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Cytomorphological, cytogenetic, and molecular biological characterization of four new human renal carcinoma cell lines of the clear cell type

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Abstract Four new permanent cell lines (RCC-A, -B, -C, and -D) derived from different human renal cell carcinomas of the clear cell type were established in tissue culture. The cell lines displayed characteristic differences in cell size and shape, which allowed individual identification by phase contrast microscopy. Ultrastructurally, the cell lines exhibited varying amounts of cytoplasmatic glycogen and lipid. Immunohistochemistry revealed co-expression of vimentin and cytokeratin in all cell lines. The mean population doubling time ranged from 27 h (RCC-A) to 104 h (RCC-D). RCC-B and -C cells produced slowly growing tumours after heterotransplantation into nude mice, whereas RCC-A and RCC-D cells were non-tumorigenic. The modal chromosome number was either near-diploid (RCC-A, -B, and -C) or near triploid (RCC-D). Clonal abnormalities affecting the short arm of chromosome 3 were seen in all cell lines. Northern blot analysis revealed no expression of the proto-oncogenes c-*fos*, c-*ros*, and c-*mos*, whereas c-Ki-*ras* expression was observed in all cell lines. Expression of c-*myc* was observed in RCC-A, RCC-B, and RCC-D cells, whereas c-*raf* expression could be detected in RCC-B and RCC-D. Tumour suppressor gene *p53* mRNA was observed in the cell line RCC-D.

Key words Human renal cell carcinoma · Cell line
Proto-oncogene · Tumour suppressor gene · *p53*

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Dedicated to Professor Dr. Wolfgang Thoenes (†1992)

Introduction

Thoenes et al. (1986) introduced a subtle subclassification of human renal cell carcinomas (RCC) based on distinct cytomorphological criteria. The main cell type present in the majority of all human RCCs is the clear cell showing a highly transparent and structureless ("empty") cytoplasm due to an abundance of glycogen and lipid (Tannenbaum 1971; Bennington and Beckwith 1975; Mostofi 1981). Another type of human renal cell carcinoma designated as chromophobe carcinoma (Thoenes et al. 1985) resembles the clear cell carcinoma, likewise showing tumour cells with a highly transparent cytoplasm. In contrast to the clear cell carcinoma, however, closer inspection reveals a finely reticular (not "empty") cytoplasm in chromophobe tumour cells. The cytomorphological separation between clear and chromophobe RCCs was further substantiated by differences of ultrastructural appearance, cytoskeletal architecture and enzyme synthesis (Thoenes et al. 1986, 1988; Pitz et al. 1987; Störkel et al. 1989; Bonsib and Lager 1990). Furthermore, convincing arguments have been accumulated in the meantime suggesting that the clear cell carcinoma originates from the proximal tubulus system, whereas the chromophobe tumour cells closely resemble the intercalated cells of the cortical collecting duct system (Bachmann et al. 1983; Holthöfer et al. 1983; Waldherr and Schwechheimer 1985; Störkel et al. 1989; Oosterwijk et al. 1990). The difference in histogenetic derivation of clear and chromophobe RCCs is also reflected by differences in the biological behaviour of these tumour types, the clear cell carcinoma exhibiting a markedly worse prognosis (Störkel et al. 1990).

This distinction between clear and chromophobe RCCs has not been applied to the characterization of renal carcinoma cell lines described previously (Hoehn and Schroeder 1978; Matsuda et al. 1979; Naito et al. 1982; Sytkowski et al. 1983; Grossman et al. 1985; Ebert et al. 1990; Anglard et al. 1992). In an ongoing project, we are trying, therefore, to establish a panel of different cell lines, derived from the different types of human RCC as

defined by Thoenes et al. (1986). In this report, we describe the establishment and characterization of four different cell lines originating from strictly defined clear cell carcinomas.

Materials and methods

To establish cell lines, the tumour samples were obtained immediately after nephrectomy and minced under aseptic conditions with paired scissors. The resulting mechanically macerated tissue mass was repeatedly washed by centrifugation and finally seeded into 25 cm^2 Nunclon culture flasks (Gibco, Karlsruhe, FRG) with Dulbecco's modified Eagle's medium (DMEM, Gibco), supplemented with fetal calf serum, penicillin and streptomycin. The cultures were maintained at 37° C in an atmosphere with 5% carbon dioxide. For subculturing, cells were disaggregated by exposure to 0.05% EDTA (Biochrom, Berlin, FRG).

The tumour cells became adherent within 7–10 days after seeding, forming small colonies during the next days. Fibroblastic contamination did not prove to be a major problem, since fibroblasts could be removed by selective trypsinization during the next passages.

In order to determine the doubling time 15 replicate 25 cm^2 culture flasks received inocula of 2×10^5 cells each. Cells from 3 culture flasks were harvested separately each day for 5 days and cell counts with the Neubauer haemocytometer were performed. The results were plotted on semi-logarithmic paper and the mean population doubling time was determined during the exponential growth phase.

The maximal number of tumour cells present in 25 cm^2 culture flasks was determined during the plateau phase of growth.

Chromosome preparations were obtained using standard hypotonic pretreatment procedures at early passages (4th to 6th). G-banding was performed applying the technique of Seabright (1971). Description of karyotypes was done according to ISCN (1991).

