59. Establishment of Cell Lines from Human Osteosarcoma

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Extensive research has been reported on establishing *in vitro* cell lines derived from human tumor cells. However, a few research activities have been done on cell lines from human osteosarcoma (Ishii *et al.* 1978; Tsang *et al.* 1981; Eilon *et al.* 1983; Sekiguchi *et al.* 1983; Yamane 1985). Consequently, we tried to establish cell lines from human osteosarcoma through the transplantation into nude mice because it is difficult for osteosarcoma cells to obtain cell lines by directly culturing biopsied materials.

Materials and methods. The materials were sampled from the bone tumors of patients or from metastasized lung lesions. These materials were washed with PBS and minced with scissors after being deprived of the necrotic parts. The cell suspension was injected subcutaneously into the back of nude mouse to transplant a tumor. The tumor tissue was sequentially transferred to other mice at least five times. At every passage, the doubling time was from five to eight days. A grown tumor was excised out and digested with collagenase to prepare the single cell suspension. The cell suspension was plated onto a double-layered (0.5 and 0.33%) soft agar medium and cultured to form colonies. This procedure was to remove intermingled murine fibroblasts. Individual colonies were repeatedly transferred to double-layered soft agar media for cloning. The most proliferative of these clones was selected and cultured in a liquid media through serial passages. Four kinds of cell lines were established according to this method (Table I). OST1 and OST1-M cells were respectively derived from primary and metastatic lesions of the parosteal osteosarcoma of a patient. Moreover, the original cells for OST1 were sampled before the therapy, while those for OST1-M were extracted after chemotherapy and radiotherapy. OST2 and OST3 cells were respectively derived from primary and metastatic lesions of different osteosarcoma patients.

The chromosomes of these cell lines were analyzed according to Q-banding method. Metaphase cells were collected by subjecting the cells to colcemid for one hour at a final concentration of 0.05 $\mu\text{g/ml}$. The cells were then harvested through the trypsinization, treated with the hypotonic solution (0.075 M KCl) for 15 min, and fixed with methanol-acetic acid (3:1). Cells suspended in the fixative were dropped onto slides and air-dried. The slides thus prepared were stained with the Giemsa solution and quinacrine mustard-Hoechst 33258 solution (Yoshida *et al.* 1975).

Results and remarks. Sarcoma cells were effectively isolated from tumor tissues by repeating the transplantation in mice, and murine fibroblasts were also successfully removed by repeatedly cloning on double-layered agar media. All four biopsied materials (capable of forming tumors in mice when transplanted) yielded *in vitro* cell lines. Chromosomal analysis showed that these cell lines all originated from human tumors. The chromosomes in these cells were 43 for OST1 and OST1-M, 52-56 for OST2, and 74 for OST3 (Table I). Fig. 1

Table I. Established osteosarcoma cell lines

Cell lines	Age (years)	Sex	Origin · Diagnosis	Modal no. of chromosomes
OST 1	27	F	Tibia · paro. os.*	43
OST 1-M			Lung	43
OST 2	15	M	Lung · osteo.#	52-56
OST 3	11	M	Femur · osteo.#	74

* Parosteal osteosarcoma. # Osteosarcoma.

Fig. 1. Q-banding karyotype of an OST 1 cell consisting of 32 intact chromosomes with 11 marker chromosomes.

shows a Q-banding karyotype in one of OST1 cells. Of the total of 43 chromosomes, 32 are intact human chromosomes and 11 are marker chromosomes. A OST1-M cell, though having 43 chromosomes like the OST1 cells, contained 28 intact human chromosomes and 15 marker chromosomes (Fig. 2). Consequently, a comparison between the OST1 and OST1-M cells yielded the following results: The total numbers of chromosomes were the same, the fraction of intact human chromosomes was less in the OST1-M cells, and monosomies and deletions were somewhat common. In other words, the chromosomal composition was different between the two cell lines. As described above, OST1 was a descendant from primary tumor cells before therapy, while OST1-M was derived from metastatic locations after the chemotherapy and radiotherapy. Thus, the above karyotype difference between the two cell lines most likely reflects the effects of the drugs

Fig. 2. Q-banding karyotype of an OST 1-M cell consisting of 28 intact chromosomes with 15 marker chromosomes.

and/or radiation due to the therapy.

The marker chromosomes in the OST1-M cells contain a number of abnormally stretched ones, which, when analyzed cytogenetically, are inexplicable based on the simple translocation, but probably result from gene amplification. For the gene amplification involved in human tumor tissue, the following have been reported: N-myc gene amplification for neuroblastoma cell lines (Brodeur *et al.* 1984), and c-myc gene amplification for various tumor cell lines (Alitalo *et al.* 1983; Little *et al.* 1983; Michitsch *et al.* 1984). We look forward to analyzing the gene amplification observed in the marker chromosomes in the OST1-M cells, and clarifying the relationship between such genetic aberrations and the malignant function of cells.

Summary. Four kinds of cell lines (OST1, OST1-M, OST2, OST3) were established from human osteosarcoma through the transplantation into nude mice of biopsied tumors. OST1 and OST1-M cells were respectively derived from primary and metastatic lesions of a patient. The original cells for OST1 were sampled before the therapy, while those for OST1-M were extracted after the chemotherapy and radiotherapy. Q-banding analysis showed that the chromosomal composition was different between the two cell lines. Also, the marker chromosomes in OST1-M cells contained a number of abnormally stretched one which probably resulted from gene amplification.

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