Abnormal Growth and Clonal Proliferation of Fibroblasts Derived from Kidneys with Interstitial Fibrosis (43118)

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Abstract. **Renal fibroblasts from normal kidneys (NKF cells) and from kidneys with interstitial fibrosis (FKIF cells) were established from biopsy material. In primary and passage 1 cell cultures, the amount of fibroblasts was increased by a factor of 5–10 in cultures derived from kidneys with interstitial fibrosis as compared with cultures of normal origin. As tested by clonal growth and growth kinetic experiments, FKIF cells showed significant alterations in the proliferation capacity and generation time resulting in a hyperproliferative growth in primary and secondary fibroblast cultures *in vitro*. Two-dimensional gel electrophoresis experiments of [^{35}S]methionine-labeled intracellular polypeptides revealed that FKIF cells express two proteins, p53/6.1 and p48/7.5, that are not present in normal kidney and skin fibroblasts. In addition, as analyzed by two-dimensional gel electrophoresis of medium supernatants of FKIF cells, two secreted proteins specific for FKIF cells could be demonstrated. Cross-feeding experiments using conditioned medium of FKIF cells on cultures of normal human skin fibroblasts (NSF cells) revealed that FKIF cells may secrete proteins into the medium or may modify preexisting serum factors that can induce hyperproliferation in normal dermal fibroblasts. As tested by serial subcultivation and clonal analysis, FKIF cells exert significant changes in the differentiation pattern of potentially mitotic fibroblast populations.**

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Renal interstitial fibrosis is the major cause of uremia and progression of interstitial fibrosis is correlated with the loss of postglomerular capillaries (1, 2). The pathogenesis of renal fibrosis as a consequence of acute glomerulonephritis is not understood at present. Collagen-producing cells in the renal cortex include at least epithelial, endothelial, and mesangial cells of the glomerulus and interstitial fibroblasts (3). In glomerulonephritis accompanied by interstitial fibrosis, an increased activity of "collagen-I-fibroblasts" has been assumed (4). However, until now nothing was known about the proliferation rate and growth behavior of renal fibroblasts during the development of renal interstitial fibrosis (4).

Cultured fibroblasts obtained from explants of various organs have proved to be very useful for investigations of numerous metabolic disorders and inherited diseases. Fibroblasts of the connective tissue of various organs of vertebrate and mammalian species including man have been demonstrated to be differentiating cells. Three mitotic (MF I, MF II, and MF III) and four postmitotic (PMF IV, PMF V, PMF VI, and PMF VII) differentiation states of fibroblasts can be characterized by morphologic and biochemical properties (5–8). As tested by clonal growth, mitotic fibroblasts show a differentiation-dependent decrease in the proliferation capacity of cell types MF I to MF III (6).

In the present study we report the establishment of fibroblast cultures from kidneys with interstitial fibrosis (FKIF cells) and the use of these cells for the analysis of their growth and differentiation behavior *in vitro*. We will provide evidence that FKIF cells show an abnormal growth, i.e., hyperproliferation *in vitro* under various culture conditions. Furthermore, FKIF cells analyzed by two-dimensional gel electrophoresis are characterized by the expression of two intercellular and two secreted proteins which are not present in normal fibroblasts of either renal or dermal origin.

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Materials and Methods

Primary Cell Cultures. Primary renal fibroblast and epithelial cells were established from biopsies of

three normal kidneys offered for transplantation, which were not grafted because of vascular abnormalities, and of five kidneys with interstitial fibrosis (Table I). Biopsies were cut into 1-mm^3 fragments and placed into culture flasks (Falcon, 25 cm^2), covered with Dulbecco's modified Eagle's medium (DMEM) supplemented with 20% fetal calf serum (FCS) and antibiotics (5, 6), and incubated in humidified 95% air:5% CO_2 at 37°C. Medium was changed once weekly. After 4–5 weeks cells grown out from the explants were subcultured using 0.05% trypsin and 0.1% EDTA. Cell numbers were determined by means of a Fuchs-Rosenthal hemocytometer (5). Cells were designated as passage 1 cells.

