LONG-TERM HUMAN BREAST CARCINOMA CELL LINES OF METASTATIC ORIGIN: PRELIMINARY CHARACTERIZATION

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SUMMARY

Nineteen human breast carcinoma cell lines have been established as continuous cultures during the past 6 years in our laboratory. This preliminary report is designed to list the lines by their designated code numbers (MDA-MB) and present a brief summary of their morphological, cytogenetic and biochemical characteristics. Sixteen of our lines were obtained from pleural effusions, two from brain metastases, and one from pericardial fluid. All lines have been shown to be distinct entities and are uncontaminated by HeLa cells or each other. A lq marker chromosome is present in all but one of the lines examined.

Key words: cell lines; metastatic; human breast carcinoma.

INTRODUCTION

The establishment of continuous cell lines from human breast carcinomas has been difficult and rare. Since Lasfargues and Ozzello (11) first obtained their line BT-20 in 1958, only a handful of authenticated continuous lines have been reported (2-5).

We have found the use of pleural effusions or other metastatic sites to be more fruitful. The advantages are (a) possible yield of large amounts of viable cells; (b) lack of contaminating fibroblasts; and (c) occasional sequential pleural effusion samples from the same patient.

All of the 19 lines described here have been isolated and established in continuous culture from patients at M. D. Anderson Hospital and Tumor Institute. These women were originally diagnosed as having carcinoma of the breast, and additional pathological evidence after surgery or needle biopsy confirmed this diagnosis. Because our lines were established from metastatic sites (16 pleural effusions, 2 brain metastases, and 1 pericardial fluid), it was necessary to study several parameters to verify their breast, epithelial and/or tumor origin. Previous reports on the first four lines isolated from pleural effusions describe most of the procedures used (6-8), and only newer modifications will be presented in their report. Additional information on a unique marker chromosome present in seven lines also has been published (9).

We describe or list briefly here the salient features of our 19 lines. Detailed analyses of the chromosome markers and karyotypes, comprehensive isozyme patterns, histological and pathological characteristics, immunological reactions, tumorigenicity in nude mice and biophysical studies are being prepared and submitted for publication.

MATERIALS AND METHODS

Pleural effusions or pericardial effusions. These were prepared as described in detail by Cailleau and Reeves (8).

Brain metastases. Pieces of the tumor removed at surgery were sent to the pathology frozen-section laboratory. Line MDA-MB-361 was established from a nonsterile specimen which was placed in sterile medium containing 3X antibiotics, then scraped, transferred, and washed three times in petri dishes containing similar medium. The exterior portions of the piece were removed with sterile scalpels and the presumably sterile inner portion transferred to another sterile medium. This portion then was sliced into small pieces about 1-mm square and transferred to a final rinse in medium with 3X antibiotics. Flasks then were inoculated and cultured as previously de-
scribed. MDA-MB-461 was obtained aseptically and, except for fewer rinses, handled in a similar fashion.

**Media.** While a variety of media were used or tested in our early studies, for the last few years we have concentrated on three variations: (a) Leibovitz's Medium L-15 supplemented with 16 μg per ml glutathione (reduced); 10 μg per ml insulin (zinc-crystalline) and antibiotics; 50 μg per ml gentamicin; and 100 μg per ml disodium carbenicillin. (b) Fetal bovine serum from two sources, at a level of 10% or 15%, obtained from Grand Island Biological Co. and Reheis Chemical Co. (c) Bovine amniotic fluid (Grand Island Biological Co.) used in conjunction with 10% fetal bovine serum for the isolation of cell lines or to try to accelerate the growth of slow growing lines.

Usually our media consisted of: (a) L-15 plus 10% or 15% GIBCO fetal bovine serum; (b) L-15 supplemented with 10% or 15% Reheis fetal bovine serum; and (c) L-15 supplemented with 10% Reheis FBS plus 10% bovine amniotic fluid.

All lines were carried in duplicate by two or more investigators. Both glass and plastic flasks were used, and at least one flask from each line was on a different serum. Three separate incubators were used (one joint and two separate ones).

**Freezing.** Cells were frozen at −70°C or in liquid nitrogen starting, if possible, with the original fresh sample of washed and packed cells. Alternately 10% DMSO or 10% glycerol was used in the slow freezing procedure. Thereafter and at frequent but irregular intervals after the cultures were established, they were frozen (at least twice a year).

**Nude mice tests.** When enough material was available, fresh pleural fluid or pieces from the brain metastases were transported to Dr. B. Giovannella’s laboratory at St. Joseph’s Hospital and Cancer Institute as soon as possible and injected by him into nude mice. Otherwise the cultures were grown in several large flasks until there was enough material (1 × 10⁸ cells per mouse) for use in two to four mice.

**Isozyme studies.** The study of the most important isozyme, G6PD, and the basis of much controversy, is reported here. A detailed study of several other important isozymes which includes PGM₁, PGM₂, ES₉, ME₉, GLY₁ and 6PGD is in progress and has been able to differentiate all our lines from each other and from HeLa; this study will be submitted for publication shortly.

**Chromosomes.** This report presents the modal chromosome numbers obtained from the fresh pleural effusion or from an early passage of each line. A detailed study of one of our lines (MDA-MB-468) has been accepted for publication (10) and describes the various methods used for G- and C-banding, the karyotype and the markers present.

**Electron microscopy.** If possible, a pellet of packed cells was obtained from the fresh pleural effusion as were packed cells or small colonies from the cultures after they had been established. These were prepared as previously described (11).

**Staining.** Routine papanicolaou and hematoxylin and eosin stained slides were made from our fresh samples by the pathology department. Similar slides were made of the tumors produced in the nude mice and compared with the slides made from the primary breast tumor.

**Tests for PPLO.** Chen’s method (12) which uses the Hoechst fluorescent dye staining technique, was used on all cultures when they were first established and thereafter at irregular intervals. A number of our cultures grown in one of our sera became heavily contaminated, possibly due to one batch of contaminated fetal bovine serum. However, our precautions of separate maintenance in different media in different incubators by different people, along with freezing the cells at different passages that had been found PPLO-free, provided us with uncontaminated lines with one exception IMDA-MB-461). As this line was of recent origin and very lightly contaminated, and had not been frozen, we treated it as recommended by Dr. Chen: chlorotetracycline from a 50X solution (5000 μg per ml) was added twice per week for a period of 10 to 12 weeks at every medium change after which treatment then was stopped. After 2, 3 and 4 weeks, the cells were tested for PPLO and found to be negative.

**EXPERIMENTAL RESULTS**

The data in Table 1 briefly summarizes some characteristics of these 19 lines designated by letters MDA-MB.

1. All lines appear epithelial in origin as examined by light and electron microscopy and show typical desmosomes, microvilli and large irregular nuclei (Fig. 1). The abundance of these features is greatest in the colonies or plaques characteristic of some of the lines and least in the lines MDA-MB-134, 453, 468, 469.