For Northern blot analysis aliquots of the renal carcinoma cell lines A, B, C, and D were kept at −70° C until RNA preparation. Total cellular RNA was isolated by the guanidine-thiocyanate method (Chirgwin et al. 1979). The RNA concentration was measured by photometry at 260 nm. The quality of the total cellular RNA was verified in an ethidium bromide stained agarose gel. Northern blot analysis was carried out with 25 µg RNA of each sample under denaturating conditions with an 1% formaldehyde-agarose gel. Before the transfer of the RNA to nylon membranes, the gel was stained with ethidium bromide and the equality of RNA amounts loaded in each lane was verified under UV-light and photographed. In a second control step, the complete transfer of the RNA from the gel to the nylon membrane was again verified under UV-light. Afterwards, the RNA was hybridized with specific DNA probes. The DNA was labelled by incorporation of ^{32}P-dCTP using the oligolabelling kit (Pharmacia, FRG). The probes were obtained from the purified inserts of the following plasmids: p-c-*fos* (exon IV), insert *PvuII*/*Sac* I; p-c-*myc*, insert *Eco* RI/*Hin*d III; p-c-Ki-*ras*, insert *Eco* RI; p-c-*ros*, insert *Eco* RI/*Pvu*II; *p53*, insert *Eco* RI; p-c-*raf*, insert *Xho*I/*Sst*II; p-c-*mos*, insert *Xba*I/*Hind* III. Hybridizations were performed in 5× SSC (1× SSC=0.15 M sodium chloride/0.015 M sodium citrate)/50% formamide/1× Denhardt's solution (1× Denhardt's solution=0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone) and 100 µg denatured salmon sperm DNA per ml at 42° C for 18 h. Filters were washed in 2× SSC/0.1% sodium dodecyl sulphate for 30 min at room temperature and in 0.1× SSC/0.1% sodium dodecyl sulphate for 60 min at 60° C. Fluorography was carried out by exposure of Kodak X-Omat films for 10 days to dried filters at −70° C, in conjunction with intensifying screens.

For light microscopy tumour tissue of the original tumour was fixed in 4% formaldehyde and embedded in paraplast. The tumour cells cultured were seeded on microscope slides and fixed in situ by immersion into 4% formaldehyde. The slides were stained with haematoxylin-eosin (H.E.) and PAS haemalum. Lipid staining with Sudan IV was performed on cryostat sections of the formalin-fixed original tumour.

Table 1 List of antibodies used (*mab* mouse monoclonal antibody)

Antibody	Specificity	Source
mab CK-7	cytokeratin no. 7	Boehringer, Mannheim, Germany
mab CAM 5.2	cytokeratin no. 8	Becton-Dickinson, Neckargemünd, Germany
mab K_s18.174	cytokeratin no. 18	Progen Biotechnics, Germany
mab K_s19.2.105	cytokeratin no. 19	Progen Biotechnics
mab IT-K_s20.3,5 and guinea pig antibodies	cytokeratin no. 20	Progen Biotechnics
mab VIM-9	vimentin	Viramed, Martinsried, Germany

Formalin-fixed, paraffin-embedded tissue samples of the original tumours had to be used for immunohistochemistry of intermediate filaments. After trypsinization, the paraffin sections were stained for simple-epithelial cytokeratins and vimentin (for primary antibodies, see Table 1) using the avidin-biotin-complex (ABC) peroxidase method (for details, see Moll et al. 1991). The cultivated tumour cells were seeded on microscope slides, fixed in situ by exposure to methanol (5 min) and acetone (10 sec) at −20° C and then air-dried. Primary antibodies (Table 1) were applied to the slides and allowed to incubate for 30 min at room temperature in a moist chamber. The visualization of the primary antibody was achieved by the indirect immunoperoxidase method (cf. Thoenes et al. 1988); reagents were from Dako (Hamburg, FRG).

For transmission electron microscopy, small pieces of the original tumour (1 mm^3) and tumour cells seeded on glass cover slips were fixed by exposure to 2.5% sodium cacodylate buffered glutaraldehyde solution (0.1 M; pH 7.4) and post-fixed in 1% sodium-cacodylate buffered osmium tetroxide solution (0.1 M; pH 7.4) prior to Epon embedding. Thin sections were contrasted with uranyl acetate and lead citrate. Electron photomicrographs were taken with an EM 410 Philips transmission electron microscope.

For in vivo studies tumour cells were injected subcutaneously into the right flank of nude mice (n=3 per tumour cell line) at an inoculum size of 1×10^7 cells each. The tumours were fixed in 4% formaldehyde and characterized by conventional light microscopy using haematoxylin-eosin (H.E.) and PAS staining.