Clonal Cell Cultures. Passage 1 cells were transferred into clonal cultures in order to separate renal fibroblasts from renal epithelial cells by clonal growth. Therefore, passage 1 cells were seeded at a density of 40 cells/cm^2 and incubated in DMEM containing 20% FCS. After 12–14 days of culture, pure clonal populations of either one differentiation state of fibroblasts (5, 6) or epithelial cells were surrounded by silicone-sealed glass rings and trypsinized as described above. Depending upon the cell number of pooled identical clones, cells were either seeded in 2-cm^2 multiwell dishes or 7-cm^2 culture dishes (Falcon) with a density of 2×10^4 cells/cm^2. Cells grown to confluency in DMEM supplemented with 10% FCS were subcultured routinely at 7-day intervals. In the experiments described in this study, a minimum of at least 90% of the cells classified as fibroblasts were negative for Factor VIII antigen as tested by the use of a monoclonal antibody directed against human Factor VIII (Amersham) (data not shown). For further analysis of fibroblasts derived from kidneys with interstitial fibrosis, only subpopulations of Factor VIII-negative cells were propagated as pure fibroblast cultures.

Clone Type Frequency and Size Determination of Fibroblast Clones. After 14 days of culture for normal skin (NSF) and normal kidney fibroblast (NKF) clones and after 12 days for FKIF clones, clonal populations were fixed and stained as described elsewhere (5, 6). Three pure clone types, CTF I, CTF II, and CTF III, resulting from the proliferation of fibroblasts MF I, MF II, and MF III, and two mixed clone types CTF I/II and CTF II/III, due to a differentiation divisions of MF I or MF II during clonal growth, were classified according to morphologic characteristics (5, 6). Clone type frequencies were determined by classifying at least 200 fibroblast clones as recently described (6).

The size of the pure clone types CTF I, CTF II, and CTF III, i.e., the number of cells per clone, was determined by counting the number of nuclei in 10 random microscopic fields (0.093 mm^2). This number was multiplied by a Factor F (total area of the clone, mm^2, divided by 0.093 mm^2), resulting in the average cell number per clone. For each clone type at least 50 clones were analyzed.

Low-Density Mass Cultures. Passage 1 cells transferred as described above were seeded into low-density mass cultures with cell densities of $1.5–2.0 \times 10^3/cm^2$. Four days later cells were fixed and stained as described elsewhere (5). The frequencies of the different mitotic fibroblast cell types and epithelial cells were determined by morphologic criteria (5, 6).

Growth Kinetics. Pure fibroblast populations of FKIF cells selected as described above were seeded at passage number 6 (cumulative population doublings, CPD 16-22) at a density of 2.5×10^3 cells/cm^2 in medium supplemented with 20%, 10%, or 5% FCS. As controls, normal skin fibroblasts (NSF cells, CPD 20-24) were seeded at identical density and incubated in media supplemented as described above. In addition, NSF cells were incubated in medium (DMEM + 10% FCS) conditioned for 24 hr by either logarithmically growing cultures of NSF cells or FKIF cells. At various times thereafter, cell numbers of three parallel cultures for each culture condition were determined. Data were plotted as growth kinetic curves.

Chromosomal Analysis. The karyotype and G-banding pattern of mass populations of FKIF cells (five cell lines) at passage levels of 3–5 were analyzed after incubating logarithmically growing cells in medium containing 0.1 μg/ml of colcemid (9). For each cell line, at least 50 metaphases were analyzed.

Two-Dimensional Gel Electrophoresis of [^{35}S] Methionine-Labeled Intracellular and Secreted Proteins. Pure renal fibroblast clones (CTF I and CTF II) of NKF and FKIF cells selected as described above were labeled with 50 μCi of [^{35}S]methionine in L-methionine-free medium (DMEM) supplemented with 1 mg/liter of L-methionine and 20% FCS for 18 hr as described elsewhere (6, 8, 10). After labeling, radioactive medium was removed and centrifuged to remove cells and cellular debris. Medium was lyophylized in 200-μl samples and reconstituted in 50 μl of modified O'Farrell lysis buffer (6, 8, 10). Clonal cell layers were washed three times with phosphate-buffered saline and lysed in 100 μl of buffer (6). After determination of radioactivity, aliquots of the medium and cell samples containing 200,000 cpm and 500,000 cpm, respectively, were analyzed by two-dimensional gel electrophoresis and subsequent computerized video gel scanning (10) as described in detail elsewhere (6, 8, 10).

