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Expression of cell adhesion molecules in an established and characterized new human renal cell cancer line, *CCF-RC7*

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Abstract In order to investigate the importance of cell adhesion molecules (CAMs) in renal cell carcinoma (RCC), a cell line, designated as CCF-RC7, was established from a human RCC of the clear cell type. CCF-RC7 was passaged over 50 times in vitro for $3\frac{1}{2}$ years. The cell line has an epithelial morphology and a doubling time of 30 h, forming colonies in soft agar with an average efficiency of 10.4% and producing clear cell tumors in athymic nude mice. CCF-RC7 cells have an aneuploid-hypotetraploid karyotype with a modal chromosome number of 82 and rearrangements in chromosomes 9, 12 and 14. Immunohistochemical and flow immunocytometric analyses revealed high expression of ICAM-1 (CD54), and Hermes antigen (CD44), which was significantly upregulated by cytokine and PMA treatment. VLA-4 was expressed on approximately 20% of tumor cells and could not be altered by cytokine or PMA stimulation. High expression of sialyl Lewis X was also demonstrated by immunohistological examination. This newly characterized cell line will serve as a useful model for the study of CAMs during hematogenous metastasis and host defense mechanisms in human RCC.

Key words Kidney neoplasms · Tumor cells · Cultures · Cell adhesion molecules

The ability of renal cell carcinomas (RCC) to develop tumor metastases is dependent upon the capacity of the tumor cells to migrate in blood or lymph vessels from the primary tumor, adhere to vascular endothelial cells and then exit the venous or capillary bed of the target organ. Extensive studies over the past decade in acute inflammatory diseases have characterized three superfamilies of cell adhesion molecules (CAMs), which play an important role in cell-cell and cell-matrix interactions: the selectins or lectin-epidermal growth factor related cell adhesion molecules [e.g., endothelial leukocyte adhesion molecule-1 (ELAM-1)], which interact with carbohydrate ligands; the heterodimeric integrins [e.g., very late activation antigen-4 (VLA-4)]; and the immunoglobulin superfamily [e.g., intercellular adhesion molecule-1 (ICAM-1)] [10, 14, 16, 20]. Recent reports of human melanoma cells and human colon carcinoma cells have demonstrated that several cell adhesion molecules, which have important functions in leukocyte adherence to and migration across vascular endothelial cells, are also an important mediator in the adhesion pathway of tumor cells (23, 29). Furthermore, studies of ICAM-1 on renal cell carcinomas in our laboratory suggest that tumor-associated CAMs appear to be important in tumor cell killing mediated by autologous tumor infiltrating lymphocytes [30]. However, the function of different CAMs in renal cell carcinoma in the metastatic cascade and the interaction between tumor cells and tumor-infiltrating lymphocytes is still unknown and requires further investigation. In order to perform the relevant studies, it is necessary to have RCC cell lines which stably express different kinds of CAM in long-term culture.

The subject of this report is a newly characterized continuous cell line, designated as CCF-RC7, derived from tumor cells of a patient with renal cell carcinoma, and characterized with regard to clinical origin, karyotype, DNA content, doubling time, morphological characteristics, tumorigenicity and expression of various CAMs in resting tumor cells and in response to stimulation with biological response modifiers.

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Materials and methods

Clinical history

CCF-RC7 was derived from a specimen of primary renal cell carcinoma obtained from a 33-year-old white female who underwent left radical nephrectomy in July 1990. The preoperative evaluation including computer tomography of the abdomen, bone scan, renal angiography and chest X-ray revealed a hypervascular mass in the left kidney consistent with a renal cell carcinoma and no metastatic lesions. Pathological examination identified an adenocarcinoma in the upper pole of the kidney, which was 5 cm in diameter. It was sharply demarcated from the renal parenchyma and did not invade the renal capsule, renal pelvis or renal vessels (Robson stage I). The tumor was a highly differentiated renal cell cancer (nuclear grade I) of clear cell type and solid growth pattern. There were no nodal metastases. The postoperative course was uneventful and the patient is alive without evidence of disease years later.

Primary culture

Surrounding fat, vessels, connective tissue and capsule were excised from the fresh operative specimen. The tumor was finely minced with a scalpel and the tissue fragments were placed in 0.1% collagenase solution (CLSIII, Cooper Biomedical, Malvern, Pa., USA) and 0.01% deoxyribonuclease solution (type IV, Sigma, St. Louis, Mo., USA) dissolved in Dulbecco's phosphate-buffered saline (PBS, Gibco-BRL, Grand Island, Ind., USA) for 1 h in a water bath with slight agitation. After digestion, the cell suspension was diluted in equal volumes of RPMI 1640 with L-glutamine. The suspension was washed twice and viability was determined by trypan blue staining. The tumor cells were plated into T-25 flasks (Primaria grade, Falcon Labware, Lincoln Park, N.J., USA) in tissue culture media consisting of RPMI 1640 with L-glutamine supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco), 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 100 mM sodium pyruvate, 0.1 mM non-essential amino acids, 5×10^{-5} M 2-mercaptoethanol, and 100 units of penicillin/streptomycin (Gibco). Flasks were placed in a 37 °C incubator and maintained in a 5% CO_2 atmosphere.

