Abstract. Background/Aim: Although the 5-year survival rate for localized prostate cancer is nearly 100%, prognosis for patients with metastases, of which the bone is the most common site, is poor. In order to evaluate efficacy of treatments against metastatic prostate cancer, experimental tibia-bone-metastasis mouse models of prostate cancer have been previously established. In the present study, we used a novel procedure for establishment of an experimental tibia-bone metastasis mouse model, with human PC-3 prostate cancer expressing green fluorescent protein (GFP), that more closely matches prostate cancer growing in the bone.

Materials and Methods: PC-3 human prostate cancer cells, labeled with GFP, were initially subcutaneously injected into the flank of five male nude mice to obtain tumor tissues. Once the tumor tissue grew larger than 10 mm in diameter, the tumor tissue was harvested and minced into fragments of 1 mm³. A 1-mm hole was made in the proximal left tibia of eight male nude mice, using the tip of a 5-mm blade, and a tumor fragment was implanted into the hole for an exact fit. Tumor size was measured once a week, by non-invasive imaging of GFP fluorescence. The mice were sacrificed four weeks after tumor implantation. Results: Tumors grew in 8 out of 8 mice (100%). All tumors were non-invasively detectable with GFP fluorescence, through the skin. Increased tumor growth in the tibia was observed every week. Conclusion: The establishment in the tibia of the novel experimental bone-metastatic mouse model of human prostate cancer enables facile screening, in a clinically-relevant system, of improved therapeutics for this recalcitrant disease.

Prostate cancer is the second most common cancer in men worldwide. The 5-year survival rate for localized prostate cancer is nearly 100%, in contrast, the 3-year survival rate for patients with metastatic disease is 60-70% (1-4). About 85-100% of patients who die of prostate cancer have bone metastasis, which is the most common metastasis site (5, 6).

The therapeutic strategy for metastatic prostate cancer comprises first-line therapy of androgen-deprivation therapy alone/or with docetaxel and/or prednisone. However, further development is needed.

Evaluation of metastasis by detection of green fluorescent protein (GFP) fluorescence is a very powerful technique (7). Experimental tibia-metastasis mouse models of PC-3-GFP prostate cancer, with cell injection into the bone marrow, have been previously established (7-10). However, the establishment ratio is inconsistent, depending on the technical level of the procedure and the quality of cells which are injected. The present study uses implantation of prostate-tumor fragments, that fit exactly into a hole made in the tibia, to closely model bone-metastatic prostate cancer in the clinic.

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Key Words: Prostate cancer, PC-3, GFP, imaging, metastasis, tibia implantation.

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for the Care and Use of Animals, was approved under Assurance Number A3873-1, as previously described (11-14).

**Cell culture.** The PC-3 human prostate cancer cell line, obtained from the American Type Culture Collection (Manassas, VA, USA), was previously labeled with GFP (7). Cells were cultured in Dulbecco’s Modified Eagle Medium, supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin, and incubated with 5% CO₂ at 37°C.

**Novel procedure to establish a PC-3-GFP tibia-bone experimental metastasis mouse model.** PC-3-GFP cells (2.5×10⁶ cells/100 μl PBS) were initially subcutaneously injected into the flank of five male nude mice. Once the tumor tissue grew larger than 10 mm in diameter, it was harvested and minced into fragments of 1 mm³. Eight male nude mice were used to establish a PC-3-GFP tibia-bone metastasis model. A 5-mm incision was made in the skin over the proximal part of the left tibia. The left knee joint was bent, to expose the tibia, and a 1-mm-diameter hole was made in the proximal tibia. A 1-mm³ PC-3-GFP tumor fragment, harvested from a subcutaneous tumor, was prepared for insertion into the hole. The tumor fragment was implanted into the tibia. The white arrow shows the hole, into which the tumor fragment was inserted. See Materials and Methods for details. Scale bar: 10 mm.

**Figure 1.** Tumor-fragment tibia-implantation method for establishment of a PC-3-GFP experimental bone-metastasis mouse model. (A) A five-mm incision was made in the skin over the proximal part of the left tibia. (B) The exposed proximal part of the left tibia is shown. (C, D) A 1-mm-diameter hole was made in the proximal tibia. (E) A 1-mm³ PC-3-GFP tumor fragment, harvested from a subcutaneous tumor, was prepared for insertion into the hole. (F) The tumor fragment was implanted into the tibia. The white arrow shows the hole, into which the tumor fragment was inserted. See Materials and Methods for details. Scale bar: 10 mm.

**Figure 2.** Representative images of growing PC-3-GFP tibia-bone experimental metastasis visualized non-invasively with GFP fluorescence. (A) Two weeks after tumor implantation. (B) Three weeks after tumor implantation. (C) Four weeks after tumor implantation.
a 5-mm blade (Medipoint, Inc., Mineola, NY, USA). Once a hole was made, the blade was tilted along the tibia-bone axis and rotated several times to make the hole 1 mm in diameter. A 1-mm 3PC-3-GFP tumor fragment was inserted into the hole (Figure 1). The wound was sutured with 5-0 nylon sutures. The procedure was performed according to an osteosarcoma-PDOX tibia implantation model previously reported (11-14).

Non-invasive imaging and measurement of PC-3-GFP tibia growth. Tumor size was measured once a week, by non-invasive detection of GFP fluorescence (15-17) with a FluorVivo fluorescence imaging system (INDEC Systems, Inc., Los Altos, CA, USA), using the following formula: tumor volume (mm$^3$)=length (mm) × width (mm) × width (mm) × 1/2. All mice were sacrificed four weeks after tumor implantation. Data are shown as mean±standard deviation (SD).

Results

PC-3 prostate tumors grew in the tibia in 8 out of 8 mice (100%). All tumors were non-invasively imaged with GFP fluorescence, through the skin (Figure 2). Tumor volume determined at 2, 3, and 4 weeks after implantation increased with time and the growth became more rapid after 3 weeks (Figure 2 and Figure 3).

Discussion

In the present study, we established an experimental tibia-bone metastasis mouse model of prostate cancer. Compared to previous tibia-bone metastasis models (7-10), in which cell injection was performed, this new procedure proved to be more accurate and efficient, since tumors grew in all the mice, a 100% establishment rate, compared to an approximately 90% in a previous study (8). The lower establishment rate by cell injection may be due to cells leaking out of the medullary cavity of the bone. In contrast, in the case of tumor fragment-implantation in the present study, in which the fragment size is the same as the bone hole, the fragment could be fixed in the medullary cavity of the bone, which could guarantee accurate localization.

The non-invasively-imageable model of experimental prostate-cancer bone metastasis will be useful for identifying more effective drugs for this recalcitrant disease.

Conflicts of Interest

The Authors have no conflicts of interest to declare in relation to this study. AntiCancer Inc. use uses orthotopic mouse models of cancer for contract research.

Authors’ Contributions

YA, NM, YT and RMH were involved in study conception and design. YA, NM and YA were involved in acquisition of data. YA, NM, YT, YK and RMH analyzed and interpreted data. YA, YT and RMH wrote the manuscript. All Authors reviewed and approved the manuscript.

Acknowledgements

This study was funded in part by the Robert M. Hoffman Foundation for Cancer Research. This paper is dedicated to the memory of A. R. Moossa, MD, Sun Lee, MD, Professor Li Jiaxi, Masaki Kitajima, MD, Joseph R. Bertino, MD and Shigeo Yagi, PhD.

References
