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Establishment of three human renal cell carcinoma cell lines (SMKT-R-1, SMKT-R-2, and SMKT-R-3) and their characters

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Summary. We have established three new cell lines of human renal cell carcinoma (RCC), designated as SMKT-R-1, SMKT-R-2 and SMKT-R-3. These cell lines were derived from a primary lesion of the tumor or a tumor initially xenotransplanted in nude mice. These cell lines have maintained a stable growth in vitro for more than a year. They also exhibited characteristics showing a lack of contact inhibition of cells, colony formation in soft agarose and tumor formation in nude mice by a xenotranaplantation of cells, all of which suggested an epithelial origin. The tumors produced in nude mice by the innoculation of cell lines were demonstrated by light an electron microscopy tobe derived from RCC. The doubling time of these cell lines were 180.0 h, in SMKT-R-1, 56.4 h in SMKT-R-2 and 55.7 h in SMKT-R-3. The cell lines were aneuploid in their chromosomal analysis, and SMKT-R-2 and SMKT-R-3 also had three and two marker chromosomes, respectively. The different biological characters of these cell lines from the others so far established would be of benefit in the future study of human RCC.

Key words: Human – Renal cell carcinoma – Established cell lines – Epithelial

Introduction

In spite of the recent progress in chemotherapy and immunotherapy for malignant diseases, advances in the area of treatment for renal cell carcinoma (RCC) have not been clinically effective. Vinblastine as a single chemotherapeutic agent or interferon as a biological response modifier has achieved only limited clinical efficacy in RCC [2, 15, 16]. Surgery has been the only choice available at this time for RCC.

CT or ultrasonographic scanning incidentally finds small renal cell carcinoma, which has lead to an increase in the number of RCC patients. It is therefore necessary to investigate the more defined biological nature of RCC, such as mechanism of carcinogenesis, specific cell surface antigens and chemosensititvity. Human RCC cell line was used in the study of the biological nature of these factors. In this paper, we report the establishment of three new cell lines of human RCC (SMKT-R-1, R-2, and R-3) and their biological nature.

Materials and methods

Sources of cell line

A primary lesion or the xenotransplantable tumor of RCC was employed in an establishment of cell line.

SMKT-R-1 was derived from a specimen of the primary lesion of a 63-year-old female who had surgery performed on May 23, 1986. The primary lesion was revealed histopathologically as RCC of the alveolar type, clear cell subtype, pT2b, pV0 and grade 2 (UICC, TNM classification) (Fig. 1A).

In SMKT-R-2, the xenotransplantable tumor at passage 2 in a nude mouse was the source for the cell line. The tumor was produced by xenotransplantation of the primary lesion surgically removed on Dec. 13, 1986, in a 79-year-old male patient. Histopathological study of this primary lesion showed an alveolar type and clear cell predominant-mixed subtype of RCC with pT3, pV1a and grade 2, to which the xenotransplantable tumor at passage 2 had similar histopathological features (Fig. 2A).

The third established cell line, SMKT-R-3, was derived from the xenotransplantable tumor at passage 1 in a nude mouse, the tumor specimen being originally obtained at surgery on June 17, 1987, from the primary lesion of a 64-year-old male. Histopathological features of the xenotransplantable tumor was similar to that of the original papillary type and granular cell subtype of RCC with pT3, pV1a and grade 2>3 (Fig. 3A).

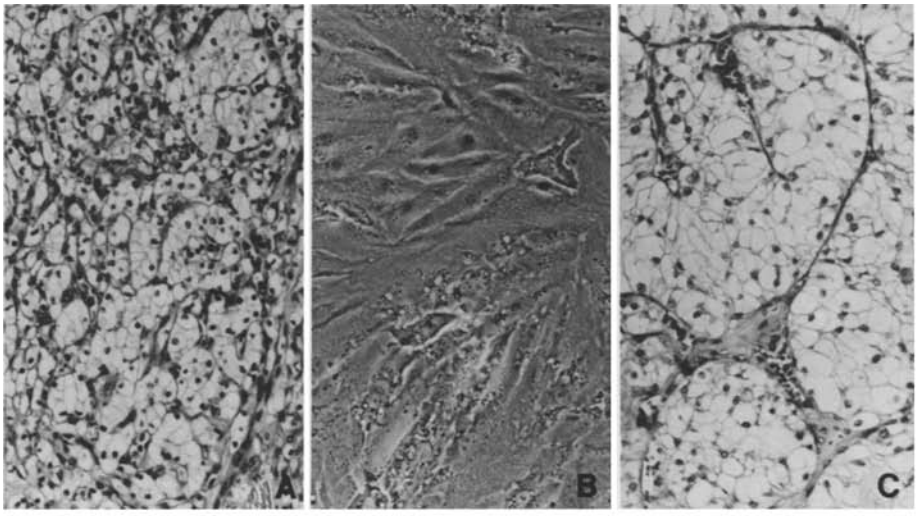

Fig. 1A–C. Microscopic features of SMKT-R-1. **A** Histopathological featuares of the original tumor (H & E, ×211), **B** morphological features in the monolayer culture (×106), **C** histology of the tumor by xenotransplantation of the cell line (H & E, ×211)

