ESTABLISHMENT AND CHARACTERIZATION OF A CELL LINE DERIVED FROM A SPONTANEOUS MURINE LUNG CARCINOMA (M109)

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SUMMARY

The Madison lung (M109) tumor cell line, initiated from a “spontaneous,” anaplastic murine lung carcinoma, has been propagated continuously in vitro for more than 300 cell generations. Cytogenetic analysis revealed a mouse karyotype with a mode of 78 chromosomes (2n = 40). Three distinct marker chromosomes were identified by trypsin-giemsa banding. The cells piled up in culture and had a short generation time and high plating efficiency. Electron microscopy revealed highly undifferentiated cells with little rough endoplasmic reticulum, an abundance of free polysomes, the presence of few and often odd-shaped mitochondria, lipid bodies and phagocytic vacuoles. Virus particles of the C-type were found frequently. The subcutaneous transplantation of M109 cultured cells at a relatively low cell inoculum produced highly metastatic tumors in syngeneic BALB/c mice.

Key words: cell culture; lung carcinoma; C-type virus.

INTRODUCTION

The Madison alveolar carcinoma (M109) arose from the lung of an 18-month old female BALB/c mouse at Microbiological Associates, Bethesda, Maryland, in 1964. The tumor was considered to have arisen “spontaneously,” although the mouse previously had received a cell-free extract of a human testicular tumor. We endeavored to establish this tumor in continuous cell culture since well characterized tumor cell lines of spontaneous etiology are of immense value in biochemical and immunologic studies of neoplasia.

The M109 cell strain has been carried continuously in this laboratory for over 50 passages and has retained high tumorigenicity with the ability to metastasize to a distant site when injected into syngeneic BALB/c mice. In this report, we present ultrastructural, karyologic and growth characteristics of the M109 in vitro cell line.

MATERIALS AND METHODS

Cell source and cultivation. M109 cells were derived from a poorly differentiated lung carcinoma in a BALB/c mouse. The tumor was kindly supplied to us by Ruth L. Geran, Drug Research and Development, National Cancer Institute, NIH, Bethesda, Maryland, and has been maintained as a transplantable line in BALB/c mice. A subcutaneous tumor in its 100th in vivo passage was dissected aseptically, washed three times in Dulbecco’s PBS and trypsinized (0.25% trypsin) at 23°C. The dispersed cells were collected after 30
min, washed once in PBS and plated in 250-ml plastic flasks. Primary cultures were initiated and maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (treated at 56°C for 30 min), 50 μg Gentamicin per ml and 2.5 mg Fungizone per ml (RPMI-FBS). The medium was buffered with 0.075% NaHCO₃ and 10 mM HEPES solutions. Fresh medium was added to the culture 24 hr after plating and at 3-day intervals thereafter.

**Growth curves and plating efficiency.** Cultures of M109 and a clone derived from the M109 cell strain were used at low passage (passage 6) and high passage (passage 21) generations for growth curve studies. At each passage level semiconfluent cultures were trypsinized (0.25% trypsin), washed and resuspended in medium for counting. For each time point, 1 x 10^4 cells per 25 cm² tissue culture flask were plated in 4 ml RPMI-FBS. Cells, which were removed from the flasks by trypsin treatment at 24-hr intervals after plating, were resuspended in RPMI 1640 and counted in a hemacytometer. The counts from triplicate cultures at each interval were averaged. The plating efficiencies of M109 and the Madison clone were determined from a portion of the culture used in the growth curve experiments. Three sets of plastic flasks were plated in triplicate with 125, 250, 500 and 1000 viable cells and incubated at 37°C. After 6 days, the cultures were washed three times with Dulbecco's PBS, fixed with absolute methanol and stained with Giemsa stain. Colonies consisting of greater than four cells were counted with a stereoscopic microscope.

**Growth of colonies in soft agar.** Cultures of M109 were tested for their ability to grow on semisolid agar using a modification of the technique of Macpherson (1). Briefly, 2 ml of a mixture of NCTC-135 medium (2X) and 1% agar was poured into 25-cm² tissue culture flasks and allowed to gel. This basal layer was overlayed with either 125 or 250 viable M109 cells suspended in equal volumes of NCTC-135 medium (2X) containing 30% fetal bovine serum and 0.6% Noble agar. The flasks then were incubated at 37°C for 6 days. Colonies consisting of >5 cells were examined and counted with the aid of an inverted microscope.

**Cytogenetic analysis.** M109 cells were studied at the 19th passage for cytogenetics. The chromosomes were identified by trypsin-Giemsa (T-G) banding according to the method of Wurster (2).

**Histopathology.** Part of the tumor which was used for establishment of the cell line was fixed in 10% neutral formalin. The tissue then was sectioned and stained with hematoxylin and eosin.

**Tumorigenicity of parent line and clone.** For determination of tumor-producing capacity, M109 and clone cell suspensions containing log₁₀ dilutions from 10⁰ to 10 cells per 0.2 ml were inoculated subcutaneously in the right inguinal region of BALB/c mice. The animals were monitored twice weekly for tumor size and checked daily for survival. The lowest dose producing 50% lethal tumors (LD₅₀) was determined.

**Electron microscopy.** For electron microscopy examination, the cells were fixed in situ with 2.5% glutaraldehyde, buffered to pH 7.2 with 0.1 M sodium cacodylate and scraped from the flasks. The cells then were pelleted and postfixed with chrome-osmium (3). They were embedded in Epon-Araldite, sectioned with an LKB Ultrrotome III, stained with uranyl and lead salts, and examined in a Siemens Elmiskop 1A equipped with double condenser, 50-μ objective aperture and an accelerating voltage of 80 kV.

**RESULTS**

Histologically, the M109 tumor represents a poorly differentiated lung carcinoma (Fig. 1). The tumor cells contained eosinophilic cytoplasm without distinct cytoplasmic borders. The large nuclei were round or oval-shaped, contained up to three prominent nucleoli and had fine granular distributed chromatin. Many nuclei had one or two deep indentions. Mitotic figures were abundant.

The M109 cells in vitro were markedly similar in appearance to histologic sections of the original tumor. Colonies at early passages exhibited a strong tendency to clump and pile up (Fig. 2), and have retained this quality at later passage levels (Fig. 3). Very few fibroblasts were seen in the primary culture and unexpectedly none were evident by the third passage. Morphologically, these cells have not altered over repeated passage generations.

M109 cells were tested for population doubling times. Under the conditions of the experiments described above, M109 and Madison clone cell cultures showed an initial growth lag of approximately 24 hr, and then an exponential phase of growth between 24 and 96 hr after plating. The mean doubling times (Table 1) were calculated by averaging the doubling times determined at several intervals over the exponential phase of the growth curve. M109 cells had a mean population doubling time of 18.7 hr when tested at passage 6.