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Expression of the fibroblast growth factor receptor 1–4 genes in glomeruli in anti-Thy1.1 mesangial proliferative glomerulonephritis

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Abstract Basic fibroblast growth factor (FGF2) is generally known to induce proliferation of cultured mesangial cells and is expressed in proliferative mesangial cells in anti-Thy1.1 mesangial proliferative glomerulonephritis (anti-Thy1.1 GN). The distribution of the FGF receptor (FGFR) has not been studied in anti-Thy1.1 GN, so we used in situ hybridization to determine whether cells expressing FGFR1–4 mRNAs could be detected. In normal rats, all glomeruli were negative for FGFR1–4 mRNA, but those of the mesangial proliferative phase expressed FGFR1–4 mRNA in proliferative mesangial cells. Proliferation of mesangial cells has not been observed in normal rats injected with FGF2 but it has been noted in anti-Thy1.1 rats injected with FGF2. These data and our results demonstrate that mesangial cells produce and release FGF2 after injury and that during the proliferative phase these cells upregulate FGFR in vivo. This study is the first to demonstrate expression of FGFR1–4 mRNAs in pathological glomeruli of anti-Thy1.1 GN. The FGF2 and FGFR1–4 genes were expressed in the proliferative mesangial cells. Upregulation of FGFR is necessary for mesangial proliferation by FGF2.

Key words Growth factor · Experimental mesangial proliferative glomerulonephritis

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Introduction

Fibroblast growth factor (FGF), found in the bovine brain and pituitary gland, provokes initiation of DNA synthesis in resting 3T3 cells [10, 11]. Fibroblast growth factors are the family of polypeptide growth factors (of which nine have been identified to date) that are capable of binding to any of a family of tyrosine-kinase receptors called FGFR1 through FGFR4 [14, 19, 27]. FGF is found in almost all organs of the body and has been reported to play a fundamental role in various physiological processes, including growth, differentiation, and the repair of injury. Basic FGF (FGF2), in particular, has been studied extensively. A major biological effect of FGF2 is induction of proliferation in cultured cells, including fibroblasts, endothelial cells, and vascular smooth muscle cells [18]. Previous studies have demonstrated that FGF2 induces the proliferation of cultured mesangial cells and glomerular epithelial cells [7, 26]. In addition, FGF2 has been observed to act as the initial growth factor after mesangial cell destruction in a model of mesangial cell proliferation [7]. Four FGFRs have so far been reported [1, 16]. Each of these has a small number of immunoglobulin-like domains in the extracellular region that are connected via transmembrane domains to cytoplasmic domains having tyrosine-kinase activity. In a recent study, expression of FGF2 mRNA was detected in glomeruli by means of Northern blotting, but the distribution of neither FGF2 nor FGFR mRNAs has been determined. Moreover, it has been reported that the podocytes of FGF2-injected passive Heymann nephritis (PHN) rats showed evidence of injury and developed glomerulosclerosis when compared with control PHN rats. These effects were explained in terms of upregulation of glomerular FGF2 receptor expression.

In the present study, the expression of FGF2 and FGFRs 1–4 was investigated in normal rat kidneys and in a model of mesangial proliferative glomerulonephritis (anti-Thy-1.1 GN) by means of in situ hybridization [17, 29].

Materials and methods

Male Wistar rats (Clea Japan, Osaka, Japan) weighing 180–200 g were used. The animals were housed in metabolic cages, given food and water ad libitum, and separated into a normal control group (n=4) and a treated group, which received the monoclonal antibody OX-7 (Cosmo Bio, Tokyo, Japan) against Thy1.1, a surface marker of mesangial cells. We injected 0.25 µl ascites/100 g body weight of the antibody into the caudal vein as reported previously [17, 29]. After injection, the rats were sacrificed on days 4 (n=4), 7 (n=4), and 11 (n=4). All surgery and infusions were performed under general anesthesia with ether.

Kidneys were perfused and fixed with 4% paraformaldehyde buffered with 0.1 M phosphate-buffer saline (PBS), then rinsed three times for 15 min with 0.1 M PBS and dehydrated using increasing concentrations of ethanol (50%, 70%, 80%, 90%, 95%, 100%, 100%, 100%) for 15 min. After dehydration, they were cleared three times with toluene and immersed in molten paraffin. Next, they were embedded in the paraffin, sectioned at 2 µm for periodic acid–Schiff staining and immunohistochemical staining and sectioned at 5 µm for in situ hybridization. They were mounted on 2% silane-coated slides.

Urinary protein levels were determined using the Kingsbury-Clark's method for 24 h before the rats were sacrificed.