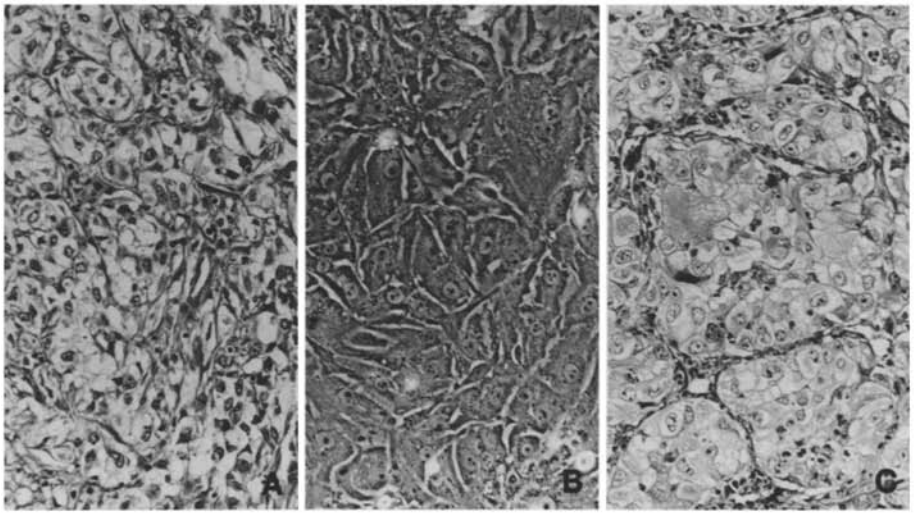

Fig. 2A–C. Microscopic features of SMKT-R-2. **A** Histopathological featuares of the original tumor (H & E, ×211), **B** morphological features in the monolayer culture (×106), **C** histology of the tumor by xenotransplantation of the cell line (H & E, ×211)

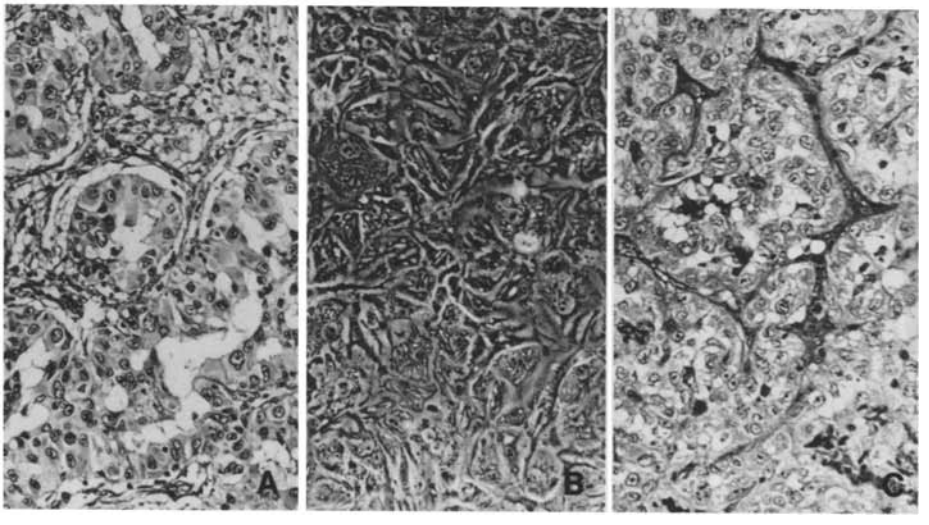

Fig. 3A–C. Microscopic features of SMKT-R-3. **A** Histopathological features of the original tumor (H & E, ×211), **B** morphological features in the monolayer culture (×106), **C** histology of the tumor by xenotransplantation of the cell line (H & E, ×211)

Cell culture method

An aseptically obtained tumor was incised into 1×1 mm pieces. They were, then, incubated for 30 minutes at 37°C in McCoy 5A medium (Sigma Chemical Co., St. Louis, MO) containing 3.0 mg/ml of collagenase, type I (Sigma Chemical Co.) and 0.3 mg/ml of DNase I (Sigma Chemical Co.). The cell suspension was filtered through a steel mesh and a 75 μm pore-size filter. After adding culture medium (described later) containing 10% fetal bovine serum (FBS, Flow Lab., Australia), the suspension was then centrifuged. Viable cell were counted with 0.4% trypan blue exclusion.

