ESTABLISHMENT OF TWO NONMETASTASIZING AND ONE METASTASIZING RAT MAMMARY CARCINOMA CELL LINES

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SUMMARY

Two continuous rat mammary tumor cell lines have been established in culture from the lymphogenously metastasizing rat mammary carcinoma TMT-081 and one from the nonmetastasizing MT-100 and some of their in vitro and in vivo characteristics studied. Cell line TMT-081-MS was established as a free-floating cell suspension from the metastasis-free spleen of a rat bearing TMT-081 in the ascites form and is characterized by a high level of mammary tissue specific antigen (MTA), an antigen present on lactating or hormonally stimulated rat mammary tissues but not detected on normal mammary tissue. This line metastasizes in the syngeneic host but is rejected by the nude mouse without metastases. Cell line TMT-081-NM is a line derived from the ascites of a rat also bearing TMT-081 ascites. Cell line MT-100-TC is a line derived from the ascites of a rat bearing the ascites form of MT-100. Neither TMT-081-NM nor MT-100-TC has ever shown metastases in the syngeneic host but they are lethal; in the nude mice they grow rapidly, are lethal, and sometimes show hematogenous metastases. Both grow in small clusters and show a low level of MTA. These cell lines have been in continuous culture for a year and have proliferated and maintained their individual in vitro and in vivo growth characteristics during more than 100 consecutive subcultivations.

Key words: mammary tumor cell culture; lymphogenous metastasis.

INTRODUCTION

Although a number of well-established rodent mammary tumor cell lines are available (1-3), only rarely do they metastasize when inoculated back into syngeneic animals and then metastasis is usually confined to the lung. However, an experimental mammary tumor cell line suitable for the study of lymphogenous metastasis has been reported recently (4). There are also a number of well-established human breast cancer cell lines, but their biological characteristics can only be gleaned in vivo by xenografting them into athymic nude mice (5-8). Therefore, many of these cell lines have limited usefulness for studying the mechanism of metastasis in general and of lymphogenous metastasis in particular.

Here we report the in vitro establishment of three mammary tumor cell lines of W/F rats; one spontaneously metastasizes via the lymphatic route in the syngeneic rat and two are nonmetastasizing. To establish a long-term culture of tumor cell lines directly from solid carcinomas is frequently difficult on account of the low viability of the epithelial tumor cells and the frequent outgrowth of stromal fibroblasts overtaking these cells. Many of the successfully established tumor cell lines, especially human breast carcinoma lines, have been derived either from the cultivation of tumor cells isolated from malignant effusions or from successful xenografts of carcinomas in athymic nude mice (5,6,8,9). Although some rat mammary tumor cell lines have been established successfully from the solid tumor (4), we have not be able to establish any of our metastasizing and nonmetastasizing rat mammary carcinomas in vitro from the solid tumors.
However, we were successful when rats bearing the ascites forms of these tumors were used.

The tumor cell lines described here should be particularly useful in the multidisciplinary investigation of the mechanism of tumor invasion and metastasis because there is already a substantial knowledge of the biological, biochemical, and immunological characteristics of the original solid tumors (10–13). Some of the properties and characteristics of these cultured tumor lines are described in this report.

**MATERIALS AND METHODS**

Ficoll-Paque was from Pharmacia, Piscataway, NJ. Dulbecco’s modified Eagle’s medium with high glucose (0.45%) (DMEM-HG) and RPMI 1640 medium were prepared in the media facility of the Department of Molecular Immunology using Powders 430-2100 and 430-1800, respectively (Grand Island Biological Co., Grand Island, NY); both were supplemented with 10% heat-inactivated fetal bovine serum (FBS). Culture flasks were from Corning Plastics Div., Corning, NY. Linbro 24-well culture plates were from Flow Labs., Inc., McLean, VA.

**Tumors.** The lymphogenously metastasizing rat mammary carcinoma, TMT-081, and the nonmetastasizing tumor, MT-100, were originally developed in W/F rats by Kim (14) as was TMT-081-AS, the ascites form of TMT-081 (Fig. 1). The TMT-081-AS was developed by the malignant effusion method, i.e. by transplanting TMT-081 intraperitoneally and repeatedly passing the intraperitoneal grown tumor cells until the ascites form was developed. Like the parent solid tumor, TMT-081-AS is highly metastatic.

**Establishment of the metastasizing rat mammary tumor cell line, TMT-081-MS, from TMT-081-AS.** The TMT-081-MS cell line was established from the spleen of a rat bearing TMT-081-AS (Fig. 1). The spleen, with no apparent metastatic lesions, was cut into small fragments under aseptic conditions. The tissue fragments were suspended in 10 ml of DMEM-HG-FBS, gently disrupted in a loose fitting Dounce glass homogenizer and put through a 50 mesh screen. A one milliliter portion of the cell suspension was transferred into each well of a 24-well Linbro culture plate and the plates incubated at 37°C in humidified air containing 5% CO₂. In about 30 d, a cell outgrowth was clearly present in most of the 24 wells, after which the cultures were fed twice a week with fresh medium for 3 wk. Two of these cultures were expanded in 10 ml of DMEM-HG-FBS in 75 cm² culture flasks and maintained by feeding with fresh medium every 3 d. A cell line established from one of these cultures was found to be metastasizing in W/F rats and was therefore designated as TMT-081-MS.

This line has been growing well in culture for more than a year in 75-cm² culture flasks with the volume maintained at 20 ml. It has therefore been through about 100 transfer generations with no apparent decrease in growth capacity. Cells have been frozen away, and tests show that the tumor can be recovered readily.

**Establishment of the nonmetastasizing tumor cell line, TMT-081-NM, from TMT-081-AS (Fig. 1).** Ten milliliters of freshly drawn TMT-081-AS ascites fluid was layered on 20 ml of