Maintenance of cell culture

Cultures were fed twice weekly and passaged shortly after becoming confluent. Confluent flasks were subcultured by pouring off the old medium from the flask, washing the cells with sterile Hank's balanced salt solution (HBSS, Ca^{2+} and Mg^{2+} free, Gibco) and adding 0.25% trypsin/0.1% ethylenediaminetetra-acetic acid (EDTA) (Gibco) for 5 min at 37 °C to produce a single cell suspension. Complete medium was added and the cells were washed by pelleting at 1000 rpm for 5 min before resuspension in complete medium and seeding to new flasks. In general, cells were passaged at a split ratio of 1:5. In addition, aliquots of cells were cryopreserved in FBS/10% dimethyl sulfoxide (DMSO, Sigma) and stored in liquid nitrogen after every five to ten passages.

In vitro growth rate determination

Passage 37 was used to determine in vitro growth rate of CCF-RC7. T-25 flasks (Corning) were set up in triplicate and each flask was plated initially with 3×10^4 cells. The cells were cultured in RPMI 1640 with 10% FBS and 25 mM HEPES buffer and observed daily with an inverted microscope. The culture medium was changed every 3 days. After 24, 48, 72, 96 and 120 h three flasks were trypsinized and the number of cells in each flask counted using a hemocytometer. A growth curve was constructed and the doubling time of the cell population was calculated during the logarithmic growth phase.

Soft agar culture of CCF-RC7

CCF-RC7 in passage 36 was grown to subconfluency for this experiment. A bottom layer of 0.5% agar was prepared with tissue culture media RPMI 1640 supplemented with 20% heat-inactivated FBS and Bacto-Agar (Difco Laboratories, Detroit, Mich., USA); 1 ml of this solution was placed into 35 × 10-mm plastic Petri dishes (Nunc, Rosklide, Denmark) and allowed to solidify. After trypsinization and washing, the cells were resuspended as a single-cell suspension in 0.3% agar solution containing RPMI 1640 supplemented with 20% heat-inactivated FBS. One milliliter of each agar/cell solution was placed over the lower layer of agar, allowed to solidify and incubated at 37 °C in a 5% CO_2 atmosphere. Cells were plated in triplicate in a concentration of 0.5×10^4/ml. Cell aggregates 60 μm or greater in diameter were defined as colonies. Colonies were counted 2 weeks after plating using an Omnicon computerized image analysis system (Bausch & Lomb, Rochester, N.Y., USA).

In vivo growth determination (tumorigenicity)

A subconfluent culture of CCF-RC7 at passage 34 was trypsinized, resuspended in PBS and injected s.c. into two 8-week-old male athymic nude mice (Charles River, Laboratories, Wilmington, Mass., USA) with concentrations of 0.5×10^6 cells and 5×10^6 cells in 0.1 ml PBS, respectively. In a control animal only 0.1 ml PBS was injected. The formation of tumors was evaluated after 3 weeks, the animals put to death and the tumors excised and measured. Pieces of the tumors were frozen in isopentane and histologically examined after staining with hematoxylin and eosin.

Electron-microscopy

Tumor cells from CCF-RC7 (passage 38) were grown in 50 × 10-mm plastic Petri dishes (Thermonox) to confluency and fixed in 0.1 M cacodylate-buffered 3.75% glutaraldehyde containing 6% sucrose (pH 7.4). This step was followed by postfixation in 0.1 M cacodylate-buffered 1% osmium tetroxide, dehydration in graded ethanols and embedding in Spurr resin. Plastic sections were taken at a thickness of 1 μm, stained with a mixture of toluidine blue and basic fuchsin, and examined by light microscopy. Appropriate blocks were selected and corresponding thin sections were cut at 60–80 nm, stained in uranyl acetate and lead citrate, and studied with a Philips 400T electron microscope.

DNA ploidy analysis by flow cytometry

DNA ploidy analysis was performed on passages 3 and 33 by flow cytometry. The tumor cells were harvested in a status of subconfluency. Approximately 1 million cells were then washed, incubated with trypsin and ribonuclease A, and stained with propidium iodine staining solution according to the method of Vindelov et al. [34]. Between 15 000 and 30 000 cells from each sample were analyzed using the FACScan flow cytometer (Becton Dickinson, Mountain View, Calif., USA). Ethanol-fixed human lymphocytes served as diploid controls. Designation of tumor populations as demonstrating "DNA aneuploidy" was based upon the criteria outlined by Hiddemann et al. [11].

Karyotype analysis

Karyotype analysis was performed on passages 3 and 50 for CCF-RC7. Colcemid, 0.01 μg/ml, was added to the culture and incubated overnight. The tumor cells were harvested after 6 days in culture by removing the media and adding hypotonic solution (HEPES, EDTA, and KCl) for 30 min at 37 °C. The cells were scraped from the flask, fixed with a mixture of methanol:acetic acid (3:1) for 20 min and stored overnight at 4 °C. Slides were prepared by dropping cell suspensions onto cold wet slides. The slides were incubated for 24 h in an oven at 60 °C and placed in a desiccator at room temperature for at least 1 week prior to banding. G-banding was performed by a previously described method [33].