We determined the number of positive cells for PC10 (a cell proliferation marker; Dako), the anti-proliferating cell nuclear antigen (PCNA) antibody as a marker of proliferating cells, and the number of positive cells for ED-1 (Serotec), which is a monocyte/macrophage specific antigen. The sections were rehydrated and exposed to a 3% methanol–hydrogen peroxide solution for 20 min to inactivate the endogeneous peroxidase. Then, they were treated for 20 min with 10% normal horse serum in PBS to prevent non-specific staining. They were then incubated with the primary antibody diluted to 1:100 in PBS for 2 h, followed by the avidin-biotin complex method [13], using biotinylated horse anti-mouse immunoglobulin (Vector Laboratories, Burlingame, Calif.) as a secondary antibody and 3.3'-diaminobenzidine as the peroxidase substrate. The negative controls consisted of substitution of an equivalent murine monoclonal antibody or normal rabbit IgG for the primary antibody.

In each sample, 30 sequential glomerular cross-sections were examined and two groups of data were measured: total of glomerular cells, the mean number of nuclei per glomerular cross-section in periodic acid–Schiff-stained tissue sections; proliferating glomerular cells, the mean number of PCNA and ED-1 positive cells.

Results were analyzed using the Student's t-test or one-way analysis of variance with modified t-tests using the Bonferroni correction. A P value of less than 0.05 was considered to be significant.

Rat glomerular mesangial cells were cultured, cloned, and characterized as described previously [25]. The cells exhibited typical mesangial cell morphology. The cell clones used showed positive staining for Thy1.1 antigen, α-smooth muscle actin, vimentin and desmin, and negative staining for cytokeratin and factor-VIII-related antigen, thus excluding epithelial and endothelial cell contaminations, respectively.

The cells were grown in RPMI 1640 (Gibco, Grand Island, N.Y.) supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin, and 100 µg/ml streptomycin. For the experiments, cells of passages 10–15 were used.

Total RNA from the rat cultured mesangial cells was extracted using ISOGEN (Nippon Gene, Toyama, Japan) [4] according to the manufacturer's instructions. RNA was amplified using the Perkin Elmer Tth reverse-transcription polymerase chain reaction (RT-PCR) kit (Norwalk, Conn.), with a 10-min RT step followed by 50 cycles of three-step PCR at an annealing temperature of 52°C in a Perkin Elmer model 9600. PCR products were electrophoresed on 1.5% agarose gels in Tris acetate ethylene diamine tetraacetic acid (TAE). FGF2 primers were designed to amplify FGF2 mRNA (sense 5'-GCCGGCAGCATCACTTCGCT-3' and anti-sense 5'-TAGAGGCTACTCACGGTTCCT-3' predicted product 438 bp) [22]. In addition, FGFR1 primers were designed to amplify FGFR1 mRNA (sense 5'-CCGTGACCTCACCTCTG-3' and anti-sense 5'-GCTCATATTCAGAGACGCCA-3' predicted product 294 bp) [30], FGFR2 mRNA (sense 5'-CTGTGCCGA ATGAAGAACACGACC-3' and anti-sense 5'-CCCAAAGTCTG CTATCTTCATCAC-3' predicted product 743 bp), FGFR3 mRNA (sense 5'-GGAGGACGTGGCTGAAGACACAGG-3' and anti-sense 5'-TCTGCCGGATGCTGCCAAACTTGT-3' predicted product 295 bp) and FGFR4 mRNA (sense 5'-CTCACGGGCCTTG TGAATCTAGAC-3' and anti-sense 5'-CCCAAAGTCTGCTATC TTCATCAC-3' predicted product 560 bp) [2, 28].

A template for synthesis of the RNA probe for FGF2 was constructed by subcloning a fragment of FGF2 cDNA into the pGEM1 vector. The templates for the FGFR probe were constructed by subcloning FGFR1, FGFR2, FGFR3, and FGFR4 fragments of synthetic FGFR cDNAs into the pBluescript II SK(–) vector [31]. The fragment (*Bam*HI, *Xho*I) of FGF2 cDNA, and the FGFR1–4 fragments (*Bam*HI, *Eco*RI) of the FGFR cDNAs were subcloned into the polylinker regions of the vector with the twin promoter for SP6 or T7 RNA polymerase. RNA probes were prepared from the sense and the antisense strands of the fragment using the digoxigenin of SP6 or T7 RNA polymerase according to the manufacturer's instructions (Boehringer-Mannheim, Germany). Mainly the FGFRs obtained from the area of membrane permeation were used [24].

The sections were deparaffinized through conventional xylene and ethanol steps, and were then treated with glycine (2 mg/ml in PBS for 20 min) to quench the fixative and with acetic anhydride (0.25% v/v in 0.1 M triethanolamine at pH 7 for 15 min) to reduce non-specific binding. Next, 20 µl of a hybridization mixture, containing 50% formamide, 2×SSC, 10% dextran sulfate, 0.25% bovine serum albumin, 1 mg/ml tRNA, 1 mg/ml denatured salmon-sperm DNA, and the RNA probe, was applied to the sections. Then the sections were covered with 25×30-mm parafilm and incubated in a moisturized chamber at 42°C for 15–16 h [5]. The parafilm was floated off the slide by immersion in 2×SSC, 50% formamide. Afterward, the sections were washed three times with 2×SSC, 50% formamide at 42°C for 1 h with agitation, then treated with 20 µg/ml RNase A in 10 mM Tris (pH 8) containing 0.5 M NaCl, 1 mM EDTA at 37°C for 30 min. Then the sections were washed three times with 0.1×SSC at 42°C for 1 h. Colorimetric detection of mRNA following hybridization was accomplished with the Genius Nonradioactive Nucleic Acid Detection Kit (Boehringer-Mannheim) with modification.