The cell was cultured in MEM D-valine modification (Sigma Chemical Co.) medium containing 0.1% penicillin (1,000 U/ml)/ streptomycin (10 mg/ml) solution (GIBCO, Grand Island, NY) and 10% FBS under the condition with a humidified atomosphere of 5% Co_2 at 37°C. When the cells were passed every seven days, they were washed with solution A and trypsin/solution A [17], MEM D-Valine modification medium with 10% FBS being subsequently added. This cell suspension was centrifuged at 1,000 rpm/min for 10 minutes, resulting in a single cell pellet. The pellet was then resuspended in the medium. Viable cells of $6\text{–}10 \times 10^5$ were plated in a 75 cm^2 plastic culture flask (Corning Glass Works, Corning, NY). During a passage of cells, a half of the culture medium was exchanged every 3–4 days.

Light microscopic (LM) observation

Morphorological changes during the culture were observed with inverted phase-contrast light microscopy (DIAPHOT TMD, Nikon, Tokyo).

Xenotrasplantation of cultured cells in nude mice

After single cell suspension being obtained with the method described previously, $2 \times 10^5\text{–}2 \times 10^6$ cells of each cell line supended in 0.05–0.2 ml of medium without FBS were subcutaneously inoculated in several nude mice. When the tumor formation was noticed, it was removed, fixed in 10% buffered formalin and stained with hematoxylin & eosin for a histopathological study.

Electron microscopic (EM) observation

A tumor produced by the inoculation of cells in the nude mouse was processed for a fixation with Karnovsky's fixative solution in 0.1 M cacodylate buffer, pH 7.4, for 12 h, at 4°C. They were postfixed with 1% OsO_4-cacodylate buffer solution (0.1 M, pH 7.2), dehydrated by alcohol solutions, and embeded in Epon 812. The ultrathin sections of the specimen were counterstained with 1.5% uranyl acetate solution and lead citrate solution, followed by an EM (LEM-1200EX, JEOL, Tokyo) observation.

Chromosomal analysis

After dispersion and centrifugation of colcemid-treated confluent cells, they were treated with hypotonic KCl solution (0.075 M). Then, the cells were fixed with Carnoy's solution and treated by Gimusa-stain for chromosomal analysis.

Growth of cells

Cells being 5×10^4 in each of the cell lines suspended in 2 ml MEM D-valine modification with 10% FBS were plated in triplicate 35 mm plastic wells (Corning). The cell number was counted after removing the cells with the previouly described methods every other day. The doubling time for each of the cell lines was determined by the growth curve.

Colony formation

Double-layered soft agarose culture system was employed, in which the basal (feeder) layer was prepared with 1 ml of 0.5% agarose in McCoy 5A medium containing 15% FBS. The upper (cellular) was formulated with 0.3% agarose in MEM D-valine medium with 10% FBS containing 1×10^5 cells/35 mm well in triplicate. Colony (> 50 μm) number was counted after an incubation of 7–14 days.

Mycoplasma testing

SMKT-R-1 at passage 69, SMKT-R-2 at passage 47, SMKT-R-3 at passage 32 were tested for contamination of mycoplasma with the Bioassay systems mycoplasma detection kit (Bioassay systems Corp. Woburn, MA).

Results

In vitro growth

While in the early phase, SMKT-R-1 was contaminated by fibroblasts, which disappeared during the later culture. Then, SMKT-R-1 grew well at passage 68, for a period of 28 months after the initial culture. A phase-contrast LM study revealed that SMKT-R-1 grew in fusiform-like cells which had round nuclei and nucleoli. The cells were, in part, piled up, indicating a lack of contact inhibition (Fig. 1B). SMKT-R-2 as did SMKT-R-1 had a favorable growth at passage 56, some 17 months later. This cell line consisted morphologically of large polygonal cells and small compact cells, the former had round nuclei and an abundant cytoplasm, while the latter had small round nuclei and several nucleoli. The pile-up of cells was also observed (Fig. 2B). SMKT-R-3 achieved good growth at passage 41, some 12 months after the first plating. The cell line had two distinct types of cells, one being large cells and the other, small cells. The round or polygonal cells had a round nuclei which contained a granule-like structure and granules suggesting, in part, a lipid droplet. Contact inhibition was lacking in these cells.