Results

Frequencies of Fibroblasts and Epithelial Cells in Passage 1 Cultures. As shown in Figure 1A and C, passage 1 cultures established from renal explants represent mixed populations of predominantly epithelial and fibroblastoid cells. Among fibroblasts all three mitotic fibroblast cell types MF I, MF II, and MF III

Table I. Clinical and Laboratory Data of the Five Patients Presenting Renal Interstitial Fibrosis[a]

	Patient				
	1	2	3	4	5
	Cell lines				
	FKIF-1	FKIF-2	FKIF-3	FKIF-4	FKIF-5
Age (years)	40	39	56	50	46
Sex	F	M	F	M	F
Renal disease	IgA-N[b]	MC	MN	DBNS	RPGN type II
Duration of disease (years)	2.5	0.5	3.0	1.5	0.5
Creatinine (mg/100 ml)	2.3	1.4	2.0	2.6	11.4
Protein urea (g/day)	0.8	2.4	4.0	0.3	Unuric

[a] The kidneys of all patients were diagnosed to be affected with interstitial fibrosis by Professor A. Bohle, Institute for Pathology, University of Tübingen, FRG, by means of light microscopy, immunohistochemistry, and electron microscopy. The donors of normal kidneys that were used as a source for NKF cells were in the age range of 36–47 years (NKF-1 36 years, female; NKF-2: 44 years, male; NKF-3: 47 years, male).
[b] IgA-N, IgA nephropathy; MC, minimal changes; MN, membraneous nephropathy; DBNS, decompensated benign nephrosclerosis; RPGN type II, rapidly progressive glomerulonephritis type II.

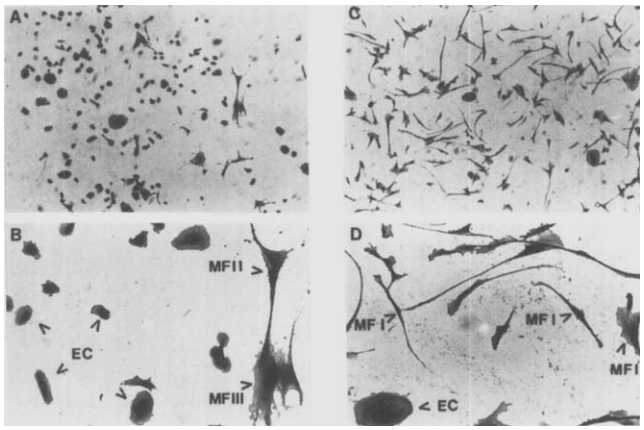

Figure 1. Low-density mass populations of passage 1 cultures of cells of normal kidneys and kidneys with interstitial fibrosis. Four days after seeding, cultures were fixed with 3.7% paraformaldehyde in phosphate-buffered saline and 70% ethanol, and stained with Coomassie and Giemsa solutions as described in detail elsewhere (5). (A and B) Cell cultures from normal kidneys. (C and D) Cell cultures from kidneys with interstitial fibrosis. EC, epithelial cell; MF I, fibroblast type I; MF II, fibroblast type II; MF III, fibroblast type III (original magnification: A and C, ×36; B and D, ×180).

Table II. Frequency of Epithelial Cells and Fibroblasts in Passage 1 Cultures of Normal Kidneys and Kidneys Affected with Interstitial Fibrosis[a]

	% total cells present	
	Normal ($n = 3$)	Fibrosis ($n = 5$)
Epithelial cells	93.3 ± 2.6	53.2 ± 9.4
Fibroblasts	6.7 ± 2.6	46.8 ± 9.4

[a] Passage 1 cultures of three normal kidneys and five kidneys affected with interstitial fibrosis were fixed and stained (as described in Fig. 1) 4 days after seeding. For each passage 1 culture at least 500 cells were classified by morphologic criteria.

Table III. Frequencies of Mitotic Fibroblast Types MF I, MF II, and MF III in Passage 1 Cultures of NKF and FKIF cells[a]

	% MF I, MF II, and MF III on total fibroblasts in passage 1 cultures		
	MF I	MF II	MF III
NKF cultures ($n = 3$)	1.6 ± 0.5	33.8 ± 7.2	64.6 ± 7.3
FKIF cultures ($n = 5$)	39.1 ± 8.0 (+2343%)	39.7 ± 3.4 (+17%)	21.2 ± 6.2 (−67.2%)

[a] Passage 1 cultures were seeded as low-density mass populations as described in Materials and Methods. Four days after seeding cells were fixed and stained as described above (Table I). For determination of the cell type frequencies of mitotic fibroblast types, a minimum of at least 200 fibroblasts were classified according to morphologic criteria (5) in each cell line analyzed.

described earlier (5–7) are present (Fig. 1). Table II shows the frequencies of epithelial cells and fibroblasts in passage 1 cultures of NKF (Fig. 1A and B) and FKIF cells (Fig. 1C and D). As compared with NKF cell cultures, FKIF cell cultures at passage 1 comprise by a factor of 6.98 ($P < 0.001$) significantly enhanced amounts of fibroblasts (Table II).