Control

Control experiments were performed to confirm the specificity of hybridization between probes and target mRNAs. The sense strand probes were used as a control.

Results

Level of urinary protein

The 24-h urinary protein excretion of normal Wistar rats was less than 10 mg. The protein excretion during the mesangial proliferative phase (day 4 and day 7) was more than 50 mg (Fig. 1).

Total glomerular cells, PCNA-positive cells, and ED-1-positive cells during anti-Thy1.1 GN

The morphological changes in nephritis concurred with those reported previously [6, 23, 29]. The total number of glomerular cells decreased on day 4 after disease induction, but on day 7 and day 11, they had significantly

increased. On day 4 and day 7, the number of PCNA-positive cells had significantly increased. However, the number of PCNA-positive cells decreased to almost normal range on day 11 (Table 1). The infiltration of ED-1-positive cells peaked on day 4, and declined on day 7 and day 11. Previous studies have reported that infiltration of macrophages peaked on day 4 and the staining score for α smooth muscle actin (αSMA) between day 4 and day 9 was higher than for other days [20]. In this model, 98% of the PCNA-positive cells were negative for ED-1 on day 5 [15]. These results suggest that the proliferative cells in the mesangial areas on day 7 were almost all mesangial cells.

Detection of FGF2 and FGFR1–4 mRNA gene expression using the RT-PCR technique

RT-PCR revealed the presence of FGF2 and FGFR1–4 mRNAs in cultured mesangial cells (Fig. 2). A single band of the expected size was amplified.

Detection of FGF2 and FGFR1–4 mRNA gene expression using the in situ hybridization technique

Expression of FGF2 and FGFR1–4 mRNAs was not observed in normal rat glomeruli (Fig. 3A, Fig. 4A, Fig. 5A, Fig. 6A, and Fig. 7A), but expression was upregulated in cells in the mesangial areas during the mesangial proliferative phase (day 7) (Fig. 3B, Fig. 4B, Fig. 5B, Fig. 6B, and Fig. 7B). The great majority of these proliferating cells have been identified as glomerular in origin [17]. Namely, it is thought that most of the cells in the mesangial areas are mesangial cells. Expression of FGFR1, FGFR3, and FGFR4 was also observed in Bowman's epithelial cells and/or podocytes during this phase. Some tubular cells and periglomerular cells were also variably positive for FGFR mRNAs during the same phase (Fig. 4B, Fig. 5B, Fig. 6B, and Fig. 7B). Furthermore, this expression decreased during the mesangial decrease phase after mesangial proliferation (day 11) (not shown). Expression of FGF2 and FGFR1–4 was not observed in the glomeruli of anti-Thy1.1 GN rats with a control sense probe on day 7 (Fig. 3C, Fig. 4C, Fig. 5C, Fig. 6C, and Fig. 7C).

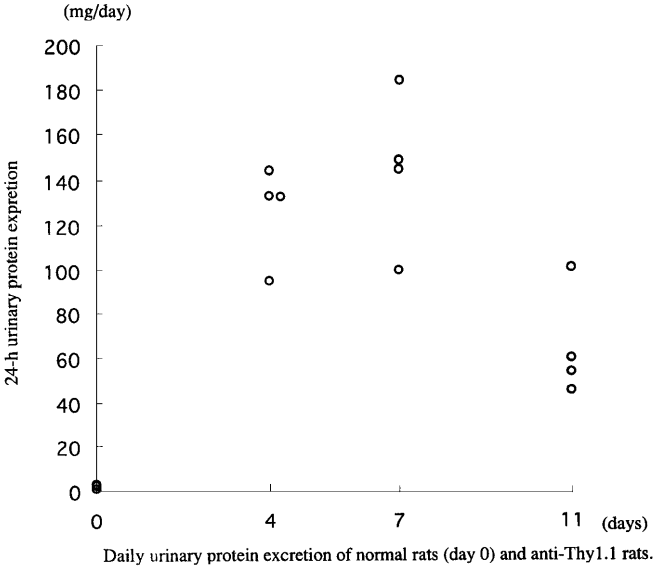


Fig. 1 Proteinuria in normal and anti-Thy1.1 mesangial proliferative glomerulonephritis (GN) rats

