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New Cell Line

EPITHELIAL CELL LINE AND SUBLINE ESTABLISHED FROM PREMALIGNANT MOUSE MAMMARY TISSUE

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SUMMARY

A cell line and subline with epithelial characteristics were established from mouse mammary hyperplastic alveolar nodules (HAN). The cells do not grow in suspension cultures in vitro or form tumors in vivo. The cells do produce significant amounts of C-type and A-type virus and low amounts of plasminogen activator.

Key words: epithelial; mammary cells; hyperplastic nodules.

INTRODUCTION

The mouse mammary system includes a class of premalignant lesions, hyperplastic alveolar nodules (HAN), which proceeds to malignancy much more readily than does normal mammary tissue (1). The determination of the fundamental properties of these premalignant cells will potentiate our understanding of the role of these lesions in the neoplastic process. Medina (2) has successfully established in vivo a series of transplantable outgrowths of mouse HAN tissue having different tumorigenic potentials in cleared (parenchyma-free) mammary fat pads. To date no in vitro cell lines of premalignant mammary origin have been developed. The cell line and subline described below were established from the D1 outgrowth line. The D1 line has a low tumorigenic potential when transplanted into cleared mammary fat pads and is believed to be made up of a variety of cell types (3).

ISOLATION METHOD

The WAZ-1 cell line was initiated on 12/6/76 from five BALB/c mammary fat pads that contained hyperplastic outgrowths of the D1 line (8 weeks post-transplantation). The minced tissue was rinsed with Ca-Mg-free saline and resuspended in basal Eagle's medium plus 0.3 mg per ml of collagenase (type 1, Sigma, St. Louis, Missouri). The suspension was plated into three small flasks (25 cm^2, Falcon Plastics, Los Angeles, California) and incubated at 37° C for 24 hr in a 5% CO_2/air environment. The digested tissue fragments were agitated by rapid pipetting and allowed to settle, at which time most of the collagenase solution was removed. The remaining cells were incubated in complete medium, which included Dulbecco's modified Eagle's medium plus 10% fetal bovine serum (GIBCO, Grand Island, New York), 10 μg per ml insulin (Calbiochem), 60 μg per ml penicillin (Sigma) and 100 μg per ml streptomycin (Sigma). Selective saline-trypsin-versene treatments were used over the next several months to remove fibroblastic cells (4). The original culture was not passaged for 1½ months. At that time the cells were passaged every 2 weeks. After several further passages, the cells were subcultured weekly 1:2. Since the original WAZ-1 cell line occasionally showed small areas of less epithelioid cells, a subline was isolated from the parental culture. This subline, C1-S1, maintains a strictly epithelial morphology (Fig. 1). Both cell cultures have been carried for over 40 passages and have been found to be free of mycoplasma (Flow Laboratories, Rockville, Maryland).

GENERAL CHARACTERISTICS

The doubling times of WAZ-1 and C1-S1 cells are approximately 22-24 hr, and the cells grow to

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a relatively low saturation density of 1–2 × 10^5 cells per cm^2. WAZ-1 and C1-S1 cells are aneuploid in chromosome number with modes in the sixties, and the chromosomes of both cell cultures are telocentric (consistent with a mouse cell origin) with an occasional metacentric chromosome present. When plated into suspension cultures using either methylcellulose or agarose as a supporting medium, neither WAZ-1 or C1-S1 cells grew into colonies. Plasminogen-activator tests showed that these cells produce considerably less plasminogen activator than do malignant cell lines of similar origin. Subsequent in vivo experiments using cleared mammary fat pads and subcutaneous inoculations have shown that neither the cell line or subline produce tumors in syngeneic mice. Cytologic analysis of Giemsa-stained cells indicated that the cells were less pleiomorphic in size and shape than were cells isolated from malignant mammary tissue.