As demonstrated in Table III, the frequencies of the two mitotic cell types MF I and MF III are significantly different between passage 1 cultures of NKF cells and FKIF cells. In FKIF cultures, fibroblast MF I is enhanced by a factor of 24 and fibroblast MF III is reduced by a factor of 3x as compared with NKF cultures (Table III).

Clone Type Frequencies and Clonal Proliferation. To test the proliferative potential of fibroblasts of NKF and FKIF cells, passage 1 cultures were analyzed for clonal proliferation. As demonstrated by the use of normal skin fibroblasts (NSF cell lines HH-4, HH-8,

and CPD 22–24) as reference cells, depending upon the differentiation state, mitotic fibroblasts give rise to morphologically and biochemically distinct clone types: clone type CTF I composed of mitotic fibroblasts MF I, a spindle-shaped cell; CTF II made up of MF II, a cell of epithelioid morphology, biochemically characterized by the expression of proteins PIIa, PIIb, and PIIc as demonstrated by two-dimensional gel electrophoresis (Fig. 2A; see also refs. 5, 6); CTF III composed of MF III, a pleiomorphic cell expressing the cell type-specific protein PIIIa (data not shown; see also refs. 5, 6) (Table IV). Due to differentiation divisions, cell type MF I and MF II can give rise to mixed clone types CTF I/II and CTF II/III.

These clone types can also be observed when renal fibroblasts are analyzed. Table IV shows the comparison of the various clone type frequencies of NKF and FKIF passage 1 cultures. In NKF cultures the predominant clone type is CTF III. CTF I or CTF I/II was not present in passage 1 clonal cultures of NKF cells (Table IV). In FKIF cultures the predominant clone type was CTF II; CTF I and CTF I/II reflected almost one fourth of all of the clone types present (Table IV).

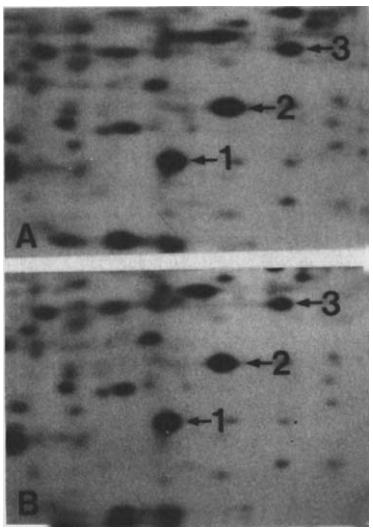

Figure 2. Expression of the MF II cell type-specific proteins PIIa, PIIb, and PIIc in clone types CTF II of NSF cells (cell line HH-6) and FKIF cells (cell line FKIF-5) analyzed by two-dimensional gel electrophoresis (acidic end to the right). 1, protein PIIa, molecular mass 32 kDa, P_i 8.2; 2, protein PIIb, molecular mass 37 kDa, P_i 7.9; 3, protein PIIc, molecular mass 40 kDa, P_i 7.2. These proteins are synthesized in NSF and FKIF cells of MF II type at approximately the same abundance.

Each pure clone type (CTF I, CTF II, or CTF III) has a characteristic cell number reflecting the differential proliferation capacity as well as the differential doubling time of the three mitotic fibroblast cell types (6).

When identical pure clone types of passage 1 cultures of NKF, FKIF, and NSF cells were compared, FKIF cultures showed a significant increase in the mean size (MS) and a significantly reduced average doubling time (ADT) of the clone types CTF I, CTF II, and CTF III reflecting the hyperproliferative growth behavior of FKIF cells (Table V).

A comparison of clone types CTF II and CTF III of NKF and NSF cells reveals similar data in regard to clone size and average doubling time, although NKF clones tended to be slightly smaller with slower doubling times (Table V).