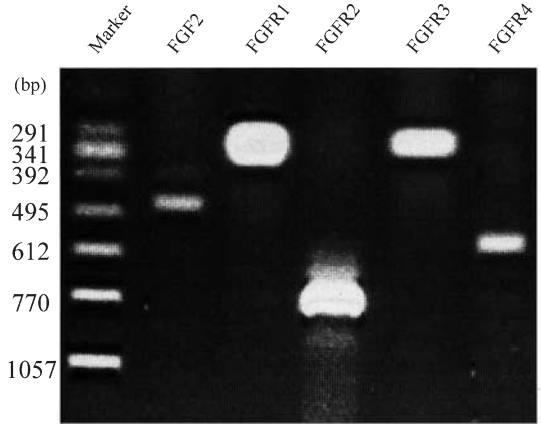


Fig. 2 Reverse-transcription polymerase chain reaction (RT-PCR) for basic fibroblast growth factor (FGF2) and FGFR1–4 mRNAs. Ethidium bromide-stained 1.5% agarose gel analysis of RT-PCR products obtained with specific FGF2 and FGFR1–4 primers for culture mesangial cells

Table 1 Natural course in the glomeruli of rats with anti-Thy 1.1 GN. Total glomerular cells and immunostaining for PCNA and ED-1.

	Total glomerular cells		PCNA positive cells		ED-1 positive cells	
	Mean±SD	*P* value	Mean±SD	*P* value	Mean±SD	*P* value
Normal	65.6±0.8		0.6±0.1		1.34±0.12	
		P<0.001		*P*<0.001		*P*<0.001
Day 4	57.7±0.7		13.3±0.9		12.4 ±1.25	
		P<0.001		*P*<0.001		*P*<0.05
Day 7	88.4±0.7		16.8±0.4		8.15±0.79	
		P<0.001		*P*<0.001		*P*<0.05
Day 11	98.8±0.7		3.7±0.5		6.16±0.92	

