ESTABLISHMENT, CHARACTERIZATION, AND RESPONSE TO CYTOTOXIC AND RADIATION TREATMENT OF THREE HUMAN MELANOMA CELL LINES

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(Received July 12, 1982; accepted February 18, 1983)

SUMMARY

Three human melanoma cell lines were derived from tumor specimens and established in culture. CAL 1 originated from a bone marrow metastasis and CAL 4 and CAL 7 were derived from solid tumor fragments. CAL 1 and CAL 7 were cloned before establishment. Ultrastructural and chromosome analysis were carried out along with the response to nine chemotherapeutic agents at various concentrations. Survival curves after irradiation were also plotted. The uncloned cell line, CAL 4, displayed some differences from the other two cell lines as regards ploidy and response to chemotherapy. Greater spread of chromosome numbers were observed with this cell line, which contained both hypoploid and a hyperploid modal numbers. All three cell lines showed a relatively high extrapolation number after irradiation, suggesting that inherent cellular properties may be partly responsible for the clinical radioresistance of malignant melanomas.

Key words: human melanoma; cell lines; karyotype; ultrastructure; chemosensitivity; radiosensitivity.

INTRODUCTION

Melanoma mortality rates have almost doubled since 1960 (1). The increasing incidence of this disease is one of the reasons justifying attempts by epidemiologists and other research workers to clarify the precise etiological factors involved and the natural history of melanoma. Malignant melanoma still carries a poor prognosis among human cancers; in particular, it is considered resistant to chemotherapy and radiotherapy, and surgery is often advocated.

Human melanoma cells can be grown readily in vitro, and numerous cell lines have been established (2-12). Although they share common properties pertaining to the tissue of origin, such cell lines present considerable variations that may reflect the individual variations encountered in clinical practice. Moreover, heterogeneity has been demonstrated within the parental tumor (13), and cloning methods have been adopted to study the subpopulations of cells that form the tumor.

The three human melanoma cell lines presented here (CAL 1, CAL 4, CAL 7) were derived from patient tissue biopsies. Two of these cell lines (CAL 1, CAL 7) were cloned before establishment. The sensitivity of these cell lines to currently used chemotherapeutic agents was studied, and cell survival curves after single doses of radiation were plotted.

MATERIALS AND METHODS

Clinical History and Tissue Culture

For all three cell lines, culture establishment and cell maintenance were conducted using Eagle’s minimum essential medium (MEM) modified with Earle’s salts, with 1% l-glutamine (200 mM), 10% fetal bovine serum (FBS) (Flow Laboratories), 400 U/ml of penicillin, and 200 µg/ml of streptomycin. Cells were kept in an incubator at 37° C with an atmosphere containing 5% CO2.

CAL 1. In April 1980, a 45-yr-old woman underwent an enlarged colpohysterectomy for a probable undifferentiated mesenchymal tumor of the cervix uteri that had metastasized to the ovary. Complementary treatment included five
courses of chemotherapy that resulted in severe tricytopenia. In view of the clinical findings, a bone marrow sample was cultured in agar in September 1980 and was found to be metastatic. After 21 d in culture, small clones could be seen with the naked eye. These clones were removed from the soft agar using a tapered Pasteur pipette and placed in 1.5 cm diam well of a 24 hole Costar culture dish containing liquid medium. The cells rapidly left the clones and spread out in a single layer. Centrifugation and staining using Fontana's silver salt technique revealed the melanin pigment and settled the diagnosis of melanoma (Fig. 1). The original biopsy specimen was revised subsequently and the diagnosis was confirmed.

**CAL 4.** A 66-yr old man presented with a painful bulky tumefaction of the right axillary region, of recent onset. At the time of excision, this mass already had diminished spontaneously in size. The laboratory diagnosed a necrotic and pigmented nodal metastasis of a malignant melanoma. A fragment of this node was removed under sterile conditions. Using tapered scissors, a cellular suspension of this tissue was obtained by immersion in culture medium in a hemolysis tube. The suspension obtained was passed through two metal filters to eliminate any large clumps, rinsed twice, then suspended in culture medium in a sterile flask with a screw top. Three months later, three foci of melanoma cells, which later gave rise to cell line CAL 4, arose within a bed of large, pigmented fibroblastic cells.

**CAL 7.** In December 1980, a 75-yr-old woman underwent surgical removal of an excavating ulceration of the left temple diagnosed as a nodular malignant melanoma. A sterile fragment was removed and placed in cellular suspension, as

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<tr>
<th>TABLE 1</th>
<th>SUMMARIZED HISTORY OF THE THREE CELL LINES</th>
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<tr>
<td><strong>Origin</strong></td>
<td><strong>Pathological Characteristics</strong></td>
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<tr>
<td>Bone marrow biopsy</td>
<td>Metastatic mesenchymal tumor. Special staining of cultured cells established the diagnosis of melanoma</td>
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<tr>
<td>Axillary lymphadenectomy</td>
<td>Metastatic necrotic pigmented malignant melanoma</td>
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<tr>
<td>Excised mass in the temporal region</td>
<td>Nodular malignant melanoma</td>
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