Establishment and Characterization of a New Cell Line from a Canine Osteosarcoma

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ABSTRACT. A new cell line has been established directly from a spontaneous osteosarcoma on the femur of a 1.5-year-old male mongrel dog and named POS. Doubling time of the cells was approximately 33 hr. Morphologically, spherical cells, fibroblast-like cells, large or small polygonal cells and multinucleated giant cells seemed to be the major component of this cell line. The transmission electron microscopic feature of most of the cells was abundant dilated rough endoplasmic reticulum. Alkaline phosphatase activity as one of the osteoblastic properties was high in this cell line. The tumor tissue produced by the inoculation of the cells into nude mice was histologically identical to the original osteosarcoma.—KEY WORDS: canine, cell line, osteosarcoma.

Osteosarcoma is one of the most malignant tumors in both dogs [6] and man [8]. Various studies including the therapeutic modalities for human osteosarcoma with in vivo nude mice model or in vitro osteosarcoma cells [1, 10] have been reported. Canine osteosarcoma closely resembles human osteosarcoma in its histological appearance and biological behavior [3, 5], and may therefore be an excellent model for studying human osteosarcoma. A few canine osteosarcoma cell lines have been established, but these cells have often lost their original characteristics as spontaneous osteosarcoma and have produced undifferentiated sarcoma without new bone formation when transplanted into nude mice [7]. We have established a new cell line derived from a spontaneous canine osteosarcoma and named it POS. This paper reports the morphological features and characteristics of this cell line.

The original osteosarcoma developed on the left femur in a 1.5-year-old male mongrel dog (Fig. 1), where the femoral neck was considerably destroyed by the tumor. Immediately after the surgical removal of this lesion, the tissue was minced with a scalpel. A cell suspension was made from the minced tissues in RPMI-1640 medium (Nissui, Tokyo, Japan) containing 20% fetal calf serum (Cell Culture Lab., OH, U.S.A.) and antibiotics (gentamicine 50 mg/ml, amphotericin-B 1.5 mg/ml) without trypsin or collagenase. Viable cells at a concentration of 4 × 10^6 cells/ml were transferred into 25 cm² tissue culture flasks (Sumitomo Bakelite, Tokyo, Japan) with 5 ml RPMI-1640 medium and incubated at 37°C in a humidified 95% air - 5% CO₂ atmosphere. Several colonies were formed within 2 weeks. These colonies were treated with 0.1% trypsin and 0.02% EDTA, then subcultured at a density of 2×10^5 cells/ml under the same conditions. During an initial 4-month period, 20 passages were routinely cultured in this manner. Doubling time of the cells at the 4th, 8th and 20th passages was approximately 33 hr. Colony-forming ability (plating efficiency) was determined by plating 10^3–10^5 cells on 80 mm culture dishes and scoring for macroscopically visible, stained colonies after 14 days' incubation. The cells in most of the passages formed grossly visible colonies, but the plating efficiency was low (0.5–1.2%).

In this cell line, the following several cell types, spherical cells, fibroblast-like cells, large or small polygonal cells, and multinucleated giant cells, were morphologically distinguished by light microscopy (Fig. 2). Scanning electron microscopy revealed that spherical cells had ruffled membranes and long attenuated processes, and that polygonal cells were flattened. Some of polygonal cells were covered with small spheres (Fig. 3). Transmis-

Fig. 1. A radiograph of the left femur of a 1.5-year-old male mongrel dog with a naturally occurring osteosarcoma. The femoral neck was destroyed by the tumor.

Fig. 2. Micrographs of the cultured cells. Spherical cells (A), fibroblast-like cells (B), small polygonal cells (C), large polygonal cells (D), and multinucleated giant cells (E) were distinguished morphologically. × 450.
sion electron micrography of the same passage cells is shown in Fig. 4. Characteristic features of the cells included abundant dilated rough endoplasmic reticulum and irregular outlines of the cell and nucleus.

Alkaline phosphatase (ALP) activity of the cells was determined with the ALP measurement kit (Sigma Chemical, St. Louis, U.S.A.). The protein concentration was determined by the method of Bradford [2]. ALP activity of the cells in 10th passage was 1.59 μmol/min/mg protein. This high activity indicates that the cells have retained some of their osteoblastic function.

Chromosome analysis was performed by incubating the cells with 0.05 μg/ml colcemide (Gibco Lab., New York, U.S.A.) for 2 hr, treating with hypotonic KCl, and fixing with 3:1 methanol-acetic acid. Chromosome preparations were stained with Giemsa stain and photographed for counting. Chromosomes of fifty cells were analysed. The cells in the 4th passage showed an average of 48 chromosomes per cell (median: 48, range: 43–52), including approximately 20 biarmed chromosomes. This hypodiploid pattern was similar to that in the previous report on canine osteosarcoma karyotypes [9].

Cells (5×10^6) from the primary tumor, 4th passage and 12th passage suspended in the same medium were inoculated subcutaneously into nine 6-week BALB/c nude mice (Nippon SLC, Hamamatsu, Japan). Each cell produced a solid tumor with a diameter of 0.5–1 cm within a month in all nude mice. These tumors mainly consisted of osteoblastic type of osteosarcoma showing characteristic osteoid trabeculae (Fig. 5) and partially contained tissues of a chondroblastic type, a fibroblastic type and an undifferentiated type. These histopathological findings were almost the same as those for the original spontaneous tumor. Metastasis to the lung occurred in a nude mouse three months after the inoculation.

Osteosarcoma tissues in man are reported to be composed of several cell types such as anaplastic cells, osteoblast-like cells, fibroblast-like cells, chondroblast-like cells, osteocyte-like cells, myofibroblast cells, angioblastic cells and osteoblast-like giant cells [4]. In a mouse osteosarcoma cell line, several cells in various grades of differentiation are observed [11]. Several tissue types observed histopathologically in the original tumor and nude mice tumors, and several cell types distinguished morphologically in this newly established cells suggest that the osteosarcoma cells of any animal species consist of several different cell types. Osteosarcoma is a tumor of osteoblastic cells which are thought to be derived from stromal cells with a potential activity for differentiation, but it is not clear whether the morphological difference in osteosarcoma cells reflect the ability for differentiation or not. Further study will be needed concerning this important point.

These results indicated that the POS cell line maintains the characteristics of spontaneous osteosarcoma and provides an ideal in vitro and in vivo model for studying the histopathological and morphological characteristics, differentiation, metastasis, and new therapeutic modalities of both canine and human osteosarcoma.
REFERENCES