Results

The original tumours (A, B, C, and D) were composed of compact cell complexes, separated by narrow septae of stroma. In H.E. stained sections, the tumour cells showed the highly transparent and structureless cytoplasm, typical for clear cell carcinomas. PAS- and lipid staining and transmission electron microscopy, however, revealed varying amounts of glycogen and lipid deposits in the different clear cell carcinomas. Thus, lipid deposition was a prominent feature of the original clear cell carcinomas A and B, whereas in the original tumours C and D glycogen deposition was much more pronounced than lipid deposition. In the tumours A and B, the nuclei were small with only slight variations in size, corresponding to a GI grade of malignancy. The tumours C and D exhibited moderately enlarged tumour nuclei with nucleoli

Table 2 Growth properties in vitro

Cell line	Time in permanent culture	Mean population doubling time	Saturation density (cells/cm^2)
RCC-A	48 months	27 h	68000
RCC-B	42 months	64 h	40000
RCC-C	48 months	51 h	28000
RCC-D	45 months	104 h	21000

Table 3 Description of karyotypes of the different cell lines in accordance with ISCN (1991) (*m* male; *f* female)

Cell line/ sex of patient	Description of karyotype
RCC-A (f)	46–47, XX, der (3)t(3; 5) (p13; q22), der (5)t(1; 5) (q12; q32), +7, der (14) ?t(9; 14) (q12; p11), +12, +16, der (22) ?t (21; 22) (q11.2; q12), +mar[cp10]
RCC-B (f)	45, XX, der (9)t(3; 9) (q11; p11), −8, +i (9) (q10), −10, +r[cp13)
RCC-C (f)	44–46, XX, +der (1) del (1) (p11), der (2) t (2; 3) (p25; p14), +der (2) del (2) (p13) del (2) (q33), der (3) t (3; 11) (p13; q13), +der (3) t (2; 3) (q22; p14), der (?) t (?; 12) (?; q12), der (13) t (13; 14) (p10; q10), +mar[cp12]
RCC-D (m)	68–71, XX, −Y, dir ins (1) (p35; q41q31), −3, t (5; 10) (q21; p15), +i (5) (p10), +5, +7, −8, +10, −14, del (16) (q22), +dir ins (16; 4) (q21; q21q28), +17, −18, +20[cp10]

and occasional mitoses, corresponding to a G II grade of malignancy. Immunohistochemically, the original tumours A, B and D exhibited a focally positive staining reaction for vimentin on paraffin sections. All original tumours showed a positive staining for cytokeratin no. 8 and the original tumour D exhibited a focally positive staining reaction for cytokeratin no. 19. Expression of cytokeratin no. 20 was not observed in any original tumour.

The RCC cell lines have been characterised extensively. Lines A, B, C, and D have been maintained in culture for up to 4 years. Most of our studies were performed with cells from passages 20–30, describing the different cell lines at an early in vitro stage. Nevertheless, the morphological features of these cell lines remained remarkably stable and were still present after prolonged culture.

The in vitro growth properties of the RCC cell lines A, B, C, and D are summarized in Table 2. The cell line RCC-A exhibited the shortest mean population doubling time, whereas RCC-D was the cell line growing most slowly, its mean population doubling time exceeding 4 days. The saturation density was lowest in RCC-D (21000 cells/cm^2) and highest in RCC-A (68000 cells/cm^2).

With exception of RCC-D, which was near-triploid, all cell lines showed a near-diploid modal chromosome number (Table 3). Complete loss of one chromosome 3 was observed only in RCC-D. The other cell lines displayed various derivative chromosomes composed of parts of the chromosome 3, mostly 3p. The chromosomes involved in those marker formations were chromosomes 2, 5 and 11. Gain of chromosome 5 or 5q was observed in RCC-D and RCC-A. One homologue of chromosome 8 was found to be absent in RCC-B and RCC-D. Further karyotypic changes affecting chromosome 9 and 14 were seen in three and two cases, respectively.

Three marker chromosomes (one ring included) failed to show typical banding patterns and could therefore not be identified. The number of subclones and also the number of chromosomal rearrangements was highest in RCC-C.

The tumour cells of all cell lines grow in a strictly anchorage dependent manner. Each tumour cell line exhibited characteristic cytomorphological features, which were stable throughout prolonged in vitro cultivation and which allowed for an individual identification of each cell line. Thus, RCC-A (Fig. 1a) and RCC-C (Fig. 1c) consisted of spindle-shaped fibroblast-like tumour cells, the cells of RCC-C being slenderer than RCC-A cells. In contrast, the cells of RCC-B (Fig. 1b) and RCC-D (Fig. 1d) exhibited a polygonal, more epithelium-like appearance, RCC-B being more voluminous than RCC-D.

PAS haemalum revealed no appreciable glycogen deposits in RCC-A cells (Fig. 1e). RCC-B (Fig. 1f) exhibited a pronounced heterogeneity of glycogen deposition, only a minority of cells showing extensive cytoplasmatic glycogen deposition. Large deposits of glycogen were observed in most cells of RCC-C (Fig. 1g) and RCC-D (Fig. 1h). These differences in glycogen deposition were confirmed by transmission electron microscopy. Lipid deposition was a prominent feature in cells of RCC-B and RCC-D. Typical desmosomes were not observed in any cell line, even when the cells were closely apposed. Immunohistochemically, all lines showed a uniform cytoplasmatic staining with antibodies against vimentin (Fig. 2a, c, f, h), but markedly differed in their cytokeratin expression. Thus, the cells of RCC-A showed a fibrillar cytoplasmatic staining for cytokeratins nos. 7 and 18 (Fig. 2b). RCC-B cells showed a fibrillar cytoplasmatic staining for cytokeratin nos. 18 (Fig. 2d) and 19 (Fig. 2e). RCC-C cells expressed cytokeratin no. 18 exclusively (Fig. 2g), whereas RCC-D cells revealed a spot-like cytoplasmatic staining with antibodies against cytokeratins nos. 18 (Fig. 2i) and 19 (Fig. 2j). Expression of cytokeratin no. 20 was not observed in any cell line.