Growth Kinetic Experiments. To study this hypoproliferation of FKIF cells more extensively, clones of the types CTF I and CTF II of five different FKIF cell lines were propagated in mass cultures. For NKF cells, due to the low starting number of clone types CTF II that could be pooled and although the cultures obtained were propagated for five additional passages, the cell number finally obtained was not sufficient to allow growth kinetic experiments with NKF cells. After 4–6 passages NKF cultures spontaneously differentiate to irreversible postmitotic cultures that could not be propagated further. Therefore, as controls, NSF cells of approximately the same relative percentage of their total life span as compared with FKIF cells were used. FKIF and NSF cells were analyzed in growth kinetic experiments using culture media with varying serum concentrations (20%, 10%, 5%).

As shown in Figure 3A, in each medium condition tested, FKIF cells showed significantly faster growth with maximum cell densities between 3.5 and 4.9×10^5 cells/dish (20 cm^2) than the comparable control (maximum cell densities between 8×10^4 and 2.1×10^5 depending on the serum concentration). However, as

Table IV. Frequencies of Fibroblast Clone Types of Passage 1 Cultures of NKF and FKIF Cells[a]

	Clone type frequency (%)				
	CTF I	CTF I/II	CTF II	CTF II/III	CTF III
NKF ($n = 3$)	—	—	13.8 ± 2.9	13.0 ± 3.2	73.2 ± 5.7
FKIF ($n = 5$)	10.7 ± 3.6	12.7 ± 3.3	33.6 ± 4.6	20.9 ± 3.6	22.2 ± 3.7

[a] Clone types of passage 1 cultures of NKF and FKIF cells were analyzed after 12 and 14 days of clonal growth. Clonal cultures were fixed and stained as described (5). The various fibroblast clone types were classified according to morphologic criteria (6). Clone type frequencies of the various clone types (percentage of total clones present) were determined by classifying at least 200 clones of the different NKF and FKIF cell lines.

Table V. Growth Characteristics of Fibroblast Clones from Normal Human Skin, Normal Kidneys, and Kidneys with Interstitial Fibrosis[a]

Clone type	NSF cells		NKF cells		FKIF cells	
	MS	ADT	MS	ADT	MS	ADT
CTF I	5.2 ± 2.1	27.2 ± 3.3	—	—	17.4 ± 3.2	20.4 ± 3.4[b]
CTF II	3.7 ± 1.7	28.3 ± 3.2	2.1 ± 0.9	30.4 ± 3.1	6.8 ± 2.9	22.6 ± 2.8[b]
CTF III	0.3 ± 0.2	40.8 ± 4.4	0.2 ± 0.1	42.1 ± 3.9	0.3 ± 0.2	34.9 ± 3.8[b]

[a] Cells were cloned as described in Materials and Methods. NSF cells, cell lines HH-4, HH-6, and HH-8 (CPD 20–24). NKF cells, cell lines NKF-1, NKF-2, and NKF-3, passage 1. FKIF cells, cell lines FKIF-1, FKIF-3, and FKIF-5, passage 1. MS, cell number per clone $\times 10^3$; ADT, hr. ADT was determined by counting the cell number of individual clones (CTF I, CTF II, and CTF III) during the logarithmic growth between Days 3 and 7. ADT was calculated according to the formula $t = \log 2(t_n - t_o)/\log N\text{-}\log N_o$. At least 14 clones of each type and cell line were analyzed. Differences in the ADT's between NSF or NKF clones and FKIF clones were analyzed for statistical significance using the Dunnet test (10). Incubation time was 14 days (NSF and NKF cells) and 12 days (FKIF cells). NSF cell lines were established from skin biopsies of the left upper arm (HH-4, HH-6) of two boys and of the lower abdominal region (HH-8) of one girl, aged 7–11 years. Numbers represent the mean ± SD.
[b] $P < 0.05$; $n = 14$.