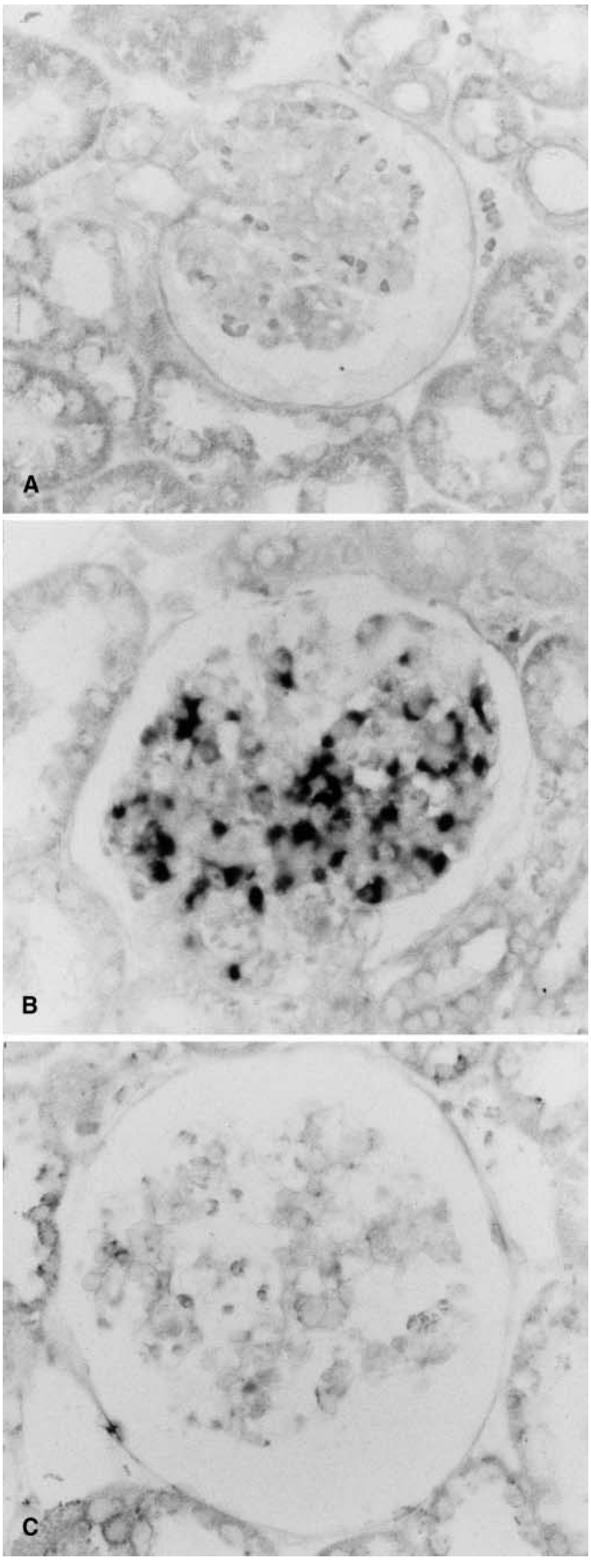


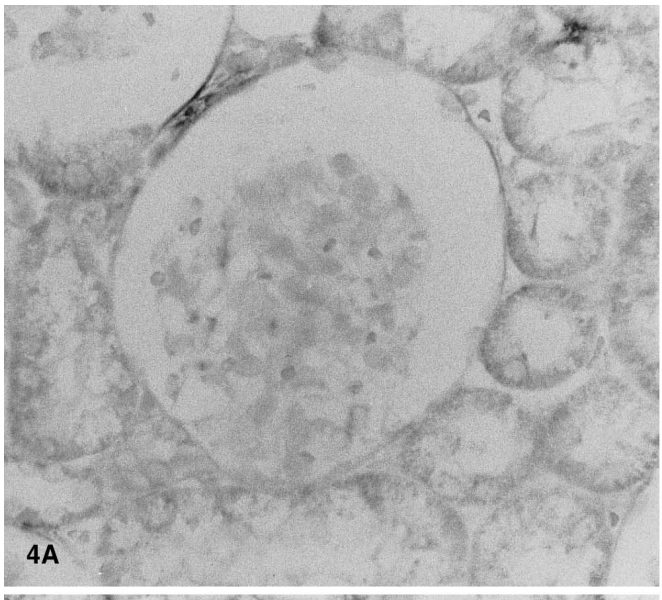
Fig. 3 In situ hybridization for basic fibroblast growth factor (FGF2). **A** Normal glomeruli, **B** Glomeruli of anti-Thy1.1 mesangial proliferative glomerulonephritis (GN) rats on day 7. FGF2 was not expressed in the normal glomeruli, but was strongly expressed in the proliferative mesangial cells. **C** Hybridization with the FGF2 sense probe in anti-Thy1.1 GN rats on day 7. FGF2 was not expressed in the glomeruli (×400)

Discussion

We have examined the presence of FGF2 and FGFR1–4 mRNAs in normal rat kidney glomeruli and in the anti-Thy1.1 GN rat kidney glomeruli. FGF2 mRNA was not detected in the normal glomeruli, but was detected in the proliferative mesangial cells in anti-Thy1.1 rats. FGFR1–4 mRNAs were not detected in the normal glomeruli but were detected in the proliferative mesangial cells in anti-Thy1.1 rats. In previous studies, FGF2 has been found in mesangial areas, the glomerular basement membrane, glomerular epithelial cells, the basement membrane of Bowman's capsule, small arterial walls, and tubules of the normal kidney using the immunohistochemical method [7, 26]. In an immunohistochemical study using frozen sections, expression of FGFRs 1–4 in normal glomeruli was observed [9]. It has recently been reported that the immunolocalization patterns for FGF2 differ according to the fixative or preparation technique used, and depending on the antibody specificity [3, 12]. We also carried out an immunohistochemical study using the monoclonal antibodies against FGF2 and FGFR1 in anti-Thy1.1 rats, but these proteins could not be detected easily. Expression of FGF2 and FGFR1 mRNA in normal rat kidneys has been detected using Northern blotting [7, 30]. Expression of FGF and FGFR isoform mRNA in whole cortex and isolated glomeruli has also been demonstrated using the RT-PCR method [9]. We were unable to demonstrate mRNA for any of the FGFRs in normal rat glomeruli using an in situ hybridization method. The difference between the present findings and those of Ford et al. [9] most likely reflects the greater sensitivity of RT-PCR than in situ hybridization. A recent study found that injured mesangial cells pro-

Fig. 4 In situ hybridization for fibroblast growth factor receptor (FGFR) 1. **A** Normal glomeruli. **B** Glomeruli of anti-Thy1.1 mesangial proliferative glomerulonephritis (GN) rats on day 7. FGFR1 was not expressed in the normal glomeruli, but was strongly expressed in the proliferative mesangial cells and was focally expressed in the Bowman's capsular epithelial cells (*arrow*), podocytes (*arrow head*), periglomerular cells, and some tubules. **C** Hybridization with the FGFR1 sense probe in anti-Thy1.1 GN rats on day 7. FGFR1 was not expressed in the glomeruli (×400) ▶

