ESTABLISHMENT AND CHARACTERIZATION OF A NEW HUMAN RENAL CELL CARCINOMA CELL LINE (KRC/Y)

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SUMMARY

A new renal cell carcinoma (RCC) cell line (KRC/Y) has been established from a surgical specimen of a 41-yr-old Japanese female patient with RCC composed of both clear cells and granular cells. This cell line has been maintained for more than 15 mo. through 45 passages with a stable growth. KRC/Y cells have clear or eosinophilic polygonal cytoplasm and round to oval nuclei with one or two nucleoli, and proliferate in a pavementlike cell arrangement with a lack of contact inhibition. By electron microscopy, these cells contain abundant fat droplets and glycogen granules or well-developed organelles or both, which were also observed in the original tumor. The doubling time of these cells at the 15th passage was 73 h. The chromosome number was from 37 to 45 with a hypodiploid modal number of 42. Tumorigenicity was identified by tumor formation after subcutaneous injections of KRC/Y cells in nude mice, which showed close resemblance to the original tumor by light and electron microscope observations.

Key words: renal cell carcinoma; cell line; human; nude mouse; establishment.

INTRODUCTION

Since the first report on cultivation of renal cell carcinoma (RCC) by Richter and Akin (20) in 1957, establishment of RCC cell lines, as well as heterotransplantation of such a tumor into nude mice, have been reported by many authors (3,4,6,7,9-14,16,17,19,20,22, 23,25). Establishment of cell lines provides many good experimental models for investigations on RCC, including histogenesis, diagnosis, therapy, and hormone dependency. Reports concerned with generations of monoclonal antibodies to human carcinomas using various cell lines have been published, and Vesella et al. (24) described monoclonal antibodies to human RCC in 1985. In this report, we introduce a new human RCC cell line, designated KRC/Y, which has been established as the first step for various studies.

MATERIALS AND METHODS

Patient. KRC/Y was derived from RCC of a 41-yr-old Japanese female patient. She had an episode of macroscopic hematuria of 2-mo. duration, and RCC was diagnosed by intravenous and retrograde pyelography, ultrasonography, and computerized tomography. Left nephrectomy with hilar lymphadenectomy was performed. The cut surface of the kidney showed that the tumor was located in the middle portion of the kidney and invaded the pelvis. The tumor seemed for the most part cystic and necrotic with hemorrhage and had fibrous capsule and septa with foci of calcification, while glistening yellow viable nodules were also observed in some areas (Fig. 1). Portions of viable nodules were immediately cut into small cubes and placed in Karnovsky’s fixing fluid for electron microscope observation, and half of the kidney was fixed in 10% formalin for light microscope observation.

Tissue culture medium. Dulbecco’s modified Eagle’s medium (DMEM) (Nissui Seiyaku Co., Japan) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin (GIBCO, Chagrin Falls, OH), 12 mM sodium bicarbonate, and 20% heat-inactivated (56 °C, 30 min) fetal bovine serum (FBS) (M. A. Bioproducts, Walkersville, MD) was used as the primary culture medium (PCM). After obtaining a stable cell growth, DMEM with 10% FBS was used as maintenance medium.

Tissue culture. A specimen for culture was obtained from a portion of viable tumor nodules aseptically, was trimmed of necrotic tissue in PCM, and then minced with scalpels into pieces approximately 1 to 3 mm³. The small tissue fragments in PCM were filtered through a 20-μm pore mesh to remove red blood cells, washed in PCM, centrifuged (800 rpm 5 min at 40 °C). Then the pellet was washed in fresh Ca- and Mg-free phosphate buffered saline (PBS) (Nissui), recentrifuged (800 rpm 5 min at 40 °C), and the pellet, suspended in 20 ml of PBS with
collagenase (type IV, 473 U/mg, 0.5 mg/ml, Sigma Chemical Co., St. Louis, MO), was incubated in a 37°C waterbath for 70 or 80 min. After removing undigested tissue in a 100-μm pore mesh, the cell suspension was centrifuged, resuspended in PCM, and seeded into 35- and 60-mm culture dishes (Falcon; Becton Dickinson Labware, Oxnard, CA) and cultured at 37°C with 5% CO₂ in air. The medium was changed two to three times a week. For subculture, the cells were detached by treatment with trypsin-EDTA (GIBCO). Cultured cells were frozen and stored in liquid nitrogen in a medium of 10% dimethyl sulfoxide in culture medium.

**Observations.** Phase contrast microscopy (Nikon) was used for daily cell observations. For light microscope observations, cells grown on Lab-Tek tissue culture chamber slides (Miles Laboratories, Naperville, IL) were washed with PBS three times, fixed in 95% ethanol or absolute methanol for 20 min, and then stained with Giemsa, hematoxylin-eosin (H&E), periodic acid Schiff (PAS) with and without prior diastase digestion, and Sudan III. For electron microscope observations, confluent monolayer cells grown on Lab-Tek tissue culture chamber slides were washed with PBS three times and fixed in Karnovsky’s fixing fluid for 1 h at room temperature. After rinsing three times with 0.1 M sodium cacodylate buffer, the samples were postfixed with 2% osmium tetroxide with Millonig buffer (1:1), dehydrated in a graded series of ethanol, and embedded in Epon 812. Cells were separated from the slides, cut on an ultramicrotome, stained with uranyl acetate and lead citrate, and examined under a Hitachi H-500 electron microscope.

**Hormone and protein studies.** The production of parathormone (PTH), prostaglandin (E₁ + E₂) (PGE), renin, erythropoietin, β₂-microglobulin (BMG), and ferritin by KRC/Y cells grown in 5 ml medium for 48 h was examined. When KRC/Y cells grew confluent in the T-25 flask (Falcon), the spent medium was obtained, centrifuged, and assayed by means of radioimmunoassay kits (PTK, BMG: Eiken Immunochemical Lab., Japan; PGE, renin, ferritin: Clinical Assays, Cambridge, MA; erythropoietin: Special Reference Laboratory, Japan).

**Growth curve.** Growth curves were obtained by seeding the KRC/Y cells at 5 × 10⁶ cells/T-25 flask in duplicate. The medium was changed every 2 d after seeding. The cells were detached with trypsin-EDTA and the average number of viable cells from the two flasks was estimated by counting cells that excluded trypan blue in a Bürker-Türk hemocytometer. The doubling time of the cell population was estimated in the logarithmic growth phase.

**Chromosome studies.** Chromosome analysis was performed with KRC/Y cells in the 12th passage. Cells were incubated in a medium containing 0.04 μg/ml of colcemid at 37°C for 2 to 4 h, detached with 0.25% trypsin, and

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**FIG. 1.** The gross finding of the resected tumor showing cystic and necrosis with hemorrhage, invading the pelvis.