, Denmark), anti-human muscle actin (M-actin) (clone: HHF35; Dakopatts), or anti-smooth muscle actin (SMA) (clone: 1A4; Dakopatts) antibodies at dilutions of 1:1, 1:100, and 1:200, respectively, in phosphate-buffered saline containing 1% bovine serum albumin.

Results

Establishment of a soft tissue sarcoma PDX model

In our experiments, three of the 10 soft tissue tumor specimens derived from the patient were subcutaneously engrafted on the mice, one of which subsequently proliferated, with the size of the tumor increasing at each passage (Fig. 1). This soft tissue tumor was identified as a type of high-grade PLMS. Most of the transplanted mice showed massive tumor growth and enlargement (Fig. 1). Moreover, tumor progression continued to persist at each passage. In contrast, the PDX tumor models displayed enhanced growth rate with successive *in vivo* passages. Following transplantation, the time until harvesting decreased from 55 days at passage 0 to 50 days at passage 1, 40 days at passage 2, and 35 days at passage 3 (Fig. 1). Finally, after re-transplantation, all the tumor foci proliferated and their volumes increased.

Histological features of the patient's tumor

Histologically, the tumor was characterized by fascicular proliferation of spindle-shaped cells. Tumor cells with anomalous giant nuclei were also scattered throughout the lesion (Fig. 2A). IHC revealed that the tumor cells were positive for SMA (Fig. 2B). Additionally, these tumor cells showed an MIB-1 index of approximately 80%. On the basis of the histological features and IHC findings, the patient was diagnosed as having a PLMS.

Histopathological characteristics of the original PLMS were maintained at each passage in mice

The storiform pattern and fibrotic elements of the patient-derived PLMS were observed at all passages in transplanted mice. The nuclei showed atypical anisokaryosis, nuclear pleomorphism with coarse chromatin, and mitotic

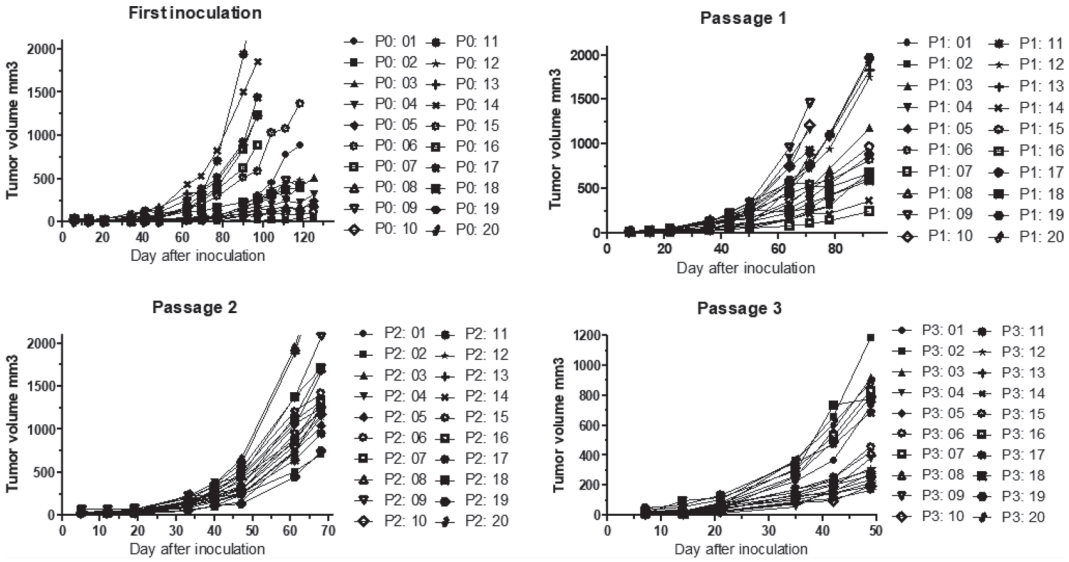

Fig. 1. Graph showing tumor volume after transplantation at each passage. Autopsy tumor samples obtained from the patient were minced (1 mm^3/tumor piece) and subcutaneously transplanted into SCID mice (first inoculation: passage 0). After some tumors had reached a volume of 2,000 mm^3, the tumor nodule was removed and sub-transplanted into the next normal SCID mice (passage 1). Sub-transplantation was conducted continually at passage 3. Volumetric measurements of xenografted tumors were performed using an external caliper, as described in the Materials and Methods section. Tumor nodules were collected at the time of necropsy and subjected to histological and immunohistochemical analyses.