Fig. 5 In situ hybridization for fibroblast growth factor receptor (FGFR) 2. **A** Normal glomeruli. **B** Glomeruli of anti-Thy1.1 mesangial proliferative glomerulonephritis (GN) rats on day 7. FGFR2 was not expressed in the normal glomeruli, but was strongly expressed in the proliferative mesangial cells and was focally expressed in the Bowman's capsular epithelial cells (*arrow*), podocytes (*arrow head*), periglomerular cells, and some tubules. **C** Hybridization with the FGFR1 sense probe in anti-Thy1.1 GN rats on day 7. FGFR1 was not expressed in the glomeruli (×400)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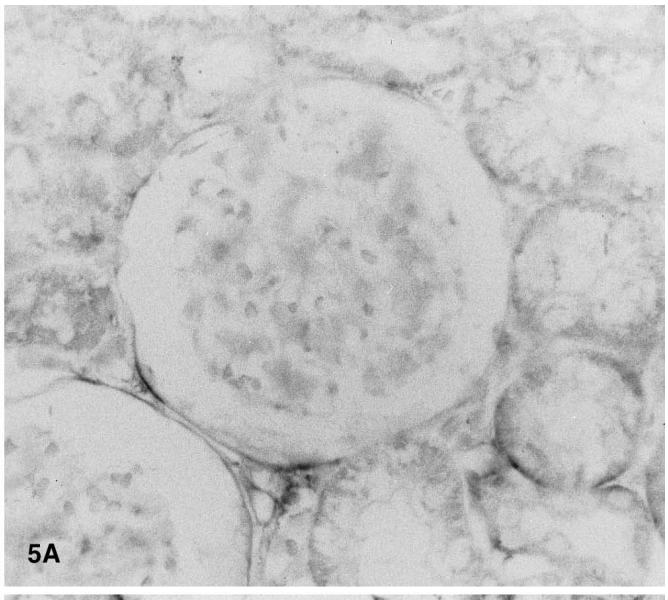
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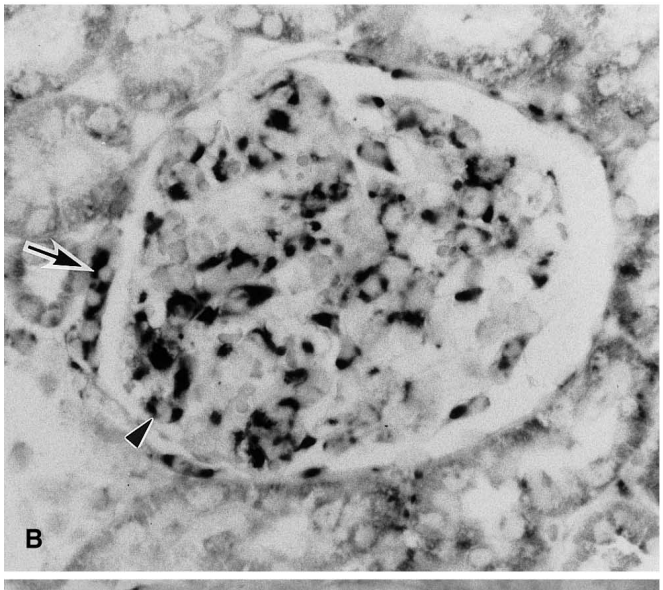
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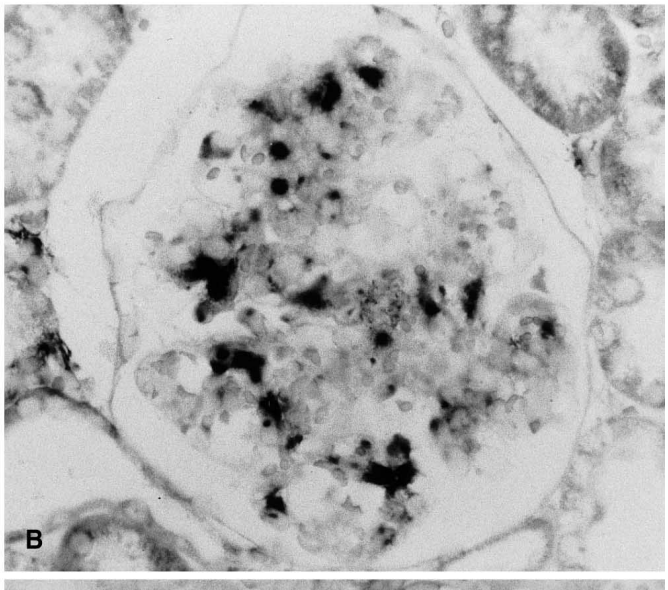
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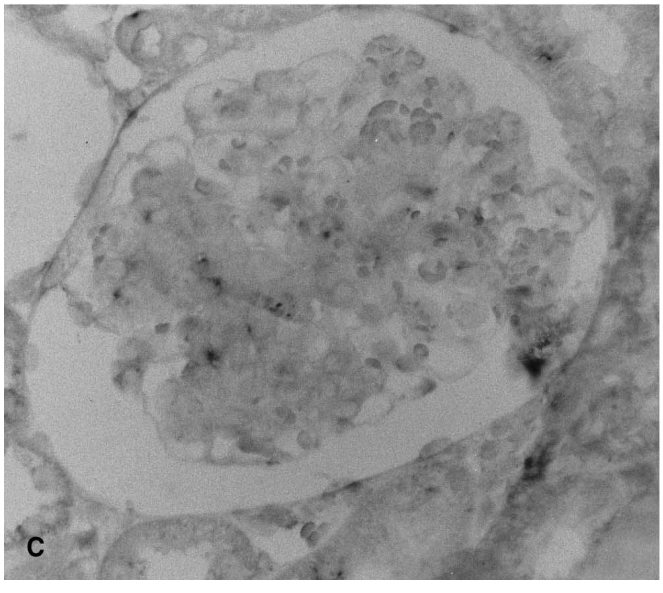
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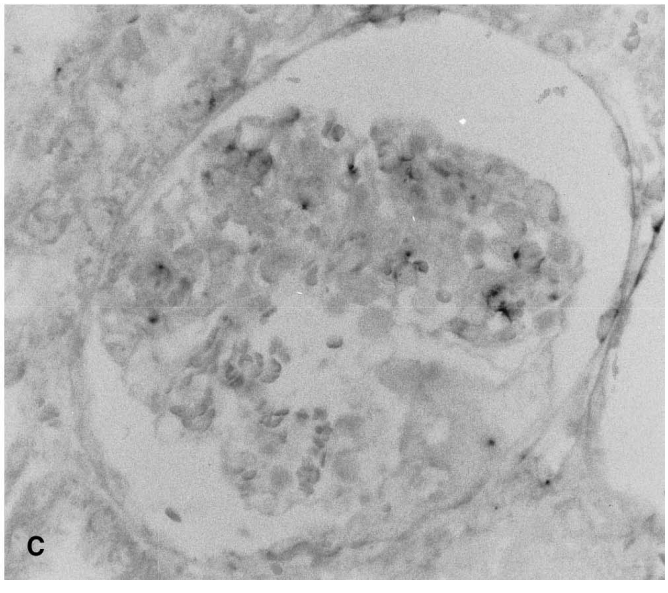
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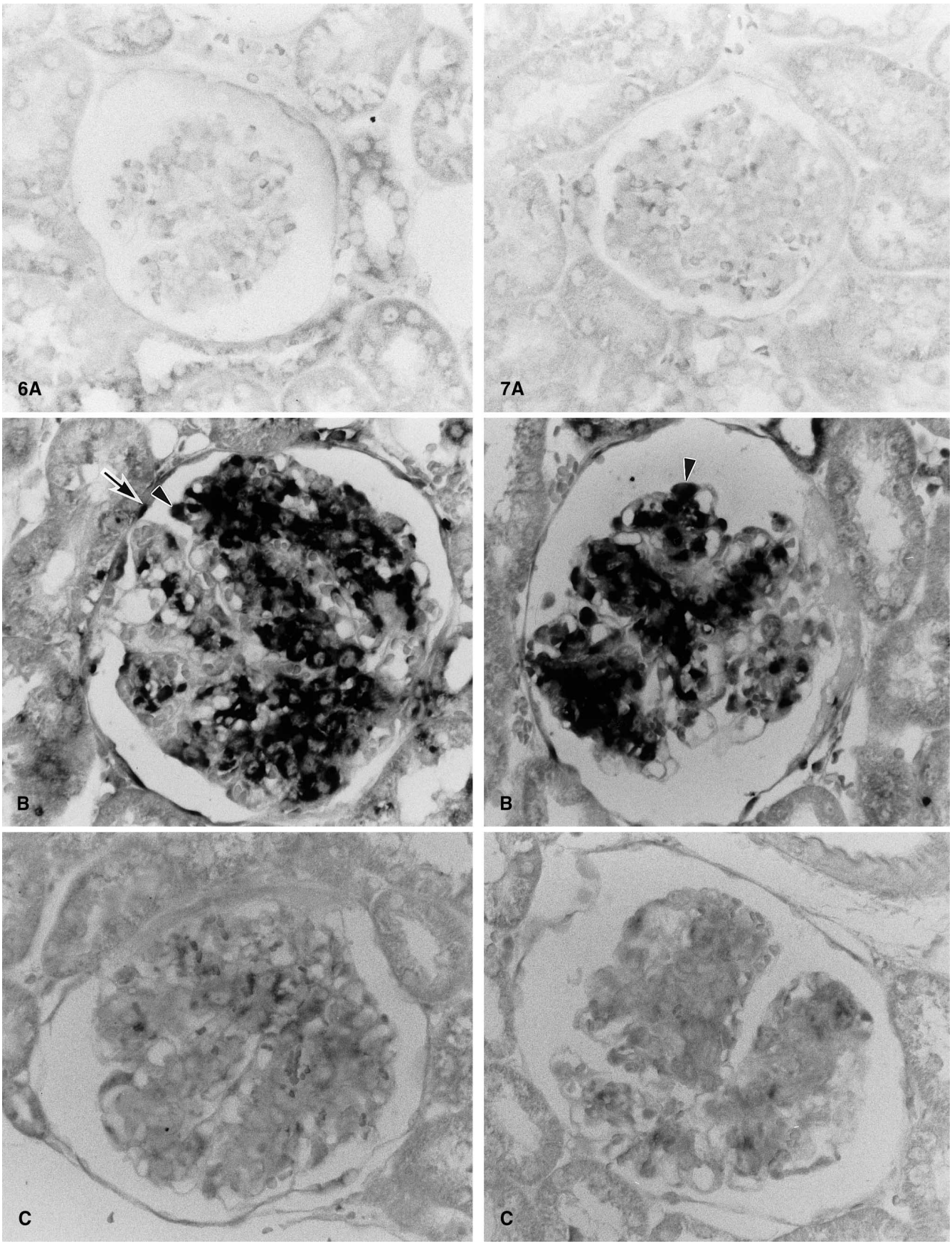
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duce and release FGF2, and that FGF2 has mitogenic action for injured mesangial cells [27]. However, in vivo localization of FGF2 or FGFR mRNAs has not been achieved.