The proto-oncogene Ki-*ras* was expressed in all cell lines. The proto-oncogene *myc* was expressed in RCC-A, RCC-B and RCC-D, whereas c-*raf* was expressed in RCC-B and RCC-D. No expression was observed for c-*ros*, c-*mos*, and c-*fos* in any cell line. Suppressor gene *p53* mRNA expression was exclusively observed in RCC-D tumour cells (Fig. 3).

Two out of four cell lines (RCC-B and -C) produced slowly growing tumours after subcutaneous transplantation of 1×10^7 viable cells in nude mice. The latency pe-

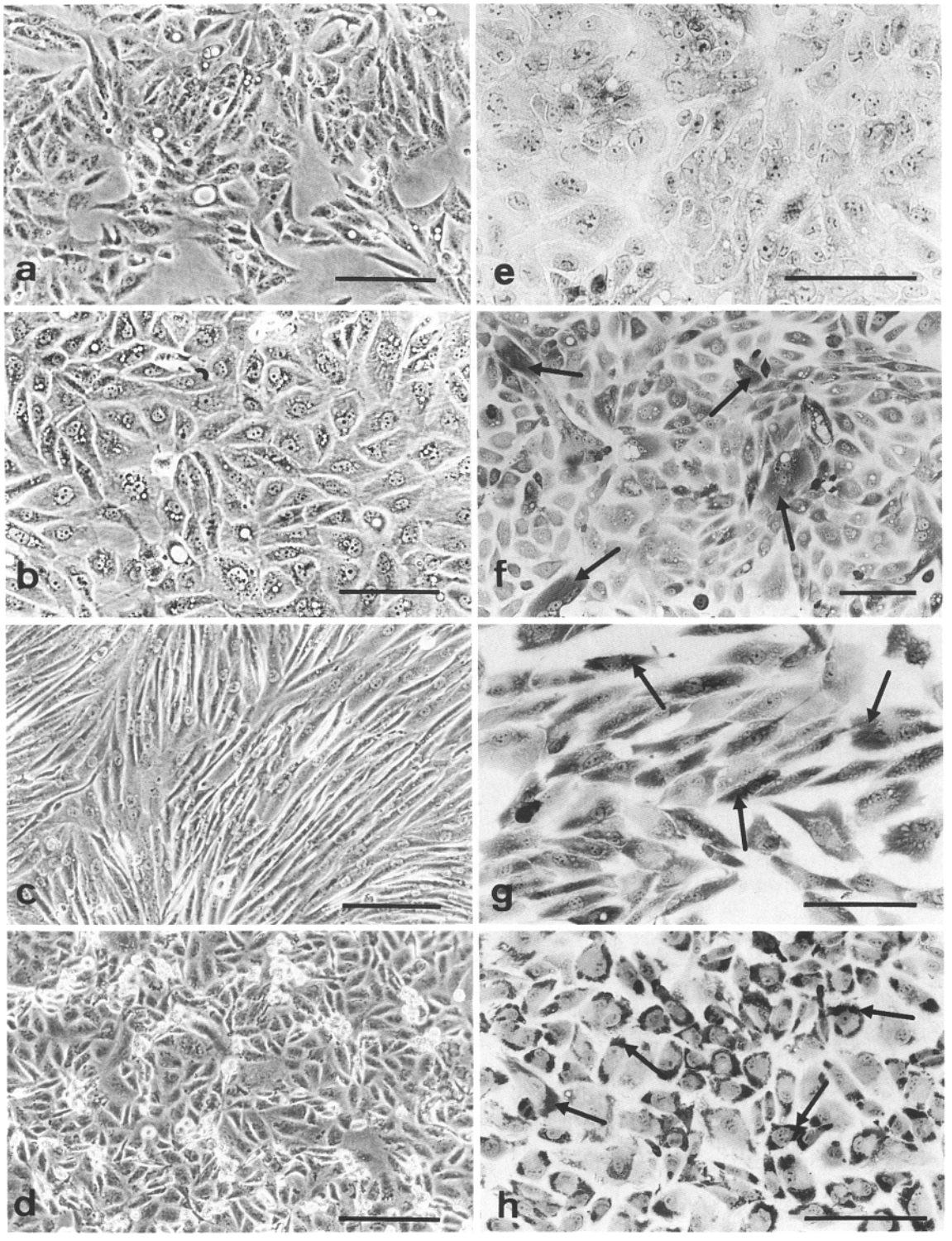

Fig. 1a–h Morphological aspects of the different RCC cell lines. Spindle-shaped, fibroblast-like tumour cells in RCC-A (**a**) and RCC-C (**c**), the cells of RCC-C being slenderer than RCC-A cells. Polygonal, epithelium-like tumour cells in RCC-B (**b**) and RCC-D (**d**), the cells of RCC-B being more voluminous than RCC-D cells. No glycogen deposits in RCC-A cells (**e**). Extensive cytoplasmatic glycogen deposits in some cells of RCC-B (**f**) and in most cells of RCC-C (**g**) and RCC-D (**h**). **a, c, e, g**: phase contrast microscopy; **b, d, f, h**: PAS staining; *bar*: 100 μm

riod between transplantation and the first positive evidence of tumour growth was 2–3 weeks. The transplants did not exceed a diameter of 4 mm after 12 weeks. Histologically, RCC-B cells (Fig. 4a) and RCC-C cells (Fig. 4b) formed clear cell carcinomas with a prominent desmoplastic reaction and markedly pleomorphic nuclei, corresponding to a G III grade of malignancy.