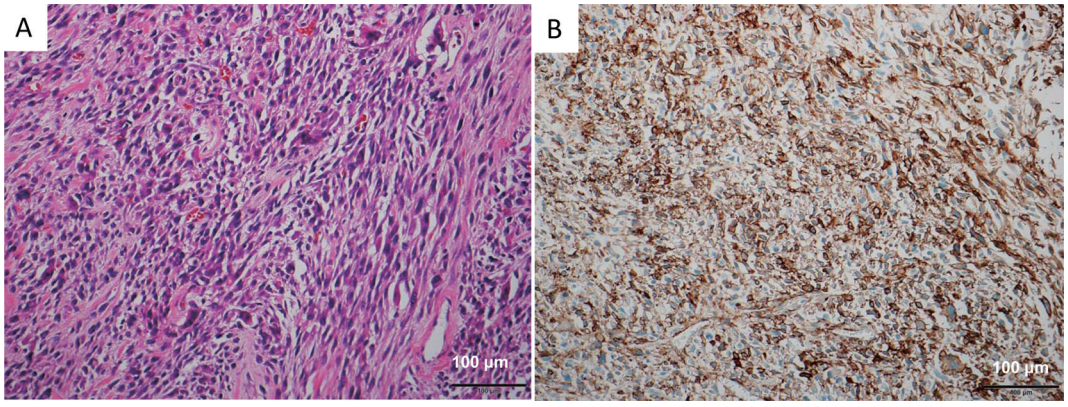

Fig. 2. Histological characteristics of the tumor derived from the patient. The tumor was characterized by fascicular proliferation of spindle-shaped cells with scattered giant cells (A). Immunohistochemically, the tumor cells were positive for myogenic markers, including smooth muscle actin (SMA) (B). The black bar indicates 100 μm.

figures, and the tissues were also characterized by small foci of fascicles consisting of smooth muscle tumor cells. A mucoid matrix was also found in the same field. The tumor tissues in mice sampled at each successive passaged showed no significant differences in structure (Fig. 3, upper panel). Therefore, structural characteristics at the third passage were histologically similar to those of the primary patient-derived tumors (Fig. 3, upper panel).

Immunohistochemical analysis of PDX tumor samples at each passage

PDX tumor samples obtained at each passage were immunologically stained for Caldesmon, M-actin, and SMA to confirm differentiation to smooth muscle in the original patient-derived leiomyosarcoma tumor. We observed that the PDX tumor was derived from the leiomyosarcoma, as indicated by the positive staining for all smooth muscle-specific markers (Fig. 3). In particular, positive staining for SMA was diffuse and significant (Fig. 3, lower panel). With respect to the expression in PDX tumors through several passages, we detected no significant differences in the levels