Immunohistochemical analysis

A Shandon Southern cytocentrifuge was used to produce cytospins of CCF-RC7 with 100000 cells/slide in equal volumes of heat-inactivated FBS and cell suspension to facilitate cell adhesion. The preparations were fixed in cold acetone for 10 min, air dried and washed in PBS. In addition, tissue pieces of original tumor were snap frozen and sections were cut at 4-μm intervals. The preparations were incubated for 30 min with mouse monoclonal antibodies against the following human antigens: Uro series (dilution 1:200, Cambridge Research Lab, Mass., USA), RC 38 (dilution 1:10, Sanbio BV Bioproducts, Uden, Netherlands), AE 1 and AE 3 (dilution 1:200, Boehringer Mannheim, Indianapolis, Ind., USA), HLA-A + B + C (dilution 1:100, Dako Corporation, Carpintera, Calif., USA), ICAM-1 [dilution 1:300, Becton Dickinson (BD), San Jose, Calif., USA], ICAM-2 (1:100, R&D, Minneapolis, Minn., USA), VLA-4 (dilution 1:3, BD), Hermes-antigen (dilution 1:80, BD), LFA-1 (dilution 1:150, BD) and sialyl Lewis X (dilution 1:3, BD) [1]. The sections were then bar coded to be stained on an automatic immunostaining machine (Ventana 320, Ventana Medical System, Tuscon, Ariz., USA), in which the slides were first subjected to an avidin-biotin flush to minimize nonspecific binding to endogenous biotin. Then biotinylated anti-mouse IgG/M and avidin-biotinylated peroxidase complex (Ventana) were applied to the preparations. The color reaction product was developed with 3-amino-9-ethylcarbazole in the presence of 0.005% hydrogen peroxide and the slides were then counterstained with hematoxylin, coverslipped and viewed.

Cytokine treatment

Subconfluent CCF-RC7 cells were treated with cytokines in culture for 18 h before harvesting for flow immunocytometry to determine the expression of various cell adhesion molecules. Cytokines used in this study are human recombinant interleukin (rIL)-1β (10 ng/ml, R&D, Minneapolis, Minn., USA), recombinant interferon (rINF)-γ (200 U/ml, Biogen, Cambridge, Mass., USA) and recombinant tumor necrosis factor (rTNF)-α (200 U/ml, Cetus Corporation, Emeryville, Calif., USA). Phorbol myristate acetate (PMA, 10 ng/ml, Sigma, St. Louis, Mo., USA) was reconstituted with DMSO/RPMI and stored at −70 °C until use.

Two-color immunocytometry

Flow immunocytometric analysis was performed using two-color immunofluorescence [15]. Fluorescein isothiocynate (FITC) and phycoerythrin-conjugated monoclonal antibodies were used to phenotypically identify and quantify tumor cell subsets. Isotypic controls were employed for each particular subclass of immunoglobulin. Analyses on the FACScan (Becton-Dickinson, San Jose, Calif., USA) were performed utilizing an argon ion laser (Cyonics, San Jose, Calif., USA) with 15 mW of 488 nm excitation. The optics in the fluorescence path included a focusing objective, beam splitters, dichroic mirrors, a 530-nm bandpass filter, a 585-nm bandpass filter, and a 650-nm-long pass/cut-on filter. Analysis was performed using multicontour, multiparameter software [Paint-a-Gate and Lysis II (Becton Dickinson)]. Results were reported as the percentage of positive cells and the relative intensity after correcting for the non-specific binding of isotypic controls as determined by measurement of the autofluorescence background.

Statistical analysis

Differences in percentage expression and relative intensity of CAM expression at baseline and in response to cytokines were analyzed by one-way analysis of variance with the multiple comparison test of mean values, according to the Tukey-Kramer method [25].

Results

Growth kinetics

The primary culture of CCF-RC7 attained confluent monolayers within 2 weeks and was subcultured as described in "Materials and methods". Currently, the split ratio is 1:5 every 7–10 days and the cells are presently in their 51st passage after 3 years in culture. CCF-RC7 does not show contact inhibition and overgrow after reaching confluency. The in vitro growth curve analysis of passage 37 indicates a cell cycle time of 30 h in the logarithmic growth phase (Fig. 1).

Tumorigenicity

After an observation period of 3 weeks, the s.c. injection of 0.5×10^6 and 5×10^6 CCF-RC7 cells into nude mice resulted in the formation of a 0.5 × 0.5-cm and

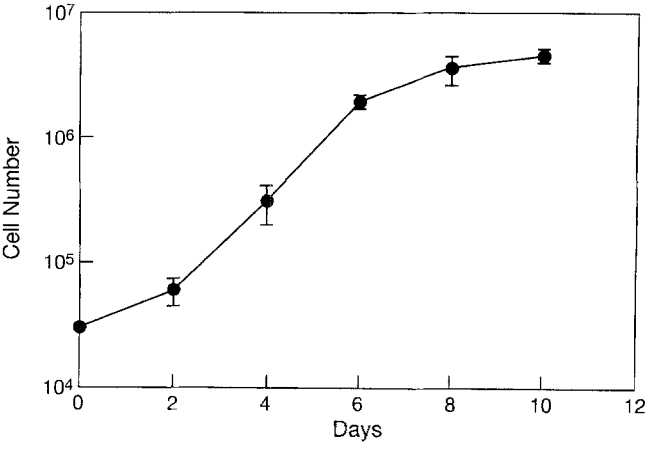

Fig. 1 Growth curve of CCF-RC7 at passage 37. An average of three counts for each time period was used to define each point. CCF-RC7 has a doubling time of 30 h in the logarithmic phase of the growth curve