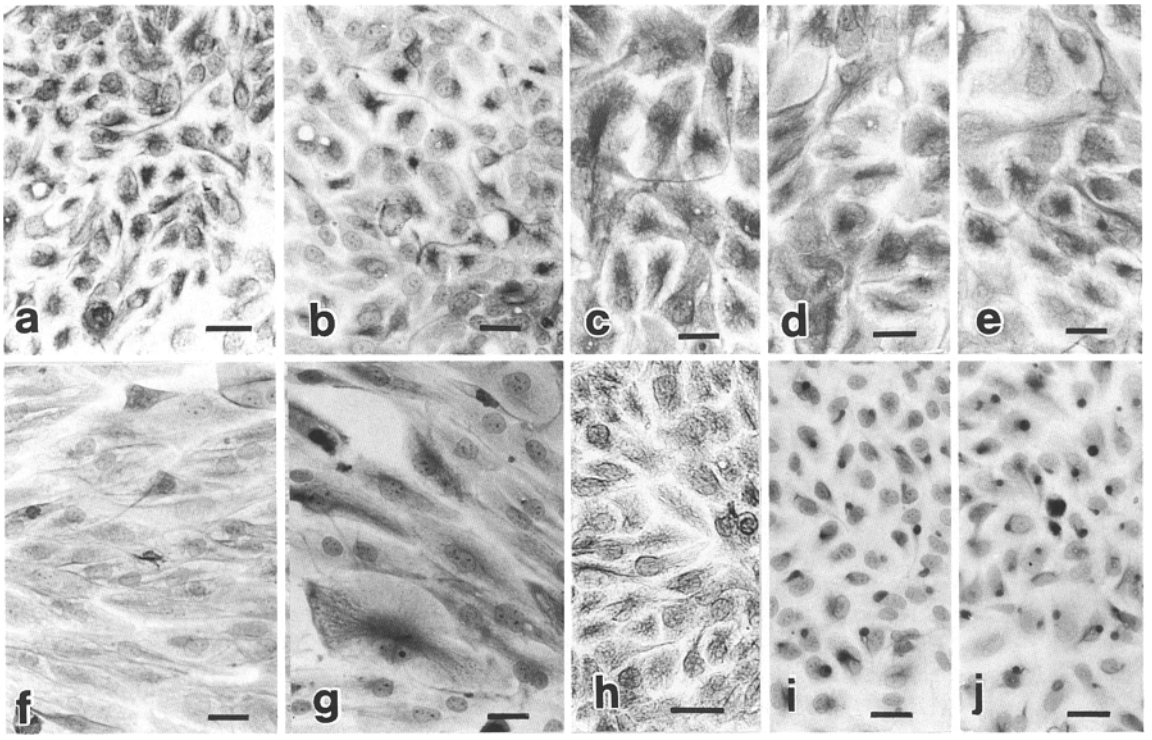

Fig. 2a–j Cytoskeletal architecture of the different RCC cell lines. RCC-A cells exhibiting vimentin (**a**) and cytokeratin no. 18 (**b**). RCC-B cells exhibiting an intensive staining for vimentin (**c**) as well as cytokeratin nos. 18 (**d**) and 19 (**e**). RCC-C cells exhibiting an intensive staining for vimentin (**f**) and cytokeratin no. 18 (**g**). RCC-D cells being positive for vimentin (**h**) as well as cytokeratin nos. 18 (**i**) and 19 (**j**). *Bar*: 33 μm

Discussion

The clinical outcome of patients with metastatic RCC is still extremely poor (Stenzl and deKernion 1989), indicating the persistent need for further investigations on the biological properties of this tumour entity. These investigations are greatly facilitated by the availability of well-characterized permanent cell lines permitting multiple and repeated studies under controlled in vitro conditions. The purpose of the present report, therefore, was to describe the cytomorphological, cytogenetic as well as molecular biological properties of four newly established cell lines derived from the clear cell type of renal carcinoma as defined by Thoenes et al. (1986).

The original tumours of our study were typical representatives of the clear cell type of renal carcinoma exhibiting extensive cytoplasmatic deposits of lipid and/or glycogen. Despite only minor cytomorphological differences of the original tumours, these tumours gave rise to phenotypically markedly divergent cell lines with characteristic traits. The individual identification of each cell line was possible by phase contrast microscopy, the cell lines differing in cell size and exhibiting either a spindle-shaped, fibroblast-like or a polygonal, epithelium-like appearance. Phenotypic divergence further became evident in vitro with respect to cytoplasmatic glycogen deposition. Whereas cytoplasmatic glycogen deposition could easily be demonstrated in cells of RCC-B, -C, and -D in vitro, RCC-A cells did not show appreciable amo-