Using the RT-PCR method, we proved that cultured mesangial cells induce FGF2 and FGFR1–4. These cells were positive for α-SMA. It is known that α-SMA-positive mesangial cells are activated mesangial cells, and that normal mature mesangial cells are negative for α-SMA in vivo. In this instance, the cultured mesangial cells were similar to in vivo proliferative mesangial cells. In contrast, FGF2 and FGFR1–4 mRNAs were detected in the mesangial proliferative lesions of anti-Thy1.1 GN. These results suggest that FGF2 may be involved in mesangial cell proliferation after injury. The mitogenic effect of FGF2 on mesangial cells may be a direct effect or could be mediated by FGF2-induced synthesis of platelet-derived growth factor (PDGF) [24]. Indeed, an upregulation of glomerular PDGF B-chain mRNA has been reported in the mesangial proliferative lesions of anti-Thy1.1 GN [32]. It has also been reported that recombinant FGF2 infusion increases mesangial cell proliferation in rats receiving a subnephritogenic dose of anti-Thy1.1 antibody but that it is ineffective in normal rats [8]. This study and our results suggest the possibility that FGF2 increases mesangial cell-mediated FGFRs. In addition, it has been reported that FGFR1 and FGFR2 bind FGF2 with high affinity, whereas FGFR3 and FGFR4 bind with low affinity [14]. Sasaki et al. also noted that the proliferation of mesangial cells in response to FGF2 could be controlled by FGFR1 but not by FGFR2 [21]. These results suggest that upregulation of FGF2 in mesangial cells may increase mesangial cell-mediated FGFR1.