WAZ-1 and C1-S1 cells clearly demonstrate several ultrastructural markers typical of epithelium. These include spot demosomes with prominent tonofibrils and numerous interdigitating microvilli at the intercellular borders (see Fig. 2*A*,*B*). Both also contain polymorphic mitochondria, a few microtubules and microfilaments,

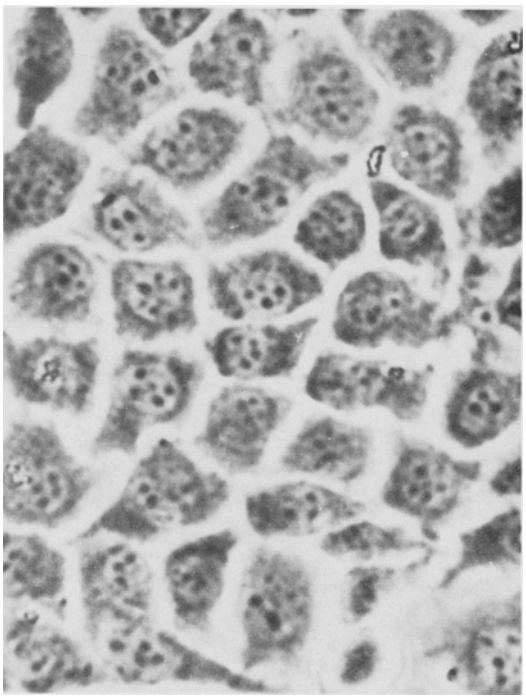
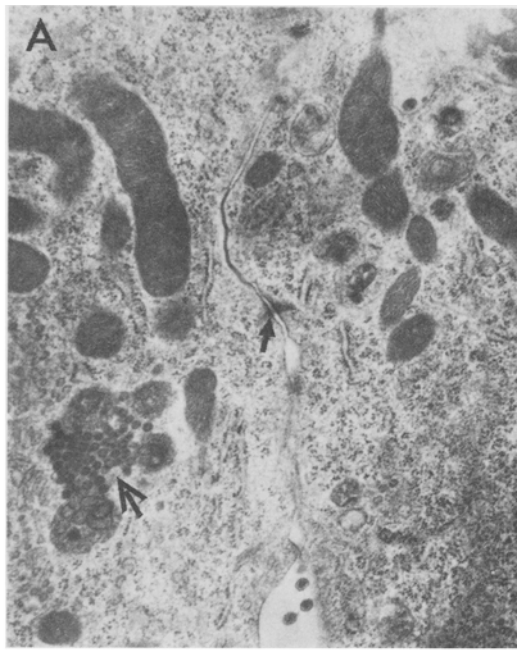


FIG. 1. Epithelioid monolayer of C1-S1 cells in culture. Phase contrast. ×560.



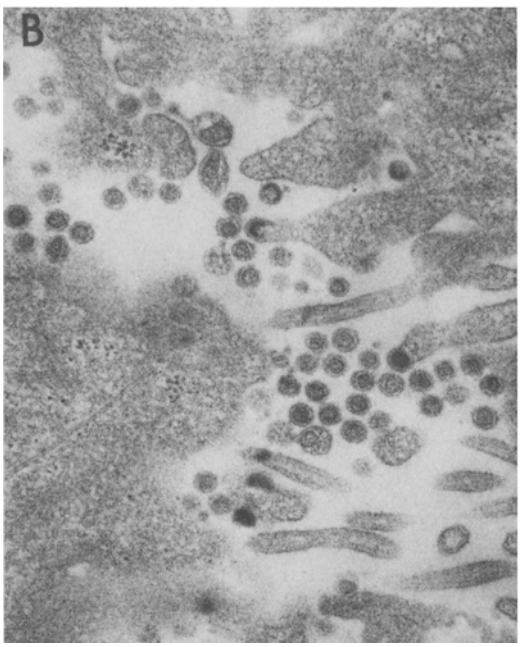


FIG. 2. Ultrastructural features of the WAZ-1 and C1-S1 cells. *A*, A desmosome (*small arrow*) and associated junctional complex between two WAZ-1 cells. Also present are numerous intracytoplasmic A particles (*large arrow*) and extracellular C particles (lower center of photo). ×6254. *B*, Numerous microvillar structures between adjacent cells and extracellular and budding virus particles. ×24,380.

and a moderately well-developed Golgi apparatus. Also present are C-type virus and a number of intracytoplasmic A particles (especially in the WAZ-1 cells). Definite B-type virus was not detected using electron microscopy, but we cannot rule out the possibility that such virus may be found using other techniques. Weibel-Palade bodies, which are characteristic of endothelial cells, have not been observed. The composite of these structural properties clearly indicates that these cells are of epithelial origin. It is our hope, therefore, that this cell line and subline will be useful in comparative studies with malignant mammary epithelia and in the study of preneoplasia.

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Note Added In Proof: The C1-S1 subline produces secretory domes at low passage in culture and does not produce endothelial Factor VIII.

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