Establishment and Characterization of Two Squamous Cell Carcinoma Cell Lines (HYVC and HMVC) Derived from Vulva

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\(<\text{Abstract}>\) Two cell lines, HYVC and HMVC, were established from cultures of the squamous cell carcinoma removed from the vulva of females of 37 and 46 years old, respectively. These cell lines were very similar in their biological characteristics, such as morphology (polygonal), growth pattern (32-43 hr of population doubling time, 50-25\% of plating efficiency), heterotransplantability (1X10\(^7\) cells produced squamous cell carcinomas in the nude mice), genetics (75-78 of modal chromosomal number), and producing the tumor markers (SCC and TPA). The HPV-DNA was not detected in either the HYVC or HMVC lines by using L1-PCR methods, however, it was detected in the HYVC using E6/E7-PCR.

Key words: cell line, epidermoid carcinoma of vulva, biological properties

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Introduction

The most common malignant tumor afflicting the vulva originates in the squamous epithelium. However, few squamous cell carcinoma cell lines (A431\(^1\), LT2\(^6\), UM-SCV-1A\(^6\), UM-SCV-1B\(^6\), UM-SCV-2\(^6\), UM-SCV-6\(^6\), UCI-VULV-1\(^6\)) of vulva were reported.

We established two human vulvar squamous cell carcinoma cell lines and investigated their biological characteristics (for example: morphology, growth pattern, karyotype, heterotransplantability and production of a tumor marker). Furthermore, we investigated whether the human papilloma virus was recognized in the epidermoid carcinoma of the vulva or not.

These cell lines are very useful for studying on the tumorigenesis and susceptibility of anticancer drugs.

Materials and Methods

1. Materials and culture techniques

The growth medium (GM) used was Ham's F12 (GIBCO) supplemented with 15\% fetal bovine serum, 50 \(\mu\)g streptomycin and 50U penicillin/ml (GIBCO) of GM, pH 7.2 to 7.4. HYVC: A tissue specimen (a squamous cell carcinoma) was obtained from radical vulvectomy conducted on a 37-year-old Japanese woman on January 22, 1997. A portion of the tumor was stored in GM at 4 \(^\circ\)C and placed in culture the following day.

HMVC: This tissue specimen was obtained from radical vulvectomy on a 46-year-old Japanese woman on November 25, 1997, stored in GM at 4 \(^\circ\)C and placed in culture on November 27, 1997.

Both Samples were rinsed twice with GM, minced
with scissors, and dissociated with 600 Pronase Units/ml of Dispase (Gohdo Shusei Co.) for 30 min at 37°C and then centrifuged at 300 Xg for 10 min. The sediments were resuspended in the GM, placed into the 6-cm plastic dishes (Falcon, Co.Ltd.) and cultured at 37°C in a 5% CO2 incubator. In the initiation of the primary culture, epithelial colonies were scattered in the fibroblast sheet in both HYVC and HMVC cultures. The epithelial colony was isolated by small filter paper soaked with 0.1% trypsin-0.02% EDTA/PBS. That is, after rinsed with Hanks' solution on the cultures, above small filter paper put on the epithelial colony under the phase contrast microscopy. Two or 3 min later, the epithelial cells were removed with small filter paper and cultured in fresh GM. The cell lines were stored in GM supplemented with 10% DMSO at -196°C in Liquid N2.

2. Morphological characteristics

Culture materials of the vulvar tumor were histopathologically interpreted as epidermoid carcinomas.

The culture materials and the grafts in the nude mice were fixed with a 10% buffered formalin solution, embedded in paraffin, sectioned at 4 μm in thickness, and stained with hematoxylin-eosin. The cultured cells were observed under a phase contrast microscope, fixed with 95% ethanol solution and stained with Papanicolaou solution.

For transmission electron microscopy, the cells were fixed in 1% glutaraldehyde in 0.1M phosphate buffer, pH 7.4 at 4°C for 1 hour, and post-fixed in 1% osmic acid in Millonig's buffer, embedded in Epon-Araldite resin. The ultra thin sections were stained with lead citrate and uranyl acetate and then observed with JEOL 1200 EX-III electron microscopy.

3. Growth characteristics

The growth characteristics were studied at the 5th and 20th passages by the previously reported techniques. For the study of the growth curve (GC), about 1X10^5 single-suspended cells were placed in 3.5cm plastic dishes (Terumo Co., Tokyo) and the preparations incubated for 8 days. Cells were counted in 3 dishes every day with the use of the Erma Counting Chamber (Erma Co., Ltd., Tokyo), and the GC was drawn as shown in Table 1. The medium was changed every other day. Population doubling time (DT) and the saturation density (SD) were determined by GC analysis. For studies of plating efficiency, 2X10^5 single-suspended cells/dish were placed in 5 plastic dishes (6cm in diameter; Terumo Co., Tokyo); the preparations were incubated for 10 days and finally stained with Giemsa solution. The ratio of the number of visible colonies (more than 10 cells) to the number of inoculated cells was calculated.

4. Chromosome analysis

Chromosomes were analyzed at 5 and 15 passages. The cells were treated with 10^-7 M colcemid (Grand Island Biological Co., Island, NY) for 4 hrs, fixed with methanol-acetic acid solution (3:1), treated 0.1% trypsin for 15 sec at room temperature, stained 3% Gimsa (ph 6.8), and analyzed for G-band karyotyping. Histograms of chromosome number distribution were determined from more than 50 metaphases.

5. Heterotransplantation

Approximately 1 X 10^7 cells of each HYVC and HMVC were transplanted into the subcutis of BALB/c/nu nude mice by using 18 G needle. The tumors were examined histologically by light microscopy at 6 weeks after transplantation.

6. Carcinoembryonic protein levels in the conditioned media.

Approximately 5 X 10^6 cells/dish were cultured in a serum-free culture medium, and the concentration of the carcinoembryonic protein (squamous cell carcinoma antigen (SCC), carbohydrate antigen-125(CA125), tissue polypeptide antigen(TPA), a-fetoprotein(AFP), carcinoembryonic antigen(CEA), human chorionic gonadotropin(hCG), neuron specific enolase(NSE)) was measured in the conditioned media by RIA or ELISA 2 days later.

7. Human papilloma virus–HPV DNA detection and typing

Polymerase chain reaction (PCR) was used for HPV detection and typing as perviously described by...