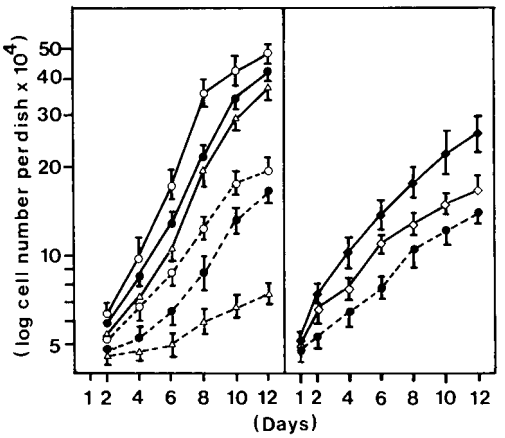

Figure 3. Growth kinetics of NSF and FKIF cells. NSF cells, normal skin fibroblasts: 3A—○– – –○, DMEM + 20% FCS; ●– – –●, DMEM + 10% FCS; △– – –△, DMEM + 5% FCS. FKIF cells: 3A—○——○, DMEM + 20% FCS; ●——●, DMEM + 10% FCS; △——△, DMEM + 5% FCS. 3B—NSF cells (see above): ◆——◆, conditioned medium (+10% FCS) from NSF cells; ◆——◆, conditioned medium (+10% FCS) from FKIF cells; ●– – –●, unconditioned medium (DMEM + 10% FCS). Data shown represent the mean ± SD of three NSF cell lines (HH-4, HH-6, and HH-11; CPD 20-24) and five FKIF cell lines (FKIF-1, FKIF-2, FKIF-3, FKIF-4, and FKIF-5; CPD 8-13).

compared with NSF cells, the differences in the growth rates of FKIF cells between 20% and 10%, and 10% and 5% serum supplement of the medium did not differ significantly, although a slight correlation of cell growth and serum concentration could be observed. When NSF cells were incubated in medium that was preconditioned by FKIF cells, NSF cells were induced to hyperproliferation, whereas with NSF cells grown in medium preconditioned by NSF cells only a slight increase in the growth rate was observed (Fig. 3B). Culture medium conditioned in the absence of cells, i.e., for 24 hr at 37°C in 5% CO_2, did not induce significant differences in the growth of either FKIF or NSF cells (data not shown).

Chromosomal Pattern. FKIF cells of the five cell lines analyzed showed normal chromosomal pattern (2n = 46). By G-banding no chromosomal aberration, such as deletion or translocation, could be observed (data not shown).

FKIF Cell-specific [^{35}S]Methionine Polypeptide Pattern of Intracellular and Secreted Proteins. Clone type CTF II of NKF and FKIF cells expresses the same MF II cell type-specific proteins PIIa, PIIb, and PIIc as recently described for normal human skin fibroblasts (5, 6, 8) (Fig. 2). When the protein patterns of total intracellular proteins of clone types CTF II and NKF and FKIF cells were compared, FKIF cells were found to express two proteins (p53/6.1 and p48/7.5) (Fig. 4C and D) which are not present in normal kidney fibroblasts (Fig. 4A and B) or in normal skin fibroblasts regardless of the differentiation state (data not shown). One protein (p54/6.2, Fig. 4C) is expressed in FKIF cells at approximately 20- to 30-fold higher levels than in NKF cells as analyzed by computerized video densitometry of the fluorograms (data not shown; ref. 8).

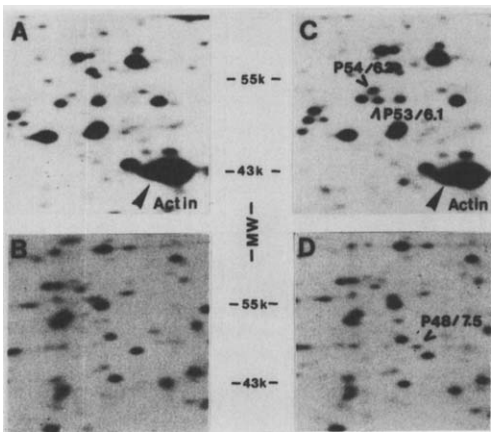

Figure 4. Two-dimensional gel electrophoresis of intracellular [^{35}S] methionine-labeled polypeptides. (A and B) NKF cells, clone type CTF II, region 1 (A), region 2 (B). (C and D) FKIF cells, clone type CTF II, region 1 (C), region 2 (D). Five-hundred thousand counts per minute were applied to isoelectric focusing gels (pH-gradient 3-10) (first dimension). Second dimension separation was performed on sodium dodecyl sulfate-15% polyacrylamide gels. Designation of indicated proteins is: P molecular mass (kDa)/isoelectric point.