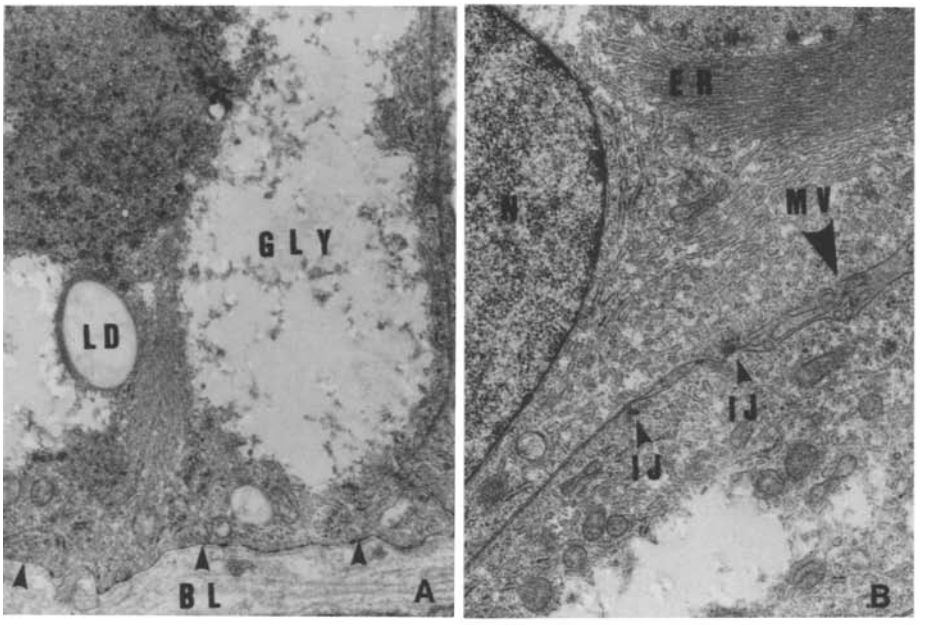

Fig. 4A and B. Electron microscopic features of SMKT-R-1. BL = basement lamina; D = desmosome; ER = endoplasmic reticulum; GLY = glycogen; IJ = intermediate junction; LD = lipid droplet; M = mitochondria; MV = microvilli; N = nucleus; RER: rough endoplasmic reticulum. A × 9,034, B × 7,137

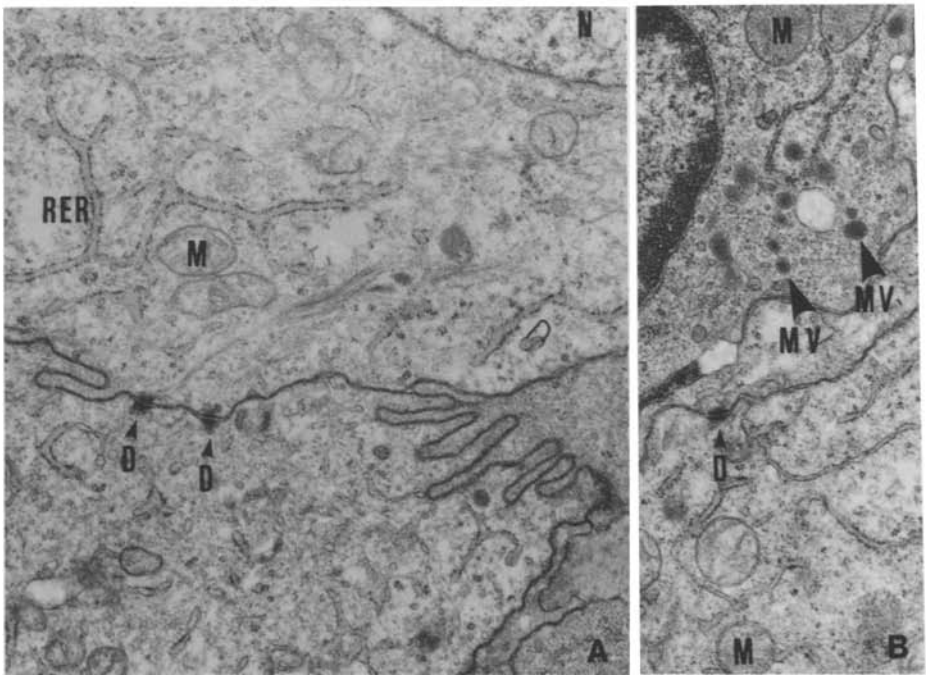

Fig. 5A and B. Electron microscopic features of SMKT-R-2. BL = basement lamina; D = desmosome; ER = endoplasmic reticulum; GLY = glycogen; IJ = intermediate junction; LD = lipid droplet; M = mitochondria; MV = microvilli; N = nucleus; RER: rough endoplasmic reticulum. A × 13,187, B × 11,537