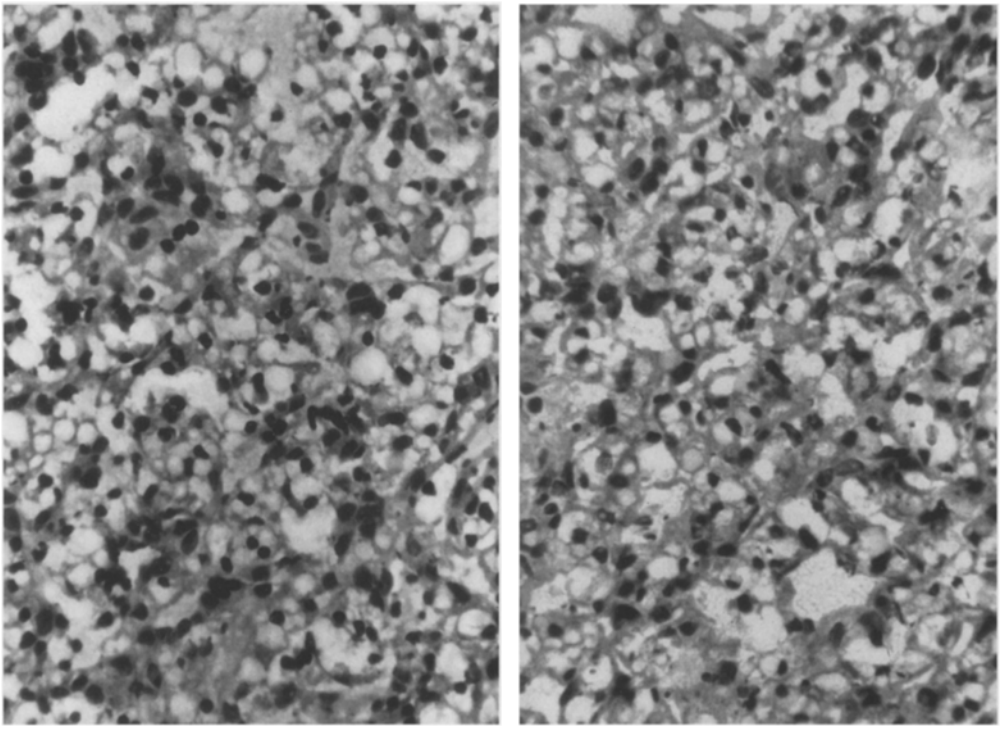

Fig. 2 Histology of the original patient tumor (*left*) and nude mice tumor (*right*) induced by CCF-RC7 cells at passage 34. The nude mice tumor resembled the clear cell morphology of the original tumor. *Left*: Tumor; *right*: Xenograft

a 1.1 × 0.9-cm tumor, respectively. No growth of tumor was observed in the control animals. Histopathological examination showed that the tumors retained the clear-cell morphology of the original renal tumor (Fig. 2). In soft agar CCF-RC7 grew into colonies 14 days after plating. The number of colonies formed 5×10^3 tumor cells was 525.9 ± 45.2, indicating that the plating efficiency was $10.4\% \pm 0.9\%$.

Morphology

Microscopic examination of the tumor cells in culture revealed an epithelioid appearance with large nuclei containing one to two nucleoli and intracytoplasmatic vacuoles. Occasionally, multinucleate cells were seen. The morphological appearance of the tumor cells has not changed despite long-term culture. Electron-microscopic analysis of CCF-RC7 cells confirmed their epithelial origin. Desmosomes, surface microvilli and perinuclear filament bundles were identified in the tumor cells (Fig. 3). Also evident were intracytoplasmatic glycogen lakes and multiple nucleoli. However, there was no evidence of intracytoplasmic viral particles or mycoplasmal contamination.

DNA ploidy analysis

The DNA ploidy analysis from CCF-RC7 in both passage 3 and passage 33 revealed an aneuploid-hypotetraploid DNA histogram. The calculated DNA index (the ratio of the modal G0/G1 peak of the cell line to that calculated for normal diploid standards) for CCF-RC7 was 1.84 and there was no significant variation in the DNA pattern between the early passage population of cells and the late passage of cells. The large number of tumor cells in the S-phase (23.1%) of the mitotic cycle, as evidenced between the G0/G1 and G2/M peaks on the histogram, indicated a high proliferative activity of this cell line (Fig. 4).

Karyotype

Karyotype analysis at passage 3 showed a modal chromosome number of 82 in CCF-RC7 and all metaphases revealed hypotetraploid tumor cells with rearrangements involving chromosomes 9, 12 and 14 (Fig. 5). Neither chromosomal deletion nor rearrangement of chromosome 3p was observed. Furthermore no unidentified marker or Y chromosomes were identified. The karyotype of CCF-RC7 cells was distinct from that of other cell lines maintained in our laboratory. Chromosomal analysis at passage 50 revealed a stable hypertriploid karyotype with a slight decrease in modal number to 79. Extra copies of all chromosomes except 13 and X were present and all cells karyotyped contained the der(14) t(9;14) and i(12q) seen in the original karyotype. Several new but unidentifiable marker chromosomes were also present.