Two-dimensional gel electrophoresis of medium samples of clone type CTF II of FKIF and NKF cells revealed that FKIF cells secrete at least two proteins (p43/7.3; and p30/7.3) (Fig. 5B) that seem to be specific for FKIF cells since they are not present in the medium of NKF cells of the clone type CTF II (Fig. 5A) or in mass cultures of normal skin fibroblasts (data not shown). Protein p30/7.5 is secreted by FKIF cells at much higher levels (approximately 10- to 12-fold as analyzed by computerized video densitometry) as compared with control cells (Fig. 5).

Discussion

In the present study we have isolated fibroblasts from kidneys affected with interstitial fibrosis (FKIF cells). As demonstrated by the use of primary and passage 1 cultures, clonal culture systems, and growth kinetic experiments, FKIF cultures *in vitro* are characterized by significantly higher amounts of fibroblast cell types MF I and MF II. Furthermore, the fibroblast cell types MF I, MF II, and MF III showed hyperproliferative behavior combined with a significantly reduced doubling time.

The growth of normal fibroblasts *in vitro* is known to be dependent on the serum concentration of the culture medium, i.e., decreasing amounts of serum result in decreasing growth rates (11). This correlation cannot be observed with FKIF cells. In several experiments using FKIF cell cultures from five different patients, the hyperproliferation of these cells was only slightly affected by the serum concentration of the medium. On the other hand, normal skin fibroblasts could be induced to hyperproliferation when cultured in medium conditioned by FKIF cells. Thus, these data may suggest that FKIF cells secrete factors into the culture medium that can induce hyperproliferation in normal skin fibroblasts. Another possible explanation could be that FKIF cells modify a preexisting serum factor, resulting in an increased mitogenic activity.

Because of the low starting cell number of NKF cells in primary cultures and because of the already advanced differentiation state of NKF cultures in passage 1 (65% of the fibroblasts present are of type MF III), NKF cultures could not be propagated to the extent that would have allowed growth kinetic experiments. Therefore, NSF cells were used as reference cells although these cells were not appropriate but the only control possible. However, the comparison of NKF to NSF cells in the clonal analyses (Table IV, mean size and average doubling time) suggests that NKF cells would show growth kinetic curves similar to those of NSF cells.

With the use of two-dimensional gel electrophoresis of medium samples from clonal populations as well as mass cultures of FKIF cells, it was demonstrated that FKIF cells secreted at least three proteins into the medium that were not present or present at only very low levels in the medium of either normal kidney or skin fibroblasts. Two of these proteins (p30/7.5 and p30/7.3) are most likely glycosylated isoforms of a protein with a molecular mass of 30 kDa and an isoelectric point of 7.8 as demonstrated by peptide mapping using protease-V8 (Rodemann, unpublished data). This protein is a major protein also present in the medium of normal NKF and NSF cells (Fig. 5, indicated by arrow). However, the third protein additionally secreted by FKIF cells, p43/7.8, seems to be specific for FKIF cells. The amount of this protein compared with total secreted proteins of FKIF cells is approximately 0.6–1.0% as determined by computerized video scanning of the fluorograms of two-dimensional gels. Experiments are in progress to purify this protein from medium samples of FKIF cells and to analyze its function as a possible inducer of cell proliferation.

Likewise, the biochemical nature and the possible function of the two intracellular proteins specific for FKIF cells remain to be resolved. However, these extra- and intracellular proteins can be used as markers for the biochemical characterization of FKIF cells.