Tumor formation in the nude mice

Subcutaneous inoculation of each cell line at passage 12 in SMKT-R-1, 13 in SMKT-R-2 and 3 in SMKT-R-3 yielded tumors in nude mice. In SMKT-R-1, the tumor was noticed at 3 months after cell injection and grew 10 × 8 mm in size at 6 months. Histopathological features of this tumor resembled that of its original one (Fig. 1C). SMKT-R-2 grew more rapidly to a 17 × 15 mm sized tumor at 2 months after cell injection. The tumor grown in the nude mouse has histopathological features of the granular cell predominant which is similar to the original tumor (Fig. 2C). SMKT-R-3 grew rapidly like SMKT-R-2, producing a mass of 11 × 8 mm in size 2 months later. The same type of histopathological feature as displayed in the original RCC was recovered in the tumor of the nude mouse (Fig. 3C).

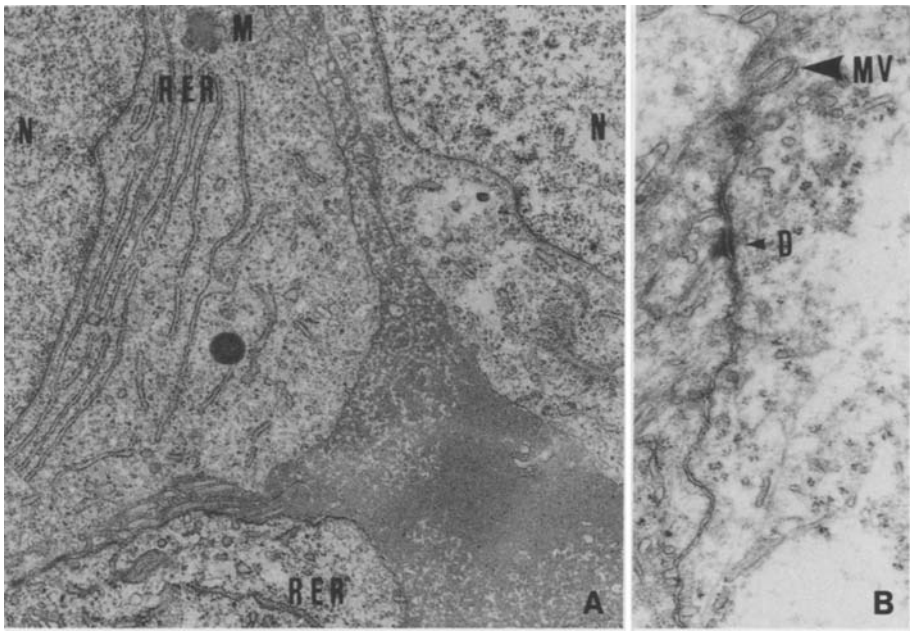

Fig. 6A and B. Electron microscopic features of SMKT-R-3. BL = basement lamina; D = desmosome; ER = endoplasmic reticulum; GLY = glycogen; IJ = intermediate junction; LD = lipid droplet; M = mitochondria; MV = microvilli; N = nucleus; RER: rough endoplasmic reticulum. **A** ×9,313, **B** ×15,274

EM findings

EM study revealed that tumors produced by an inoculation of each cell lines in nude mice had intermediate junctions in SMKT-R-1 and desmosomes in SMKT-R-2 or SMKT-R-3, as well as microvilli in all tumors. These features are typically observed in epithelial cells (Figs. 4–6). In SMKT-R-1 there were abundant glycogen granules and lipid droplets in the cytoplasm. Intracellular organelles other than endoplasmic reticulum (ER) were not well-developed. Abundant as well as swollen mitochondria and rough ER were observed in the tumors derived from the other two cell lines.

Chomosomal analysis

The typical modal number of cell lines were 86 in SMKT-R-1, 81 in SMKT-R-2 and 77 in SMKT-R-3 (Figs. 7–9). Three and two marker chromosomes were identified in SMKT-R-2 and SMKT-R-3, respectively. SMKT-R-2 and SMKT-R-3 had a Y chromosome, each of which coincided with the patient's sex.

Growth kinetics

When 5×10^4 cells/well were plated, each cell line demonstrated the growth curves shown in Fig. 10. These curves gave a doubling time of 180 h in SMKT-R-1, 56.4 h in SMKT-R-2 and 55.7 h in SMKT-R-3.