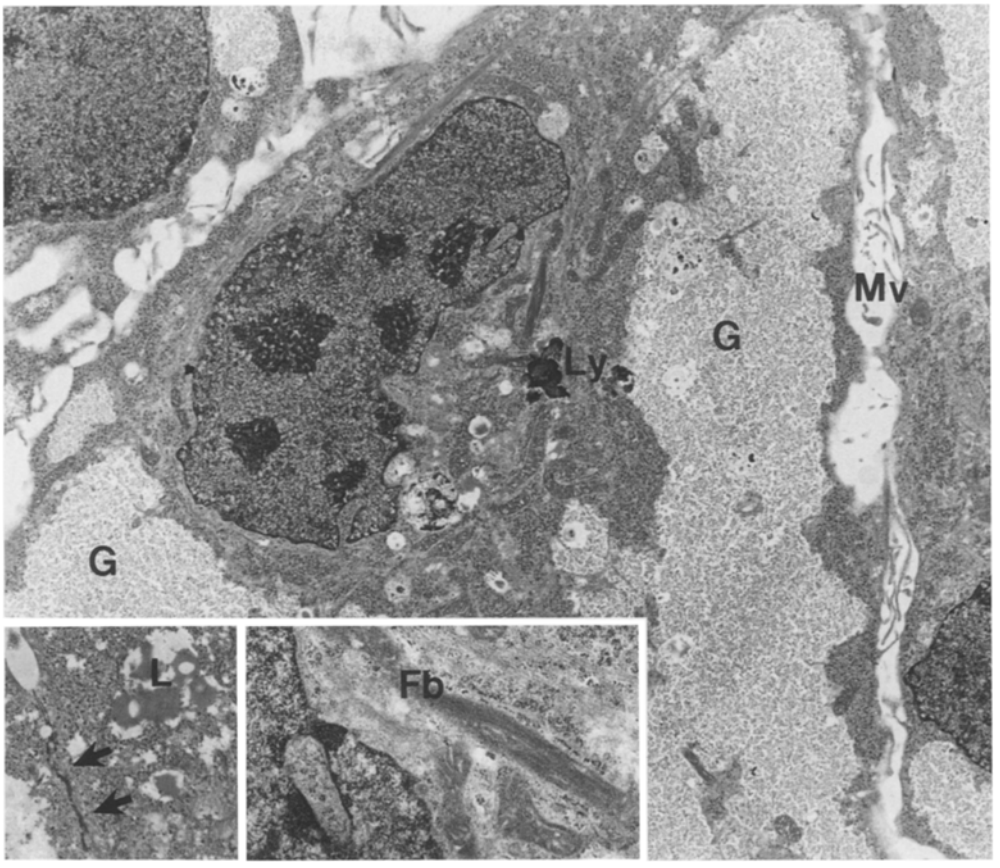

Fig. 3 Electron micrograph of cultured CCF-RC7 tumor cells at passage 38. Cells are polyglonal with pleomorphic nuclei and multiple prominent nucleoli. Cell surfaces project long microvillous processes (*Mv*) and are joined by well-developed desmosome junctions (*left insert, arrows*). Cytoplasm contains abundant glycogen (*G*), numerous polyribosomes, tubular mitochondria and short lengths of rough endoplasmic reticulum. There are a few lipid droplets (*L*) and dense lysosome bodies (*Ly*). Golgi are small and inconspicuous. Cytoplasmic filaments are plentiful and focally organized into dense perinuclear filament bundles (*right insert, Fb*). Uranyl acetate and lead citrate, ×7500

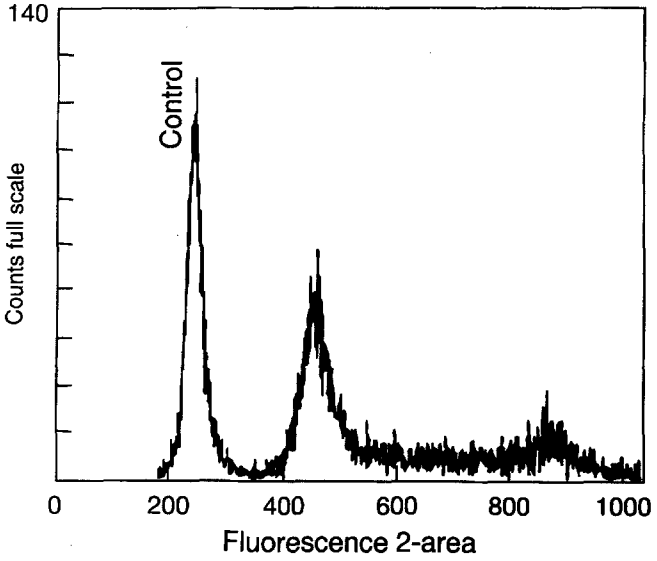

Fig. 4 DNA ploidy analysis of CCF-RC7 revealed an aneuploid hypotetraploid DNA histogram (DNA index 1, 84). Ethanol-fixed human lymphocytes were used as diploid controls

Immunohistochemical analysis

CCF-RC7 cells expressed antigens recognized by monoclonal antibodies (mAbs) AE1 and AE3, URO-1, URO-7 and URO-10. However, the tumor cells stained negatively with URO-2, URO-5 and URO-9, and slightly positive with the mAbs for URO-3, URO-4 and RC 38. These results are in accordance with those for the original tumor tissue, and suggest that CCF-RC7 originated from the proximal renal tubulus epithelium [1, 2]. Likewise, both the tumor tissue and the cell line stained strongly positive for HLA-A + B + C. The expression of the cell adhesion molecules revealed positive results for ICAM-1 (CD54), HERMES antigen (CD44) and sialyl Lewis X for the cell line as well as for the original tumor tissue. In contrast, mAbs for VLA-4 (CD49d/CD29) stained positively with the cell line, but not with the orginal tumor tissue. No positive staining was achieved with mAbs against ICAM-2 and LFA-1 for the cell line or for the original tumor tissue (Table 1, Fig. 6).

