A NEW HUMAN CHOLANGIOCELLULAR CARCINOMA CELL LINE (HuCC-T1) PRODUCING CARBOHYDRATE ANTIGEN 19/9 IN SERUM-FREE MEDIUM

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SUMMARY

A human cholangiocellular carcinoma cell line, HuCC-T1, was established in vitro from the malignant cells of ascites of a 56-yr-old patient. Histologic findings of the primary liver tumor revealed a moderately differentiated adenocarcinoma. Tumor cells from the ascites have been cultured with RPMI 1640 medium containing 0.2% lactalbumin hydrolysate and the cultured cells grew as monolayers with a population doubling time of 74 h during exponential growth at Passage 25. They had an epithelial-like morphology and were positive for mucine staining. Ultrastructural studies revealed the presence of microvilli on the cell surface and poorly developed organelles in the cytoplasm. The HuCC-T1 cell was tumorigenic in nude mice. The number of chromosomes in HuCC-T1 ranged from 61 to 80. These human cholangiocellular carcinoma cells in serum-free medium secreted several tumor markers, including carbohydrate antigen 19/9, carbohydrate antigen 125, carcinoembryonic antigen, and tissue polypeptide antigen. The carbohydrate antigen 19/9 secretion level of HuCC-T1 cells cultured in RPMI 1640 medium with 1% fetal bovine serum was sixfold higher than that with 0.2% lactalbumin hydrolysate. These findings suggest that HuCC-T1 will provide useful information to clarify the mechanism of tumor marker secretion and tumor cell growth in the human cholangiocellular carcinoma.

Key words: cholangiocellular carcinoma; human cell line; carbohydrate antigen 19/9; serum-free medium.

INTRODUCTION

Cholangiocellular carcinoma (CCC) originating from intrahepatic bile duct is lethal because the tumor is frequently diagnosed too late to be resectable. Although more information has been accumulated in recent years on both clinical and laboratory aspects of adenocarcinoma originating from the extrahepatic bile duct and hepatocellular carcinoma (HCC), the progress of study on CCC is very limited. This limitation results mainly from inaccessibility of the viable tumor-related materials derived from CCC.

To date, adenocarcinoma cell lines originating from the bile duct have rarely been established in permanent tissue culture (8,12,15,23). However, only one line of CCC origins has been adapted to grow in vitro (22) and little is known about the cell growth and the tumor marker secretion of this cancer. This article describes the establishment and characterization of a new human CCC cell line designated HuCC-T1 that grows in serum-free medium and secretes several tumor markers such as carbohydrate antigen 19/9 (CA19/9), carbohydrate antigen 125 (CA125), carcinoembryonic antigen (CEA), and tissue polypeptide antigen (TPA).

MATERIALS AND METHODS

Patient. HuCC-T1 cell line was derived from the malignant cells of ascites of a 56-yr-old male patient with a moderately differentiated adenocarcinoma originating from the intrahepatic biliary tree. Diffuse peritoneal carcinomatosis was present when the patient was first admitted to our hospital because of abdominal pain and massive ascites. On admission, the serum levels of CA19/9, CA125, CEA, and TPA were elevated (Table 1). Though palliative intraabdominal anticancer agents were given, the patient succumbed 2 mo. after the admission.

Preparation of media. RPMI-1640 medium (RPMI) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin (GIBCO, Chargrin Falls, OH), and 10% heat inactivated fetal bovine serum (FBS), and 1% RPMI (16), which was developed as chemically defined medium, were used as the primary culture medium. RPMI with
TABLE I

PLASMA TUMOR MARKER LEVELS OF THE PATIENT

<table>
<thead>
<tr>
<th>Tumor Markers</th>
<th>(U/ml)</th>
<th>(ng/ml)</th>
<th>(U/ml)</th>
<th>(ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA19/9 (ascites)</td>
<td>31800</td>
<td>13300*</td>
<td>3.2</td>
<td>N.D.</td>
</tr>
<tr>
<td>CEA</td>
<td>1060</td>
<td>1.43</td>
<td>1.43</td>
<td>1.43</td>
</tr>
<tr>
<td>CA125</td>
<td>1.43</td>
<td>1.43</td>
<td>1.43</td>
<td>1.43</td>
</tr>
<tr>
<td>TPA</td>
<td>1.43</td>
<td>1.43</td>
<td>1.43</td>
<td>1.43</td>
</tr>
<tr>
<td>AFP</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

*Plasma tumor marker levels of the patient on the day of admission are measured for CA19/9, CEA, CA125, TPA, and AFP by radioimmunoassay.
*CA19/9 levels in the ascites.
*Not detected.

0.2% lactalbumin hydrolysate (LA; Sigma, St. Louis, MO) was used as the maintenance medium.

Cell culture. Ascite fluids were obtained under sterile condition and centrifuged at 3000 ×g for 5 min at room temperature. The pellet was suspended in RPMI containing 10% FBS, 1000 U/ml penicillin, and 100 μg/ml streptomycin, and placed into 35-mm tissue culture dishes (Corning) at 37°C in a humidified atmosphere of 5% CO₂:95% air. During the subsequent 3 d of primary culture, the medium was switched to IS-RPMI. For subculture, trypsin-EDTA (0.05% trypsin and 0.02% EDTA; Sigma, St. Louis, MO) was used.

Morphologic study. The primary liver tumor obtained by needle biopsy, the tumor produced by heterotransplantation of cells in nude mice, and the cultured cells on cover slips were stained with hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), alcian blue and Mayer’s mucicarmine reaction. CA19/9 localization in periodate-lysine-paraformaldehyde (PLP)-fixed sample of the monolayer cells was stained by PAP method of Sternberger (19). Anti-CA19/9 was obtained from Tore Fuji Bionics Corp., Tokyo, Japan. The monolayer cells after fixation with 2% glutaraldehyde were processed by routine methods for transmission electron microscopy (TEM) (JEOL 200-CX) and scanning electron microscopy (SEM) (Hitachi-S-800).

Tumor markers. The tumor markers secreted by HuCC-T1 cells for 48 h, cultured in RPMI containing 0.2% LA, and several concentrations of FBS were examined. The supernatants obtained by the centrifugation (6000 ×g for 30 min) of spent medium were assayed for CA19/9, CA125, CEA, and TPA by means of radioimmunoassay (CA19/9, CA125: Centocor Inc., Philadelphia, PA; CEA: Dinabot Radioisotope Labs., Ltd., Tokyo, Japan; TPA: Daiichi Radioisotope Labs., Tokyo, Japan).

Mycoplasma detection. Test for mycoplasma contamination was performed by the Hoechst 33258 staining method (7).

Xenotransplantation. Subconfluent HuCC-T1 cells were trypsinized, centrifuged, and resuspended in

FIG. 1. A, Photomicrograph of the primary liver tumor. A, tumor showing a glandular arrangement with abundant fibrous formation. H&E. X400. B, tumor showing PAS-positive materials (arrow) in the lumina. PAS. Original magnification. X400.