Plating efficiency

Each of the cell lines formed many colonies in the double-layered soft agarose system. SMKT-R-1 yielded a plating efficiency of 0.13% with 131 ± 13 (mean $\pm$ S.D.) colonies/well, SMKT-R-2, 1.74% with $1{,}738 \pm 52$, SMKT-R-3, 1.02% with $1{,}017 \pm 116$.

Mycoplasma testing

The method described previously revealed no mycoplasma contamination in each of the cell lines.

Discussion

An established cell line can be applied to study carcinogenesis, cancer diagnosis and treatment. Although reports have been made on the establishment of human RCC cell lines, most used a short term culture. Since Jones [5] reported a long term culture in which human RCC had been cultured for 11 months, only 19 have since been established as a long term-cultured cell line [1, 3, 4, 6–10, 12–14, 18, 19, 21, 22].

In this study, we demonstrated three cell lines to be established as a new cell line. The cell lines were established by such evidence as 1) a lack of contact inhibition, 2) an anchorage independent growth with colony formation in soft agarose, 3) an epithelial cell appearance in LM and EM study, 4) an aneuploid in

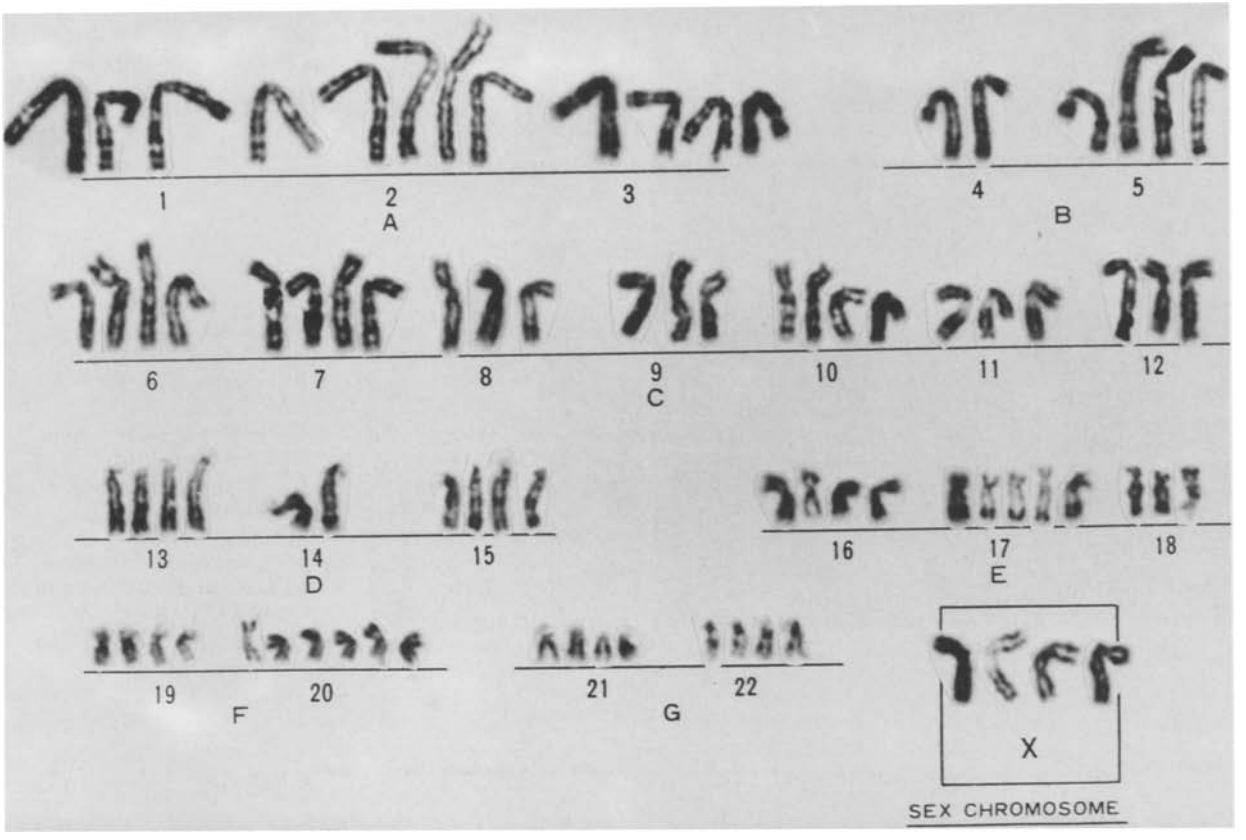

Fig. 7. G-banding karyotype of cell line SMKT-R-1: modal number 86 without a marker chromosome