Expression of cell adhesion molecules after cytokine stimulation

The percentage expression of ICAM-1 on CCF-RC7 was significantly upregulated after stimulation with rINF-γ ($P < 0.001$), rTNF-α ($P < 0.001$) and PMA ($P < 0.01$), but no effect on the percentage expression of ICAM-1 was observed after rIL-1β treatment

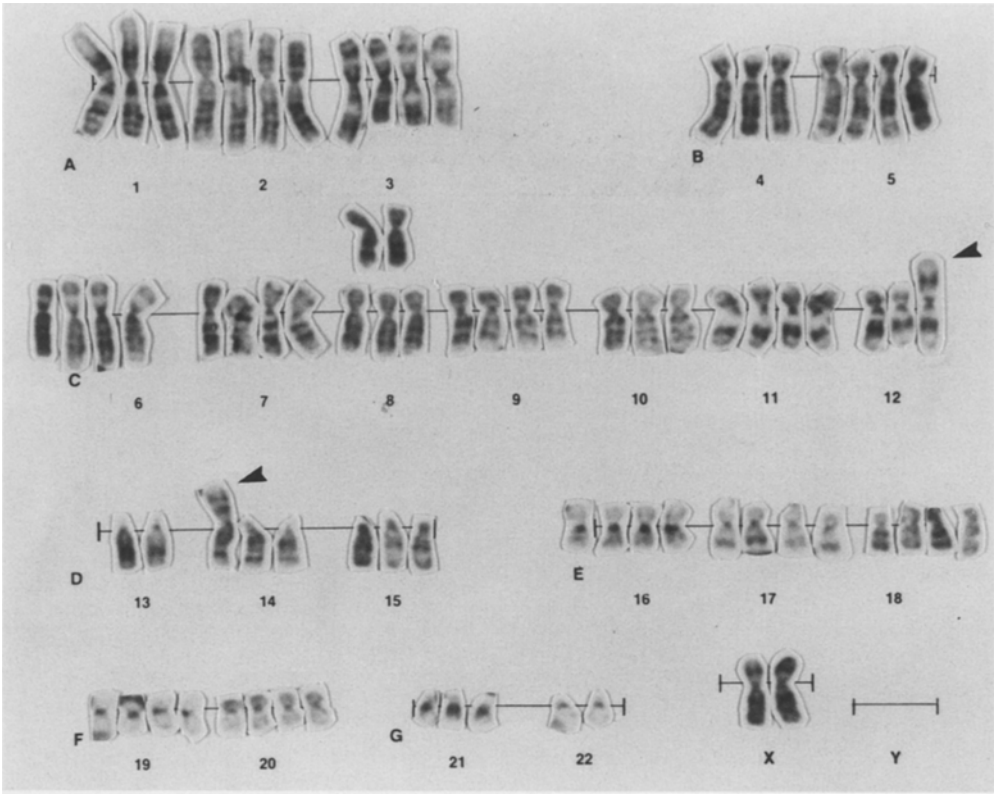

Fig. 5 G-banded karyotype of CCF-RC7 at passage 3, showing a hypotetraploid cell characterized as 80 XX, − 1, − 4, + 8, − 10, − 12, − 12, + i(12q), − 13, − 13, − 14, − 14, + der(14) t(9; 14) (q12; p11.2), − 15, − 21, − 22, − 22. Both i(12q) and der(14) t(9; 14) were present in a karyotype at passage 50

Table 1 Summary of immunochemical analysis. Only membranous staining was considered a positive result (staining was graded as *0*, no staining; *1* +, 1–24%; *2* +, 25–49%; *3* +, 50–74%; *4* +, 75–100% of malignant cells staining)

	Original tissue	CCF-RC7
URO-1	4 +	3 +
URO-2	4 +	0
URO-3	3 +	1 +
URO-4	1 +	1 +
URO-5	0	0
URO-7	1 +	1 +
URO-9	0	0
URO-10	1 +	4 +
RC38	2 +	1 +
AE1 and AE3	4 +	4 +
HLA-A + B + C	4 +	4 +
CD44	1 +	4 +
ICAM-1	1 +	3 +
ICAM-2	0	0
Sialyl Lewis X	3 +	3 +
VLA-4	0	1 +
LFA-1	0	0

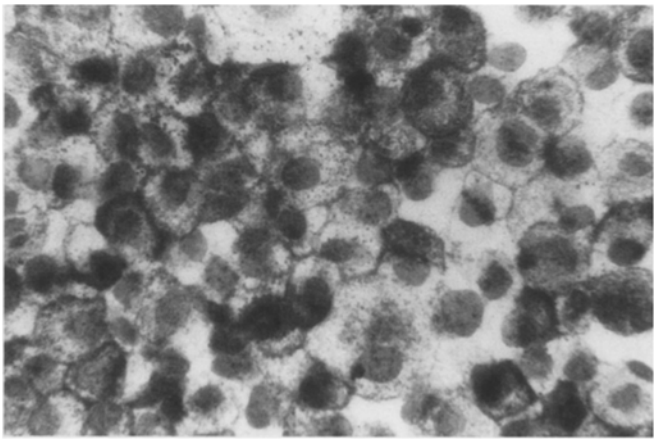

Fig. 6 Immunohistochemical staining of CCF-RC7 cells with an mAb against sialyl Lewis X revealing a high expression of this carbohydrate molecule

(Fig. 7a). The intensity of ICAM-1 molecules on the cell surface was significantly upregulated after rINF-γ ($P < 0.01$) and rTNF-α ($P < 0.001$) treatment as well as after stimulation with rIL-1β ($P < 0.05$). In contrast, PMA treatment of CCF-RC7 had no statistically significant effect on the intensity of ICAM-1 expression (Fig. 7b).