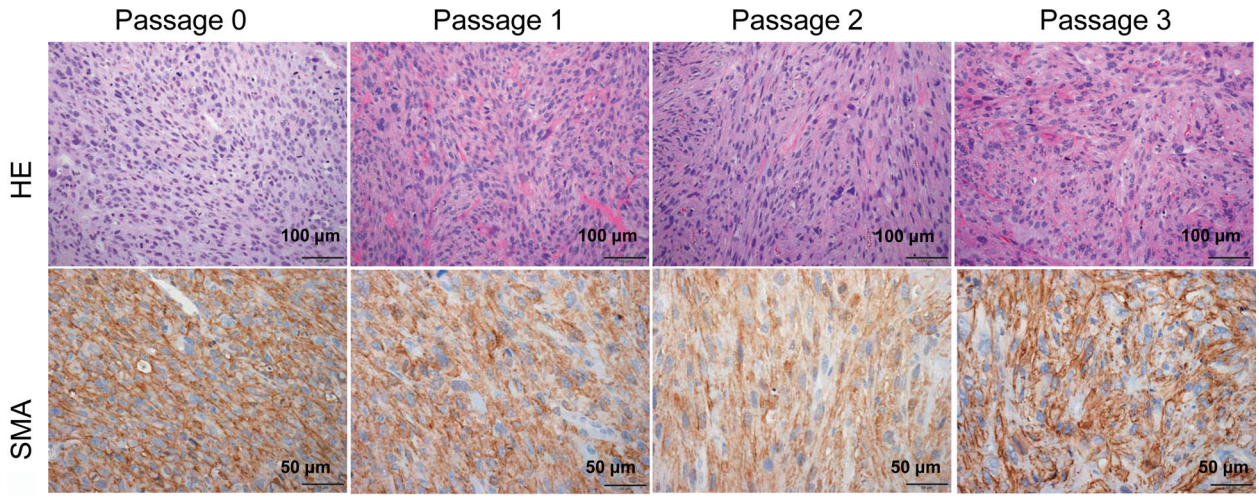

Fig. 3. Histological and immunohistochemical analyses of a patient-derived xenograft **(**PDX) at each passage. Tumor nodules were fixed in formalin-containing bottles, and tumor sections were prepared on slides. The slides were stained with hematoxylin and eosin (H&E) solution, as described in the Materials and Methods section. All sections containing tumor tissue were microscopically examined, and representative images at each passage are shown (upper panels). The expression of Caldesmon (CALD, CDM), M-actin, and smooth muscle actin (SMA) in tumors collected from recipient mice engrafted with PDX were measured using immunohistochemical staining. Staining for SMA is shown at each passage (lower panels). The black bar indicates 100 μm for sections stained with H&E, whereas the white bar indicates 50 μm for sections stained with SMA.

of Caldesmon, M-actin, and SMA staining among multiple passages (data not shown).

Discussion

Although *in vivo* sarcoma PDX models represent promising tools for drug development and elucidation of underlying pathological mechanisms, it is difficult to establish sarcoma models *in vivo*. owing to the low reproducibility of tumor restructuring and the significantly low rate of tumor engraftment in experimental mice[9, 10]. Although our PDX mouse model also showed a low rate of tumor engraftment (approximately 10%), we succeeded in establishing a PDX derived from a very rare type of tumor, PLMS. We believe that the following procedural details may have contribute to our success in establishing the PLMS PDX model. 1. Following surgery, the specimens were optimally processed prior to implantation in the mice. This optimization shortened the timeline from surgery to implantation of the specimen in mice, thereby contributing to specimen preservation. 2. The specimens were rapidly preserved during cold acclimation under refrigeration and optimal medium conditions. 3. The specimens that underwent rapid washout and cutting comprised an optimum tumor tissue for engrafting onto the subcutaneous site in the mice. Collectively, these procedures would have contributed to the prevention of sample degradation and maintenance of high cell viability. Whereas tumor tissue bricks characterized by a tumor microenvironment with scaffold stroma ensure successful engraftment of sarcoma specimens on xenograft models that mimic the characteristics of PLMS, it is also conceivable that high malignancy contributes to the success rate of PDX engraftment. Although the detailed mechanisms have yet to be sufficiently clarified, we hypothesize that the high frequency of cell division and proliferation in this type of sarcoma is conducive to good engraftment and time-dependent tumor growth in mice.

Microscopically, conventional leiomyosarcomas show a characteristic histology of smooth muscle differentiation with a low to intermediate grade potential for malignancy. In contrast, PLMS shows histological features of high-grade spindle cell sarcoma, such as variable, large, and atypical cells, and multinuclear giant cells, whilst partially retaining the features of conventional LMS, and also show distinct myogenic differentiation, as confirmed by IHC[11]. Although pleomorphic and conventional leiomyosarcomatous areas are typically intermixed within a tumor, the demarcation may be distinct or gradual in some cases[14]. Furthermore, it has been reported that PLMS contain myxoid or focally collagenous stroma, which resemble those of storiform-pleomorphic undifferentiated sarcomas[15]. The characteristics of the PDX in our model were closely comparable to the typical characteristics of PLMS, thereby confirming the establishment of an original tumor-like PLMS PDX model. However, the tumor growth rate and commencement of tumor proliferation were clearly accelerated on tumor passaging in each mouse model. In this regard, it is conceivable that a tumor microenvironment characterized by the incorporation of interstitial stroma and host animal angiogenesis created an isolated niche for human tissue-resident sarcoma foci that favored tumor survival and proliferation at successive passage. Accordingly, tumors might be adopted in the host environment and undergo immune accommodation as a foreign body. In contrast, tumor-specific marker expres-

sion and histological structure remained unchanged. Therefore, the important point to note would be the tumor niche environment for tumor growth changes. Improvements in tumor engraftment and growth rate are major factors necessary for the establishment of a rare cancer PDX model. Although the specific conditions required for PDX remain unclear, we speculate that establishing a basal scaffold will be essential for enhancing human carcinogenesis in future xenograft models.