One possible explanation for the hyperproliferative

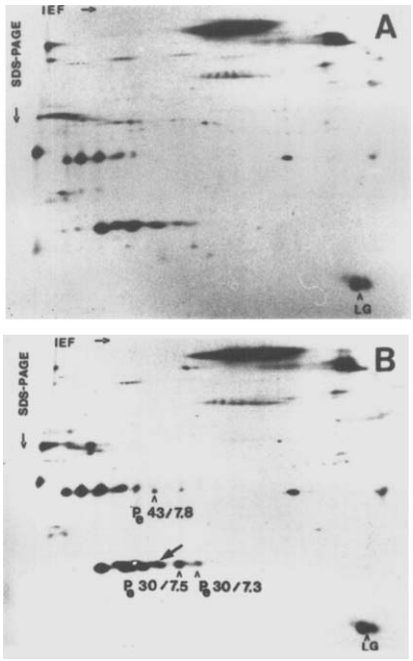

Figure 5. Two-dimensional gel electrophoresis of medium samples from NKF (A) and FKIF cells (B) of the clone type CTF II after labeling with [^{35}S]methionine. Radioactively labeled secreted proteins (p_e) were applied in aliquots of 200,000 cpm to two-dimensional gels as described in Materials and Methods and Figure 3. Designation of proteins: p_e molecular mass (kDa)/isoelectric point. Arrow indicates protein p_e30/7.8, a major protein secreted by NKF as well as FKIF cells. As radioactive marker protein for quantification of the various protein spots by computerized video densitometry (10), [^{14}C]lactoglobulin (LG, *m* 18.3 kDa; 3500 cpm) was added to the medium aliquots.

growth of FKIF cells *in vitro* could be the expression of protooncogenes in these cells (12, 13). In ongoing experiments purified mRNA from FKIF cells are tested by Northern blot analysis in order to quantitate the expression of c-*myc* and/or c-*fos* oncogenes.

As analyzed in the present study, FKIF cells have a unique chromosomal pattern (2n = 46) and do not show any sign of chromosomal aberration. Thus, chromosomal abnormalities are excluded as the cause of hyperproliferative growth of FKIF cells.

Taken together, the presented data indicate for the first time that kidneys affected with interstitial fibrosis, regardless of the renal disease, are characterized by a 7- to 8-fold increase in the total amount of fibroblasts present. Furthermore, these fibroblasts isolated from kidneys with interstitial fibrosis are characterized by abnormal hyperproliferative growth *in vitro*. By the use of these cultures and further biochemical analysis, i.e., characterization of the intra- and extracellular proteins specific for FKIF cells as well as analysis of the biosynthesis of collagen, we hope to elucidate the mechanisms triggering the fibrotic response *in vivo*.

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1. Bohle A. Zur funktionellen Morphologie der Niere. Franz-Volhard-Gedächtnisvorlesung. Nieren-Hochdruckkr **9**:253–260, 1980.
2. Spühler O, Zollinger U. Die chronische interstitielle Nephritis. Z Klin Med **151**:1–50, 1953.
3. Downer G, Phan SH, Wiggins RC. Analysis of renal fibrosis in a rabbit model of crescentic nephritis. J Clin Invest **82**:998–1006, 1988.
4. Bohle A, Mackensen-Haen S, Gise H. Significance of tubulointerstitial changes in the renal cortex of excretory function and concentration ability of kidney. A morphometric contribution. Am J Nephrol **7**:421–433, 1987.
5. Bayreuther K, Rodemann HP, Hommel R, Dittmann K, Albiez M, Francz PI. Human skin fibroblasts in vitro differentiate along a terminal cell lineage. Proc Natl Acad Sci USA **85**:5112–5116, 1988.
6. Rodemann HP, Bayreuther K, Francz PI, Dittmann K, Albiez M. Selective enrichment and biochemical characterization of seven human skin fibroblast cell types in vitro. Exp Cell Res **180**:84–93, 1989.
7. Rodemann HP, Friedrich G. Abnormal differentiation of the fibroblast stem cell system induced by alterations in protein metabolism. Eur J Cell Biol **48**(suppl 26):157, 1989.
8. Rodemann HP. Differential degradation of intracellular proteins in human skin fibroblasts of mitotic and mitomycin-C (MMC)-induced postmitotic differentiation states in vitro. Differentiation **42**:37–43, 1989.
9. Dzarlieva-Petrusevska RT, Fusenig N. Tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) induced chromsome aberrations in mouse keratinocyte cell lines: A possible genetic mechanism of tumor promotion. Cancerogenesis **6**:1447–1456, 1985.
10. Rodemann HP, Bayreuther K. Differential degradation of ^{35}S-methionine polypeptides in Duchenne muscular dystrophy skin fibroblasts in vitro. Proc Natl Acad Sci USA **83**:2086–2090, 1986.
11. Gospodarowicz D, Moran JS. Growth factors in mammalian cell culture. Annu Rev Biochem **45**:531–558, 1976.
12. Curran T, Bravo R, Müller R. Transient induction of c-fos and c-myc is an immediate consequence of growth factor stimulation. Cancer Surv **4**:655–682, 1986.
13. Verrier B, Müller D, Bravo R, Müller R. Wounding of fibroblast monolayer result in the rapid induction of c-fos proto-oncogene. EMBO J **5**:913–917, 1986.