Whereas the percentage expression of CD44 was not significantly upregulated after cytokine and PMA treatment, the intensity of CD44 on the cell surface of CCF-RC7 was significantly increased after stimulation with rINF-γ ($P < 0.01$), rTNF-α ($P < 0.05$) and PMA ($P < 0.05$). The effect of IL-1β on the intensity of CD44 was not statistically significant (Fig 7a, b). No changes in the expression of VLA-4 were observed after cytokine or PMA stimulation (Fig. 7a, b). The expression of ICAM-2 and LFA-1 ranged between 0.5% and

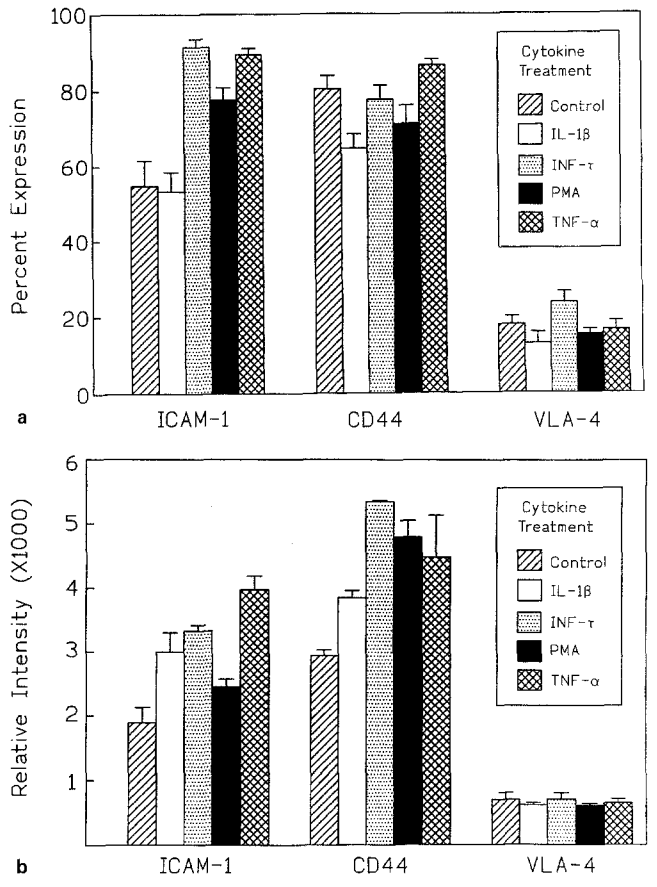

Fig. 7 **a** Percentage of positive cells for ICAM-1, CD44 and VLA-4 after treatment with cytokines and PMA, determined by flow immunocytometry. INF-γ ($P < 0.001$), TNF-α ($P < 0.001$) and PMA ($P < 0.01$) stimulation of CCF-RC7 resulted in a significant increase of ICAM-1 expression. Values represent means $\pm$ SEM of three experiments. **b** Relative intensity of ICAM-1, CD44 and VLA-4 after cytokine and PMA treatment of CCF-RC7. The relative intensity of ICAM-1 was significantly upregulated after stimulation with IL-1β ($P < 0.05$), INF-γ ($P < 0.01$), and TNF-α ($P < 0.001$). Also the relative intensity of CD44 was significantly increased in response to INF-γ ($P < 0.01$), TNF-α ($P < 0.05$) and PMA ($P < 0.05$) treatment. Values represent means $\pm$ SEM of three experiments

3%, and was not increased even after cytokine or PMA treatment (data not shown).

Discussion

In 1962 the first human renal cancer cell line was established in continuous culture; since then renal cell carcinomas have been cultured in vitro by many laboratories and used for studying the immunological, genetic and biochemical aspects of this most common renal malignancy [3, 7–9, 18]. Until now only a few reports have studied the expression and function of CAMs in renal cell cancer lines, although certain CAMs probably play an essential role in metastatic tumor spread and tumor cell lysis mediated by TIL [30–32]. To further clarify the expression and function of various CAMs in renal cell carcinoma, we characterized a renal cell cancer line, which had been established in our laboratory, and studied it with regard to the expression of CAMs known to be important. CCF-RC7 was established from a human renal adenocarcinoma of the clear cell type. The following observations suggest that this cell line is of malignant epithelial origin: (1) the tumor cells have an epithelial morphology and exhibit the absence of contact inhibition as evidenced by multilayering; (2) cultured cells of CCF-RC7 produce tumors in nude mice, with a histological appearance similar to that of the original tumor; (3) these cells grow in soft agar to form colonies; (4) the karyotype analysis revealed an abnormal chromosome number; (5) the positive reactions of CCF-RC7 cells with the mAbs URO-3 and URO-10 as well as positive staining with AE-1 and AE-3 suggested that this tumor developed from the proximal tubular cells; (6) the characteristics of epithelial cells, including the presence of cell surface microvilli and desmosomes, were demonstrated by electron microscopy; and (7) the cells have been in continuous culture for more than 3 years (over 50 passages) with no evidence of a decreased growth rate.

Contamination of the cell line with HeLa cells is unlikely, because HeLa cells have never been brought into or propagated in our laboratory. Futhermore, karyotype analysis demonstrated that there were no HeLa marker chromosomes in CCF-RC7 [19].