In summary, we succeeded in developing a PLMS PDX mouse model that mimicked the original human tissue structure at the subcutaneous site. Patients with PLMS have poor prognoses, and novel systemic therapies are being investigated to improve the survival of afflicted patients. We anticipate that our PDX model will make a valuable contribution to future drug development for PLMS through drug efficacy screening.

Disclosure of Potential Conflicts of Interest: There are no known conflicts of interest associated with this study.

Acknowledgment: We are grateful for the considerable support provided by Dr. Tamami Higuchi.

References

1. Takizawa Y, Saida T, Tokuda Y, Dohi S, Wang YL, Urano K, Hioki K, and Ueyama Y. New immunodeficient (nude-scid, beige-scid) mice as excellent recipients of human skin grafts containing intraepidermal neoplasms. Arch Dermatol Res. **289**: 213–218. 1997.
2. Tudrej P, Kujawa KA, Cortez AJ, and Lisowska KM. Characteristics of in vivo model systems for ovarian cancer studies. Diagnostics (Basel). **9**: E120. 2019.
3. Meraz IM, Majidi M, Meng F, Shao R, Ha MJ, Neri S, Fang B, Lin SH, Tinkey PT, Shpall EJ, Morris J, and Roth JA. An improved patient-derived xenograft humanized mouse model for evaluation of lung cancer immune responses. Cancer Immunol Res. **7**: 1267–1279. 2019.
4. Inoue A, Deem AK, Kopetz S, Heffernan TP, Draetta GF, and Carugo A. Current and future horizons of patient-derived xenograft models in colorectal cancer translational research. Cancers (Basel). **11**: E1321. 2019.
5. Aparicio S, Hidalgo M, and Kung AL. Examining the utility of patient-derived xenograft mouse models. Nat Rev Cancer. **15**: 311–316. 2015.
6. Gould SE, Junttila MR, and de Sauvage FJ. Translational value of mouse models in oncology drug development. Nat Med. **21**: 431–439. 2015.
7. Hidalgo M, Amant F, Biankin AV, Budinská E, Byrne AT, Caldas C, Clarke RB, de Jong S, Jonkers J, Mælandsmo GM, Roman-Roman S, Seoane J, Trusolino L, and Villanueva A. Patient-derived xenograft models: an emerging platform for translational cancer research. Cancer Discov. **4**: 998–1013. 2014.
8. Ledford H. US cancer institute to overhaul tumour cell lines. Nature. **530**: 391. 2016.
9. Wilding JL, and Bodmer WF. Cancer cell lines for drug discovery and development. Cancer Res. **74**: 2377–2384. 2014.
10. Jacques C, Renema N, Lezot F, Ory B, Walkley CR, Grigoriadis AE, and Heymann D. Small animal models for the study of bone sarcoma pathogenesis:characteristics, therapeutic interests and limitations. J Bone Oncol. **12**: 7–13. 2018.
11. Oda Y, Miyajima K, Kawaguchi K, Tamiya S, Oshiro Y, Hachitanda Y, Oya M, Iwamoto Y, and Tsuneyoshi M. Pleomorphic leiomyosarcoma: clinicopathologic and immunohistochemical study with special emphasis on its distinction from ordinary leiomyosarcoma and malignant fibrous histiocytoma. Am J Surg Pathol. **25**: 1030–1038. 2001.
12. Gargiulo S, Greco A, Gramanzini M, Esposito S, Affuso A, Brunetti A, and Vesce G. Mice anesthesia, analgesia, and care, Part I: anesthetic considerations in preclinical research. ILAR J. **53**: E55–E69. 2012.
13. Hayashi T, Saito T, Fujimura T, Hara K, Takamochi K, Mitani K, Mineki R, Kazuno S, Oh S, Ueno T, Suzuki K, and Yao T. Galectin-4, a novel predictor for lymph node metastasis in lung adenocarcinoma. PLoS One. **8**: e81883. 2013.
14. Sun M, Liu JG, Weng QY, Yu L, and Wang J. [Pleomorphic and dedifferentiated leiomyosarcoma: a clinicopathologic analysis]. Zhonghua Bing Li Xue Za Zhi. **47**: 87–93. 2018. (in Chinese)
15. Akpinar A, Ozdemir CO, Ucler N, and Inan HM. Storiform-pleomorphic type of multifocal malignant fibrous histiocytoma of the lumbar spine. Am J Case Rep. **15**: 565–568. 2014.