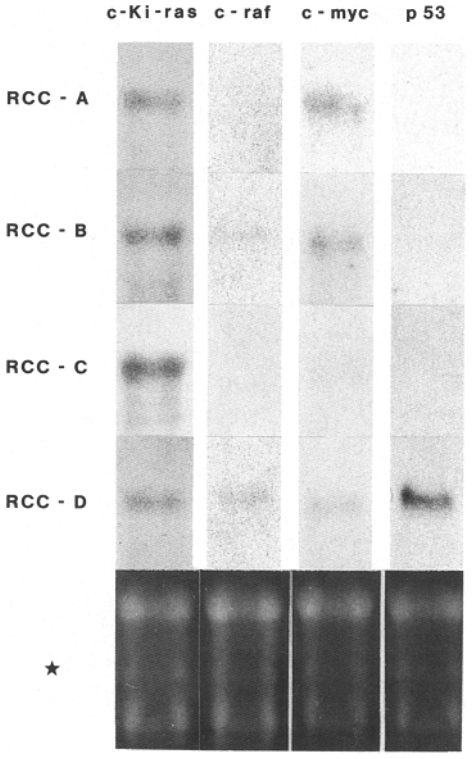

Fig. 3 Northern blot analysis of the different RCC cell lines. No expression was observed for c-*ros*, c-*mos* and c-*fos* in any cell line. Each lane contains 25 μg of total RNA. (* Ethidium-bromide stained gel showing equal amounts of RNA in each lane)

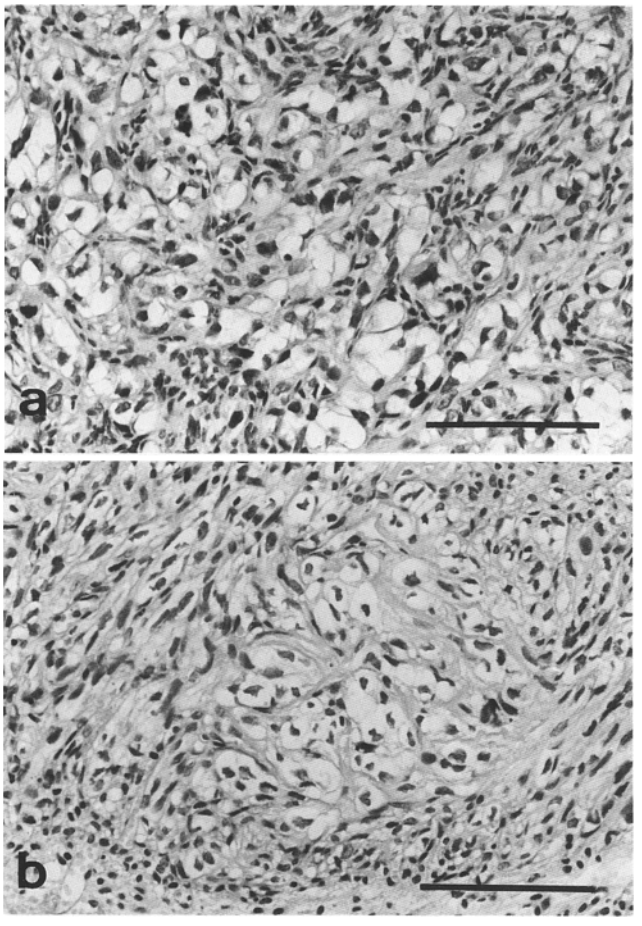

Fig. 4a, b Histomorphological aspects of RCC transplant tumours in nude mice: Clear cell carcinomas with a prominent desmoplastic reaction derived from RCC-B (**a**) and RCC-C (**b**) cells. Note marked nuclear pleomorphism corresponding to a G III grade of malignancy. *Bar*: 100 µm

unts of glycogen. In this respect, RCC-A cells closely corresponded to the original tumour, which had not shown appreciable amounts of cytoplasmatic glycogen.

The karyotypes of all cell lines consistently exhibited abnormalities of chromosome 3 or 3p. These alterations are in full accordance with the karyotype of clear cell renal carcinomas described by Kovacs and Frisch (1989). Additionally, the gain of chromosome 5 segments in two cases and the loss of chromosome 14 in one case might be seen as secondary changes in clear cell carcinomas (Kovacs and Frisch 1989; Pathak 1992). The finding of different subclones in RCC-C reflects the high degree of heterogeneity of this cell line and distinguishes it from the other lines. Furthermore, the frequency of chromosomal rearrangements was higher in RCC-C compared with the other lines, possibly indicating a more malignant genotype.