The immunohistochemical examinations of CCF-RC7 showed the expression of various CAMs, which have been reported to play an important role in hematogenous metastatic tumor spread. Both the original tumor and the cell line stained positive (3+) for the sialyl Lewis X antigen, a cancer-associated carbohydrate antigen, which has been shown to be a specific ligand for ELAM-1, a cell adhesion molecule expressed at the surface of cytokine-activated human endothelial cells [22]. Takada et al. [29] were able to demonstrate that the binding of colon carcinoma cells to activated endothelial cells was significantly lower after pretreatment of the tumor cells with anti-sialyl Lewis X antibodies. Similar findings were reported by Martin-Padura et al. [17] with colon carcinoma cell lines after stimulation of endothelial cells with thrombin. The results of this study suggest that the sialylated form of Lewis X is able to bind to thrombin-induced P-selectins. Therefore, sialyl Lewis X seems to be important in the interaction between tumor cells and endothelial cells in the metastatic cascade, and may also be important in RCC.

Recent studies indicate that the CD44 molecule, a widely distributed integral cell membrane glycoprotein, which exists in a variety of forms, may play a vital role in determining the fate of the hematogenous dissemination of tumor cells, possibly as a function of its activities as a hyaluronate receptor [4, 24]. CD44 is known to be expressed on different kinds of malignant

cells such as those in colon carcinomas, pancreatic tumors and melanomas [4, 12, 26]. However, until now very little information has been available about the expression and function of CD44 in RCC. Studies in our laboratory have established that CD44 is extensively expressed by RCC, while most corresponding normal kidney proximal tubular epithelium does not express CD44 [30]. These findings suggest that CD44 expression may be a part of the malignant phenotype of RCC. Both immunohistochemical and flow immunocytometric studies of CCF-RC7 revealed a high expression of CD44 (Fig. 7a, b). After treatment with rINF-γ, rTNF-α and PMA, the relative intensity of CD44 was significantly upregulated ($P < 0.05$), whereas the percentage of positive cells for CD44 expression was not affected, probably due to the already high (80%) expression of CD44 in unstimulated tumor cells. These findings are in contrast to results reported from different renal cell cancer lines, in which cytokine treatment did not affect CD44 expression [30]. Therefore, CCF-RC7 seems to be a suitable cell line for studying the regulation and function of this important CAM.

ICAM-1 (CD54), one ligand of LFA-1, is expressed on hematopoietic cells and nonhematopoietic cells such as vascular endothelial cells, thymic epithelial cells and fibroblasts [6]. Recently, the expression of ICAM-1 has been reported on malignant cells such as lymphomas [27], melanomas [13] and other carcinomas including RCC [30]. The presence of ICAM-1 in malignant cells seems to be important for the enhancement of metastases, and the ICAM-1 expression might augment the host immune reaction such as lymphocyte-mediated tumor cytotoxicity [13]. The authors [30] and others [32] have shown by immunohistological studies that the majority of RCCs expressed CD54, while most tubular epithelia of corresponding normal kidney were negative for CD54. These results suggest that CD54 expression is acquired as part of the malignant phenotype in RCC. In the present study, positive staining for ICAM-1 was found on the original tumor tissue, whereas the surrounding normal kidney tissue was negative for ICAM-1. Although ICAM-1 was already expressed on approximately 50% of unstimulated CCF-RC7 tumor cells, this expression was strongly upregulated by in vitro treatment with rINF-γ, rTNF-α and PMA. In contrast to findings reported by Tomita et al. [32], rIL-1β only had a significant effect on the intensity of ICAM-1 expression in CCF-RC7. Therefore, the high expression of ICAM-1 in CCF-RC7, and the possibility of modifying this expression with various cytokines, allow the investigation of the regulation of ICAM-1 protein and gene expression at different cellular levels.

Several CAMs on endothelial cells, most of which belong to the imunoglobulin supergene family, promote the tumor cell extravasation. One of these involved adhesion molecules is vascular cell adhesion molecule-1 (VCAM-1) [21]. VCAM-1 is expressed on activated endothelial cells and recognized by a member of the β1-integrin family, VLA-4 (CD49d/CD29) [5]. VLA-4 is present on most hematopoietic cells and is also expressed on cells of some solid tumors such as melanomas and sarcomas [28]. Until now only a few studies have examined the expression and function of VLA-4 in renal cell cancer. These studies suggest that VLA-4 is involved in the adhesion of a renal cancer line CAKI-1 to activated endothelial cells [28]. Immunoflow cytometric analyses of various passages of CCF-RC7 revealed a constant expression of VLA-4 on the tumor cells. However, both the percentage and the intensity of this molecule on the tumor cells could not be upregulated by cytokine or PMA treatment. Further investigations in our laboratory with CCF-RC7, including endothelial cell-tumor cell attachment assays, will determine the importance of VLA-4 in hematogenous metastasis. In contrast to the above-mentioned CAMs, LFA-1 and ICAM-2 were expressed in less than 3% and 1% of CCF-RC7 cells, respectively. The expression of both molecules could not be upregulated by cytokine and PMA treatment. Therefore, these CAMs seem to be unimportant in CCF-RC7.

In summary, CCF-RC7 is a new renal cell cancer line established and characterized from a patient with an RCC of the clear-cell type. This cell line expresses CAMs which seem to be important in hematogenous metastasis and host defense mechanisms such as lymphocyte-mediated tumor cytotoxicity, and will serve as a model system for the study of these processes in RCC.

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