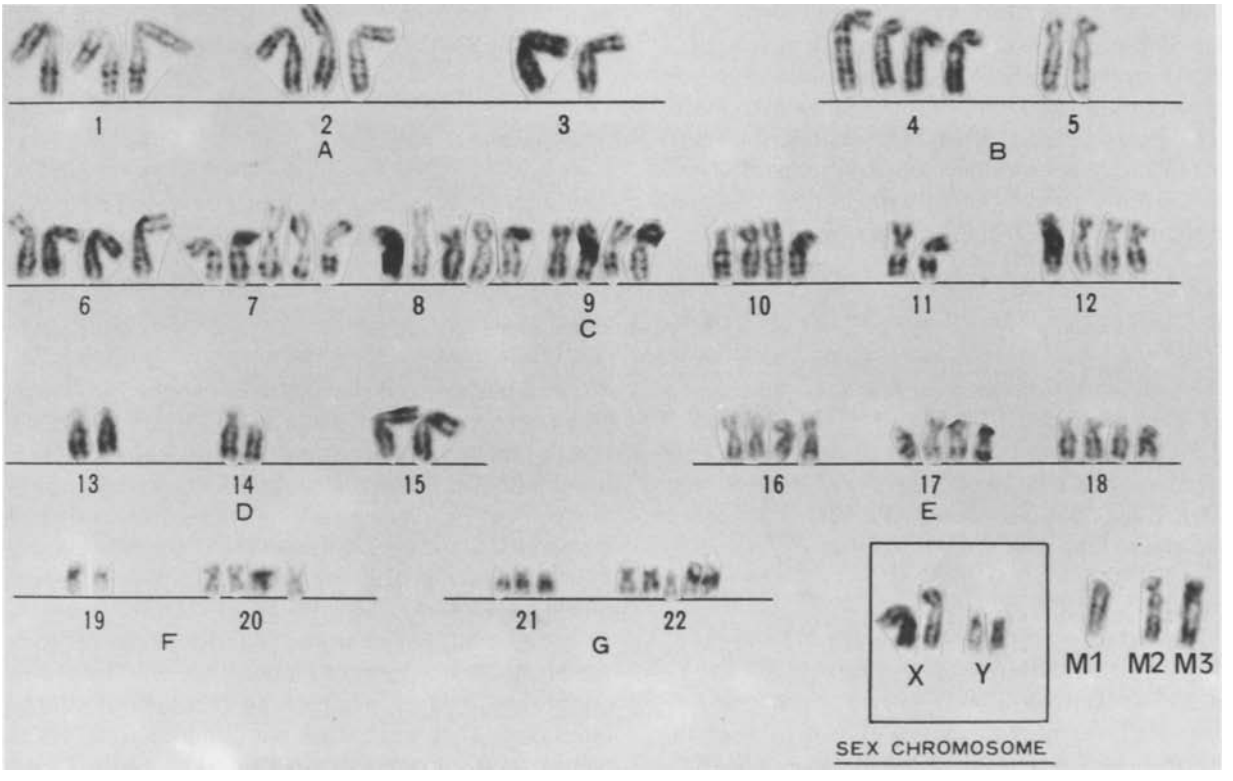

Fig. 8. G-Banding karyotype of cell line SMKT-R-2: modal number 81 with three marker chromosomes (M1–M3)