The aberrant activation of proto-oncogenes as well as the inactivation of tumour suppressor genes is considered to play an important role in the initiation and maintenance of human malignancies (Land et al. 1983; Weinberg 1991). Recent investigations on primary RCCs describe the expression of proto-oncogenes encoding cytoplasmatic (c-Ki-*ras*, c-*raf*) and nuclear (c-*fos*) proteins (Slamon et al. 1984; Karthaus et al. 1987; Yao et al. 1988; Freeman et al. 1989; Sargent et al. 1989; Weidner et al. 1990) as well as the expression of the suppressor gene *p53* (Karthaus et al. 1987). Only a few studies, however, were engaged with the Northern blot analysis of proto-oncogenes and tumour suppressor genes in renal carcinoma cell lines (Yao et al. 1988; Sargent et al. 1989; Eisenkraft et al. 1991). In accordance with previous reports on proto-oncogene expression in primary RCCs (Slamon et al. 1984; Weidner et al. 1990) we showed c-Ki-*ras* mRNA expression in all our cell lines. Members of the *ras*-gene family, i.e. Ki-*ras*, Ha-*ras* and N-*ras*, have been suspected to be implicated in the pathogenesis of a wide range of human malignancies (Burck et al. 1988; Nanus et al. 1990). Nevertheless, the actual significance of c-*ras* mRNA expression in the aetiology of RCCs is still debated. Slamon et al. (1984) reported a three- to four-fold increase in c-Ki-*ras* mRNA expression in three out of seven RCCs when compared with autologous normal renal tissue. In contrast, no c-Ki-*ras* mRNA expression at all was observed in 16 primary RCCs analysed by Yao et al. (1988). However, Nanus et al. (1989) have shown that insertion of a viral Ki-*ras* oncogene into cultured human proximal tubular epithelia resulted in cells exhibiting a renal cancer phenotype. Therefore, further investigations will have to clarify the role of *ras* gene activation for the neoplastic transformation of renal epithelia.

Interestingly, mRNA expression of the *p53* gene, which is considered to be a tumour suppressor gene (Weinberg 1991), was observed in RCC-D cells. The expression of the *p53* suppressor gene in overtly malignant cells is not a contradiction at all, since only wild-type *p53* cDNA acts as an anti-proliferative suppressor gene (Eliyahu et al. 1989; Finlay et al. 1989; Baker et al. 1990). In contrast, mutant *p53* alleles are known to act like oncogenes, stimulating growth and transformation of cells, even if these cells continue to harbour intact wild-type *p53* genes. In fact, *p53* has been considered to be the most frequently mutated gene in human cancer, often being altered by point mutations (Weinberg 1991). Therefore, RCC-D cells might carry a mutant *p53* gene supposedly implicated in the malignant transformation of these cells. The failure to demonstrate *p53* mRNA in RCC-A, -B, and C-cells might be explained either by lack of transcription or by the detection limits of Northern blot analysis.

In conclusion, the newly established cell lines represent a spectrum of RCCs of the clear cell type differing in cytomorphological, cytogenetic as well as molecular biological aspects. These cell lines will become valuable tools for further investigations on biological properties of this type of renal carcinoma. Experimental studies analysing the effects of biological response modifiers on the proliferation and invasive behaviour of these cell lines are currently in progress in our laboratory. In addition, the cell lines RCC-B and RCC-C capable of producing tumours in nude mice will facilitate complementary investigations in vivo.

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References

Anglard P, Trahan E, Liu S, Latif F, Merino MJ, Lerman MC, Zbar B, Linehan WM (1992) Molecular and cellular characterization of human renal cell carcinoma cell lines. Cancer Res 52: 348–356

Bachmann S, Kriz W, Kuhn C, Franke WW (1983) Differentiation of cell types in the mammalian kidney by immunofluorescence microscopy using antibodies to intermediate filament proteins and desmoplakins. Histochemistry 77: 365–394

Baker SJ, Markowitz S, Fearon ER, Willson JKV, Vogelstein B (1990) Suppression of human colorectal carcinoma cell growth by wild-type *p53*. Science 249: 912–915

Bennington JL, Beckwith JB (1975) Tumors of the kidney, renal pelvis and ureter. Atlas of tumor pathology, 2nd series Fascicle 12, Armed Forces Institute of Pathology, Washington DC

Bonsib SM, Lager DJ (1990) Chromophobe cell carcinoma: analysis of five cases. Am J Surg Pathol 14: 260–267

Burck KB, Liu ET, Larrick JW (1988) Oncogenes. Springer, Berlin Heidelberg New York

Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18: 5294–5299

Ebert T, Bander NH, Finstad CL, Ramsawak RD, Old LJ (1990) Establishment and characterization of human renal cancer and normal kidney cell lines. Cancer Res 50: 5531–5536

Eisenkraft BL, Nanus DM, Albino AP, Pfeffer LM (1991) α-interferon down-regulates epidermal growth factor receptors on renal carcinoma cells. Relation to cellular responsiveness to the antiproliferative action of α-interferon. Cancer Res 51: 5881–5887

Eliyahu D, Michalovitz D, Eliyahu S, Pinhasi-Kimh O, Oren M (1989) Wild-type *p53* can inhibit oncogene-mediated focus formation. Proc Natl Acad Sci (USA) 86: 8763–8767

Finlay CA, Hinds PW, Levine AJ (1989) The *p53* proto-oncogene can act as a suppressor of transformation. Cell 57: 1083–1093

Freeman MR, Washecka R, Chung LWK (1989) Aberrant expression of epidermal growth factor receptor and HER-2 (*erb*B-2) messenger RNAs in human renal cancers. Cancer Res 49: 6221–6225

Grossman HB, Wedemeyer G, Ren L (1985) Human renal carcinoma: characterization of five new cell lines. J Surg Oncol 28: 237–244

Hoehn W, Schroeder FH (1978) Renal cell carcinoma: two new cell lines and a serially transplantable nude mouse tumour (NC 65). Invest Urol 16: 106–112

Holthöfer H, Miettinen A, Paasivuo R, Lehto V-P, Linder E, Alfthan O, Virtanen I (1983) Cellular origin and differentiation of renal cell carcinomas: a fluorescence microscopic study with kidney-specific antibodies, antiintermediate filament antibodies and lectins. Lab Invest 49: 317–326

