INTRODUCTION

Although neo-adjuvant chemotherapy has greatly improved the 5-year survival of patients with osteosarcoma from 20% to about 60%, only 10% have achieved long-term disease-free interval,[1] necessitating further research on osteosarcoma. The aim of our investigations was to establish a cell line from tumor tissues resected away during reconstructive surgery, in cases diagnosed with osteosarcoma. This paper introduces a human osteosarcoma cell line, OS1 that was found to have a slow doubling time after having pass more than 80 passages. We present the initial characterization of this long-term cell line which exhibits runt-related transcription factor-2 (Runx2) gene expression and osteoblast specific markers.

METHODS

The study was approved by the Institutional Review Board, National University Hospital where informed consent for experimental use of the tissues were obtained. The biopsied tumor tissue was from a 6-year-old girl. The pathology diagnosis was osteosarcoma which was presented along the shaft of the right femur, The patient also presented with lung metastasis. Primary Culture: The tumor tissues were minced into small fragments and seeded in the cell culture flask (Nunc) and cultured at 37°C, 5% CO₂ in a mix medium comprising of RPMI 1640:DMEM (9:1), supplemented with 15% fetal bovine serum. While it was suspended, 0.25% trypsin was used. The non-tumor cells were removed with a TPP Cell Scrapper at the early stages. Target cells from the cell lines (more than 50 passages) were individually studied over a period of 4 days to observe for morphological changes. Cultured cells were seeded in twelve 96-well cell culture plates at the density of 1,000 per well. Half the medium volume was renewed daily. MTT assays (OD570) were done at intervals of 24-h and the colony doubling time calculated from a regression analysis. Immunohistochemistry (IHC) staining for Mouse originated monoclonal antibodies against type I collagen, osteocalcin, osteonectin and BMP4, were investigated. They were colored by biotinylated secondary antibody followed by ABC reagents and DAB substrate chromogen. For negative controls, PBS was used instead of primary antibody. Runx2 Gene Expression: One microgram of total RNA was used for the RT-PCR. The primer for human Runx2 (GenBank NM_004348) were 5’-cag ttg tcc cca agg tat tgg ctc cca aa-3’[2] The size of the PCR product was 443bp. Beta-actin (GenBank HSAC07) was used as an internal control. Its primer is 5’ cac act gtg ccc atc tac gag g 3’ and 5’ agt ttc gtg gat gcc aca ata tgg tcg cca aac ag -3’. The size of the PCR product was 350bp. For electrophoroses, 2.5ul of the PCR products were used in a 1.5% agarose gel. For this part, fibroblast from human skin act as the negative control. These were compared to other osteosarcoma cell lines from our local series (OS2 and OS3). Karyotype Analysis: Cultured cells at different passages (15, 21, 31 and 59) were treated with Demecolcine (Sigma) solution at the final concentration of 0.4ug/ml for 16-h, 0.075M KCl for 20- min and absolute methanol/glacial acetic acid (3:1; v/v) overnight, and then used for chromosomal analysis and to investigate their stability.

RESULTS

Currently the tumor cells have been cultured in vitro for over 22 months, passing 80 passages to form a continuous cell line which has been labeled OS1. From this cell line, 3 main cell morphology types were observed. The cell appears as a rounded cell, just after being attached, becoming polygonal as the cells come together, and then becomes spindled shaped during the initiation of cell migration. The colony growth curve was represented by $y = 0.0366e^{0.1508x}$ ($r^2=0.9371$) over 12 days, with the colony doubling time calculated as 4.6 days (Fig. 1). From the differentiation study of OS1 cells, BMP4 was slightly stained, while Collagen type I was strongly stained. Osteocalcin and osteonectin also demonstrated strong strains against the negative control. (Fig. 2). The cells also expressed the Runx2 gene (Fig. 3). Most of the karyotypes were abnormal, not only in their numbers but also in translocation (Fig 4).

DISCUSSION

The age of the patient (6-y), the location of the disease (along the shaft of the femur) and the early lung metastasis, make this an interesting case. Initially, a variety of cells proliferated, and the fast growing cells had to be removed with the cell scraper before a more stable cell line was generated. At the 50th passage, the cell line demonstrated positive staining for BMP4, collagen I, osteocalcin and osteonectin at the 50th passage, confirmed that the cell line was osteoblast specific. Runx2 gene expression further confirmed this. Interestingly, a commercial cell line (143.98.2), like fibroblast derived from the tumour tissue, did express the Runx2 gene. The complex karyotype with its polymorphism was sustained from passage 15 to 59. Given, the slow colony doubling time of about 5 days, as compared to other reports of osteosarcoma cell lines having an average of 2-3 days, this OS1 cell line becomes an important resource to study the paracrine interaction in a co-culture system with other well-differentiated cells which have similar colony doubling time (e.g. HuVEC, osteoblast, osteoclast, fibroblast).

AFFILIATED ORGANISATION

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REFERENCES