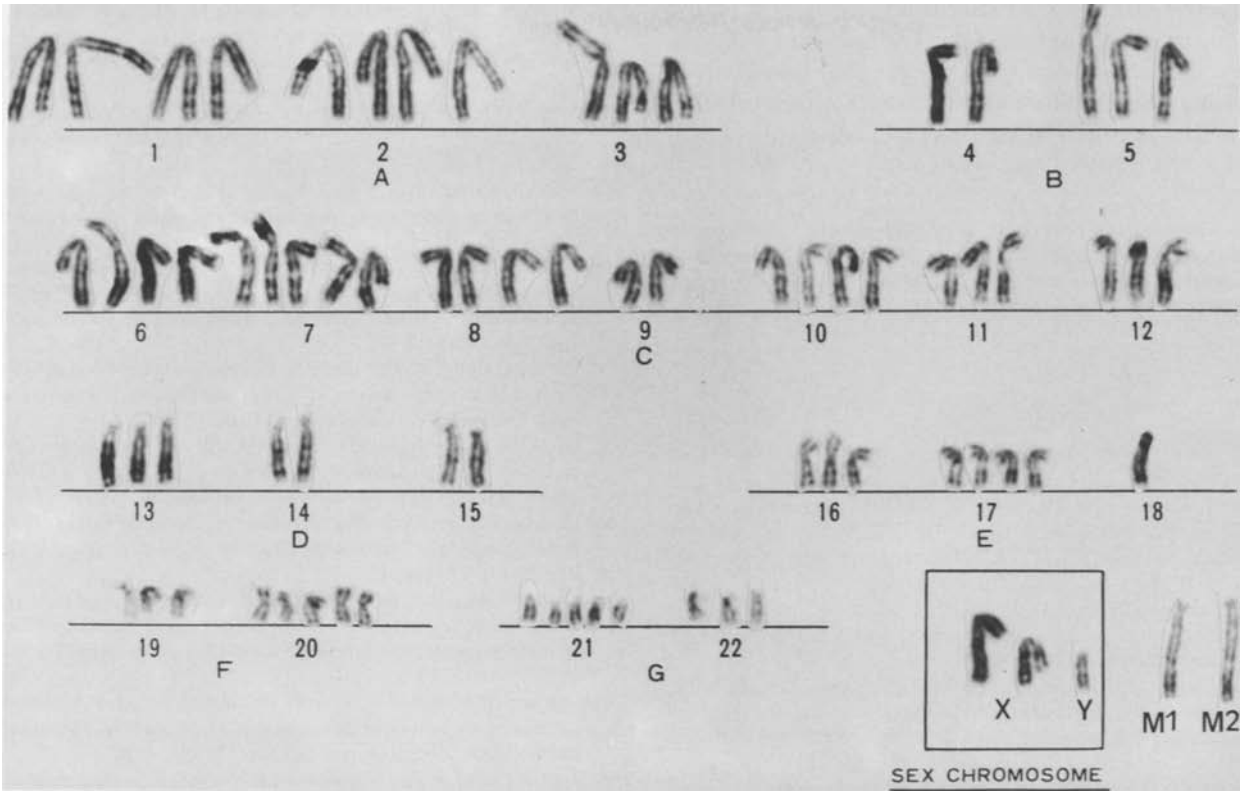

Fig. 9. G-banding karyotype of cell line SMKT-R-3: modal number 77 with two marker chromosomes (M1 and M2)

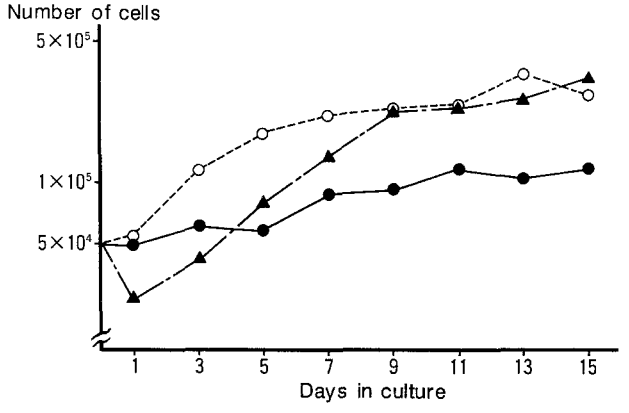

Fig. 10. Growth curves of SMKT-R-1 (●——●) at passage 58, SMKT-R-2 (▲– – –▲) at passage 28 and SMKT-R-3 (○– – –○) at passage 23

karyotype, 5) a stable growth in culture more than a year, 6) tumorigenicity in nude mouse and 7) free from mycoplasma contamination.

When compared with other cell lines reported so far, ours have several distinct biological characters. First, SMKT-R-1 has the longest doubling time among cell lines in the previous reports, in which KRC/Y has the longer doubling time of 73 h and KH-39, the shortest, 16 h [13, 22]. This characteristic of SMKT-R-1 seems to reflect, in part, the biological nature of the original RCC. Second, is the establishment of a cell line with granular cell type such as SMKT-R-3, which was confirmed by histopathological study of the tumor in nude mice. This cell line may be beneficial in a study on RCC, since only a few of such cell lines have been reported.

In the chromosomal study on human RCC, Weaver and associates [20] have suggested a possible relationship between original oncogenic transformation and chromosomal abnormality such as trisomy or tetrasomy of chromosome 7. The study on our cell lines supports his result, where each of the cell lines have an increased number of chromosome 7.

As for EM findings, all three new cell lines showed an epithelial cell origin. The findings of presence of glycogen granules, abundant lipid droplets and poorly developed cytoplasmic organelles in cytoplasm of SMKT-R-1 were consistent with the finding of clear cell type of RCC on the LM study. Although SMKT-R-2 and SMKT-R-3 had a smaller number of glycogen granules and lipid droplets, a swollen mitochondria

and a rough ER were found in the cytoplasm. These findings indicate that these two cell lines have maintained a character keeping to the original granular cell type of RCC [11]. Since only a few established cell lines have been reported, these new cell lines would contribute to the study of biology of RCC.

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