A TECHNIQUE FOR DEVELOPING ESTABLISHED CELL LINES FROM HUMAN OSTEOSARCOMAS

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SUMMARY

A method is described which has been successfully used to develop two human osteogenic sarcomas into established lines in culture. This method provides a means whereby cells growing from explanted tumor tissue can be immediately cloned and the fibroblastic (nonneoplastic) cells thus selected against. Both lines have been passaged for over 100 population doublings since cloning and have retained the ability to form colonies from single cells plated at low density without the use of feeder layers or conditioned medium. In culture, the osteogenic sarcoma cells are nonfibroblastic, pile up, and appear to retain a morphological similarity to the in vivo tumors from which they were derived. A karyotype of cells derived from one of the tumors containing a marker chromosome is also presented.

Key words: human tumor; osteosarcoma; established line; suspension cloning; karyotype.

INTRODUCTION

The methods for establishment of human tumors in tissue culture have seldom been described so as to be easily reproduced by many laboratories. Generally, only a small per cent of tumors derived from human tissue have been successfully established in culture (1). In this report we describe a method successfully used to establish two human osteogenic sarcomas into tissue culture, and we are able to show histological evidence that these lines maintain morphological similarity to the tumors from which they were derived. In addition, both lines could be cloned after only a short time in culture. After 100 in vitro population doublings, each retained the ability to form colonies derived from single cells plated at very low densities without the use of feeder layers or conditioned medium. Karyological preparations of each sarcoma line are shown as well as photomicrographs of the tumors in vivo and in vitro.

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42.5% Ham’s F-10, 42.5% Eagle’s Minimal Essential Medium (MEM) (Microbiological Associates, Inc.), 7.5% fetal bovine serum, 7.5% horse serum, and supplemented with 1X non-essential amino acids, 450 mg per l glucose, 3.3 mg per l sodium pyruvate, and 50 μg per ml Aureomycin (Lederle). Previous empirical observation suggested that medium with only fetal bovine serum favored the excessive growth of normal fibroblasts found in tumor specimens, while medium with only horse serum did not provide for optimal growth of tumor cells; thus an equal mixture of the two sera was employed. The work of others (2,3) also suggested that horse serum may favor somewhat the growth of neoplastic cells in vitro. All cultures were grown and maintained in a humidified, 5% CO₂, 95% air incubator at 37°C.

Primary cloning. When areas of obvious tumor cell morphology became visible in the explant dishes, media changes were performed every other day, and those areas which appeared most viable (generally about 1 mm in diameter) were selected for cloning by the use of stainless-steel cylinders or by scraping them off the surface of the dish with a rubber policeman. Cells from each area were then transferred to separate 35-mm-diameter plastic Petri dishes, grown to confluency, and trypsinized into a single cell suspension for cloning in the suspension medium.

Suspension cloning. Suspension medium for cloning of tumor cells was made according to the method of Stoker and co-workers (4). A suspension of 2% Methocel (Dow Chemical, 4000 centipoise) in complete medium (w/v) was prepared immediately before each use. As described under Primary Cloning, 5 to 6 × 10⁶ cells to be cloned were trypsinized from monolayer and suspended as single cells in 0.5 ml of complete medium without Methocel and mixed into a 60-mm Petri dish containing 12 ml of Methocel medium. These cultures were incubated for 8 to 10 days without media renewal at 37°C in a humidified, CO₂ incubator. When suspended colonies of approximately 3/4-mm diameter became visible, they were selected with a pasteur pipet and anchored to the bottom of a 35-mm-diameter Petri dish by overlaying with a sterile glass cover slide; these selected clones were allowed to grow to a confluent monolayer with twice weekly media changes before being subsequently passaged.

Pathology. Surgically derived tumor specimens and the pelleted samples of cells cultured from them were processed by the paraffin-embedding techniques and stained with hematoxylin and eosin for light microscopy.

Chromosome preparations. A modification of the technique of Sakse and Moorehead (5) was used for preparation of karyotypes. Exponentially growing cultures in monolayer were exposed to 0.04 μg per ml of colchicine in complete medium for 3 hr at 37°C. Cultures were then trypsinized, swollen for 30 min in 0.075 M KCl, fixed in Carnoy’s solution, and processed by the air-dry method; slides of prepared cells were stained in giemsa.

RESULTS

Within 5 days after being explanted, tumor tissues showed outgrowth of fibroblast cells; after 10 days, both explanted tumors had developed microscopically visible areas of cellular piling up in which epithelial-like cells could be clearly distinguished from the fibroblasts. These areas of epithelial-like cells could be observed to grow either on the plastic surface of the Petri dish or over an area of fibroblast cells.

Approximately 30% of those foci suspected of being tumor cells and which were therefore selected by the use of stainless-steel cloning cylinders survived when subsequently plated directly into monolayer. These clones, which may have contained both fibroblasts and tumor cells, generally reached confluency after 10 to 14 days of growth and were then plated as described above into suspension medium. Presumably, any normal (nonneoplastic) cells thus would have been selected against because of their inability to grow in suspension. Thus cells subsequently selected after growth in suspension medium had been selected first, from the original culture dish which contained the explanted tumor tissue, and secondly, as a result of their ability to grow in suspension. After surviving this technique, cells were then re-established into monolayer culture.

Once a cloned colony was returned to monolayer, a period of irregular growth occurred over a period of 2 to 3 months. This was characterized by alternate areas of sparse and rapid growth which appeared in cultures of otherwise health-appearing cells. Confluent monolayers were split at a 2:1 ratio during this time. Plating efficiencies performed on cells at this time without the use of feeder layers or conditioned medium ranged from 2 to 3%. Eventually, cells maintained in mass cultures attained stability as evidenced by higher plating efficiencies (up to 12%). These cultures