ISCN (1991) Guidelines for cancer cytogenetics. Supplement to an International System for Human Cytogenetics Nomenclature. Mitelman F. (ed), S. Karger, Basel

Karthaus HFM, Bussemakers MJG, Schalken JA, Kurth KH, Feitz WFJ, Debruyne FMJ, Bloemers HPJ, Van de Ven WJM (1987) Expression of proto-oncogenes in xenografts of human renal cell carcinomas. Urol Res 15: 349–353

Kovacs G, Frisch S (1989) Clonal chromosome abnormalities in tumor cells from patients with sporadic renal cell carcinomas. Cancer Res 49: 651–659

Land H, Parada LF, Weinberg RA (1983) Cellular oncogenes and multistep carcinogenesis. Science 222: 771–778

Matsuda M, Osafune M, Nakano E, Kotake T, Sonoda T, Watanabe S, Hada T, Okochi T, Higashino K, Yamamura Y, Abe T (1979) Characterization of an established cell line from human renal carcinoma. Cancer Res 39: 4694–4699

Moll R, Hage C, Thoenes W (1991) Expression of intermediate filament proteins in fetal and adult human kidney: modulation of intermediate filament patterns during development and in damaged tissue. Lab Invest 65: 74–86

Mostofi FK (1981) Histological typing of kidney tumours. International Histological Classification of Tumours, no. 25. World Health Organization, Geneva

Naito S, Kanamori T, Hisano S, Tanaka K, Momose S, Kamata N (1982) Human renal cell carcinoma: establishment and characterization of two new cell lines. J Urol 128: 1117–1121

Nanus DM, Ebrahim SAD, Bander NH, Real FX, Pfeffer LM, Shapiro JR, Albino AP (1989) Transformation of human kidney proximal tubule cells by *ras*-containing retroviruses. J Exp Med 169: 953–972

Nanus DM, Mentle IR, Motzer RJ, Bander NH, Albino AP (1990) Infrequent *ras* oncogene point mutations in renal cell carcinoma. J Urol 143: 175–178

Oosterwijk E, Van Muijen GNP, Oosterwijk-Wakka JC, Warnaar SO (1990) Expression of intermediate-sized filaments in developing and adult human kidney and in renal cell carcinoma. J Histochem Cytochem 38: 385–392

Pathak S (1992) Cytogenetics of epithelial malignant lesions. Cancer Suppl 70: 1660–1670

Pitz S, Moll R, Störkel S, Thoenes W (1987) Expression of intermediate filament proteins in subtypes of renal cell carcinomas and in renal oncocytomas. Lab Invest 6: 642–653

Sargent ER, Gomella LG, Belldegrun A, Linehan WM, Kasid A (1989) Epidermal growth factor receptor gene expression in normal human kidney and renal cell carcinoma. J Urol 142: 1364–1368

Seabright M (1971) A rapid banding technique for human chromosomes. Lancet ii:971–972

Slamon DJ, deKernion JB, Verma IM, Cline MJ (1984) Expression of cellular oncogenes in human malignancies. Science 224: 256–262

Stenzl A, deKernion JB (1989) Pathology, biology, and clinical staging of renal cell carcinoma. Semin Oncol 16 [Suppl 1]:3–11

Störkel S, Steart PV, Drenckhahn D, Thoenes W (1989) The human chromophobe cell renal carcinoma: its probable relation to intercalated cells of the collecting duct. Virchows Arch [B] 56: 237–245

Störkel S, Thoenes W, Jacobi GH, Lippold R (1990) Prognostic parameters in renal cell carcinoma – a new approach. Eur J Urol 16: 416–422

Sytkowski AJ, Richie JP, Bicknell KA (1983) New human renal carcinoma cell line established from a patient with erythrocytosis. Cancer Res 43: 1415–1419

Tannenbaum M (1971) Ultrastructural pathology of human renal cell tumours. Pathol Ann 6: 249–277

Thoenes W, Störkel St, Rumpelt HJ (1985) Human chromophobe cell renal carcinoma. Virchows Arch [B] 48: 207–217

Thoenes W, Störkel St, Rumpelt HJ (1986) Histopathology and classification of renal cell tumours (adenomas, oncocytomas and carcinomas). Pathol Res Pract 181: 125–143

Thoenes W, Störkel St, Rumpelt HJ, Moll R, Baum HP, Werner S (1988) Chromophobe cell renal carcinoma and its variants – a report on 32 cases. J Pathol 155: 277–287

Waldherr R, Schwechheimer K (1985) Co-expression of cytokeratin and vimentin intermediate-sized filaments in renal cell carcinomas. Virchows Arch [A] 408: 15–27

Weidner U, Peter S, Strohmeyer T, Hussnätter R, Ackermann R, Sies H (1990) Inverse relationship of epidermal growth factor receptor and HER2/*neu* gene expression in human renal cell carcinoma. Cancer Res 50: 4504–4509

Weinberg RA (1991) Tumor suppressor genes. Science 254: 1138–1148

Yao M, Shuin T, Misaki H, Kubota Y (1988) Enhanced expression of c-*myc* and epidermal growth factor receptor (C-*erb*B-1) genes in primary human renal cancer. Cancer Res 48: 6753–6757