ESTABLISHMENT OF CELL STRAINS FROM HUMAN UROTHELIAL CARCINOMA AND THEIR MORPHOLOGICAL CHARACTERIZATION

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SUMMARY

We have examined the conditions for cultivation of enzymatically dispersed cells from 34 human urothelial transitional cell carcinomas (TCC) of various types. By employing two culture methods, stationary and tapping suspension, and by using the synthetic medium DM 160 supplement with human umbilical cord serum and fetal bovine serum, six cell strains were established. In two strains the tapping suspension culture method was suitable for growth of highly malignant cancer cells that detach easily from the glass surface in stationary cultures. Each of the six cell strains has been maintained in culture for over 30 months with repeated subcultures of 32 to 128 times. The histopathological features of the original TCC were three differentiated papillary types and three anaplastic nonpapillary types. In two cell strains from TCC with low malignancy, however, the cancer masses that formed in nude mice differed from the original TCC in which they became more malignant, and one cell strain resembled the original TCC closely. In three stationary culture cell strains the epithelial nature was demonstrated by the presence of desmosomes and tonofilaments. In one cell strain only tonofilaments were present. In two tapping suspension culture cell strains the presence of desmosomes was not shown clearly, but fine tonofilaments were observed in one cell strain.

Key words: primary culture; tapping suspension culture; cell strains; human urothelial carcinoma.

INTRODUCTION

Since the pioneering work of Burrows et al. in 1917 (1), many investigators (2-13) have reported the establishment of cell lines from human transitional cell carcinomas (TCC). Although most of them tried to establish TCC cell lines from carcinoma explants, usually only one or two cell lines were obtained out of a large number of urothelial carcinomas. They emphasized the difficulty in successful cultivation of the human urothelial carcinomas in vitro. Elliott et al. (14) introduced a modified explant culture method for primary culture, depending on the size and the conditions of the cancer explants, and established eight cell lines from TCC of human urinary tract (15,16). Cohen et al. (17) obtained and characterized five papillary TCC lines and one squamous cell carcinoma line from the rats fed with a chemical carcinogen.

In the present study we have established successfully six cell strains from human TCC using Dispase-I, a bacterial neutral protease. Methods for the establishment and the characterization of each strain are described herein.

MATERIALS AND METHODS

Culture media. Synthetic medium DM 160 (18) was purchased from Kyokuto Pharmaceutical Industries Co., Japan. Eagle’s minimal essential
medium (MEM) and RPMI 1640 were from Nissui Seiyaku Co., Japan. Fetal bovine serum (FBS) was obtained from GIBCO (Grand Island, NY), kanamycin was from Banyu Pharmaceutical Co., and Geopen (disodium carbenicillin) was from Pfizer Taito Co., Japan. Human umbilical cord serum was prepared by combining sera from several donors and was heat inactivated (Table 1).

Primary dissociation of TCC cells. Carcinoma tissues were obtained from human surgical materials and minced to about 1 mm diam with sharp scalpels in a sterile petri dish. The dispersion medium was 90% DM 160 containing 10% heat inactivated FBS, 300 µg/ml of kanamycin and 500 µg/ml of Geopen. The minced tissue fragments were dispersed with shaking at 37º C for 30 min by Dispase-I (1,000 U/ml, Godo Shusei Co., Japan), a kind of bacterial neutral protease (19-21).

After the elimination of floating cells and cell debris, the precipitated tissue fragments were again placed in fresh medium of Dispase-I and incubated at 37º C for 1 h with shaking. Then the liberated floating cells were centrifuged at 1000 rpm for 5 min. The sedimented cells were resuspended in culture media and inoculated into glass tubes with a flattened surface. The remaining tissue fragments were pipetted gently, and the liberated cells and the tissue fragments were inoculated into TD-40 glass flasks (50 cm² area) (22) simultaneously.

Tapping suspension culture. This culture method was developed by Katsuta et al. (23) as an improved method for suspension culture. Briefly, the cells in suspension move by the tapping motion of the magnet bar. We utilized this culture method when the cells detached from the glass surface.

Inoculation of cultured cells into athymic homozygous (nu/nu) nude mice. Cells were harvested by a rubber policeman from the glass surface, washed three times with phosphate buffered saline, and injected subcutaneously into the middle of the back of 6 wk old nude mice. The cancer masses obtained from killed mice were fixed immediately with 10% aqueous formaldehyde, processed for paraffin sections, and stained with hematoxylin and eosin.

Cinemicrography. Inverted phase contrast microscopes (Type MD; Nikon Co.), time lapse cinemicroscopic apparatuses (Nikon), and 16 mm cameras (Type HI6RX; Bolex Co., Switzerland) were used. The Minicopy films (Fuji Co., Japan) were developed with an ultrafine-grain developer, Finedol (Fuji).

Giemsa staining. Cells cultured on the 10 x 40 mm cover slips in glass tubes with a flattened surface (24) were washed in phosphate buffered saline and fixed in absolute methanol for 10 min. Then the cells were stained with a 5% water soluble Giemsa (Merck, West Germany) solution for 30 min, washed with tap water, and dried with air.

Chromosome analysis. Cultured cells were harvested with a rubber policeman after incubation with several drops of 0.1 µg/ml of colchicine (Merck) for 3 h. Collected cells were treated with a hypotonic solution of 1% sodium citrate and fixed in alcohol-acetic acid (3:1). Fixed cells were spread on a glass slide by an air drying technique and stained with Giemsa. G banding was accom-

### TABLE 1

**Establishment of Cell Strains and Effect of Different Cultured Media**

<table>
<thead>
<tr>
<th>Cell Strain</th>
<th>Exp. Number</th>
<th>Donors</th>
<th>Media</th>
<th>DM-160</th>
<th>RPMI</th>
<th>MEM</th>
<th>Culture Time</th>
<th>Aging Time</th>
<th>Chromosomal Mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>JTC-29</td>
<td>4</td>
<td>77 M</td>
<td>FBS 10%</td>
<td>129</td>
<td>34.6</td>
<td>43(Y)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JTC-30</td>
<td>6</td>
<td>46 M</td>
<td>FBS 10%</td>
<td>101</td>
<td>69.2</td>
<td>47(Y)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JTC-32</td>
<td>15</td>
<td>73 F</td>
<td>FBS 10%</td>
<td>91</td>
<td>86.7</td>
<td>95-102</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JTC-34</td>
<td>31</td>
<td>64 M</td>
<td>FBS 10%</td>
<td>266</td>
<td>42.0</td>
<td>47(Y?)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JTC-31</td>
<td>11</td>
<td>54 M</td>
<td>FBS 5%</td>
<td>119</td>
<td>59.4</td>
<td>45(Y)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JTC-33</td>
<td>20</td>
<td>70 M</td>
<td>FBS 5%</td>
<td>115</td>
<td>43.2</td>
<td>47(Y)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Symbols represent: (△), established cell strains; (●), temporary growth of cancer cells; (X), no growth, and (−), no examination.

b HUCS = human umbilical cord serum.