During the mesangial proliferative phase, we also demonstrated that the expression of FGFR1, FGFR3, and FGFR4 is upregulated on Bowman's epithelial cells and/or podocytes, and FGFR1–4 mRNAs are expressed in some tubules and periglomerular cells. Proteinuria also increases during this phase. Therefore, these upregulations may be caused by the release of FGF2 by intraglomerular cells. It has been reported that disease limited to the mesangium leads directly to periglomerular macrophage infiltration [20]. The increase in these expressing cells in Bowman's capsule, periglomerular cells and tubular cells may be related to the composition of tubulointerstitial lesions and periglomerular fibrosis.

In conclusion, the results of the present study suggest that FGF2 plays an important role in mesangial cell proliferation after injury. Furthermore, upregulation of FGFR is necessary for stimulation of mesangial proliferation by FGF2.

Fig. 6 In situ hybridization for fibroblast growth factor receptor (FGFR) 3. **A** Normal glomeruli. **B** Glomeruli of anti-Thy1.1 mesangial proliferative glomerulonephritis (GN) rats on day 7. FGFR3 was not expressed in the normal glomeruli, but was strongly expressed in the proliferative mesangial cells and was focally expressed in the Bowman's capsular epithelial cells (*arrow*), podocytes (*arrow head*), periglomerular cells, and some tubules. **C** Hybridization with the FGFR1 sense probe in anti-Thy1.1 GN rats on day 7. FGFR1 was not expressed in the glomeruli (×400)

Fig. 7 In situ hybridization for fibroblast growth factor receptor (FGFR) 4. **A** Normal glomeruli. **B** Glomeruli of anti-Thy1.1 mesangial proliferative glomerulonephritis (GN) rats on day 7. FGFR4 was not expressed in the normal glomeruli, but was strongly expressed in the proliferative mesangial cells and podocytes (*arrow head*), periglomerular cells and some tubules. **C** Hybridization with the FGFR1 sense probe in anti-Thy1.1 GN rats on day 7. FGFR1 was not expressed in the glomeruli (×400)

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References

1. Avivi A, Zimmer Y, Yayon A, Yarden Y, Givol D (1991) Flg-2, a new member of the family of fibroblast growth factor receptors. Oncogene 6:1089–1092 (published erratum 7:823)
2. Brogi E, Winkles JA, Underwood R, Clinton SK, Alberts GF, Libby P (1993) Distinct patterns of expression of fibroblast growth factors and their receptors in human atheroma and nonatherosclerotic arteries. Association of acidic FGF with plaque microvessels and macrophages. J Clin Invest 92:2408–2418
3. Cauchi J, Alcorn D, Cancilla B, Key B, Berka JL, Nurcombe V, Ryan GB, Bertram JF (1996) Light-microscopic immunolocalization of fibroblast growth factor-1 and -2 in adult rat kidney. Cell Tissue Res 285:179–187
4. Chomczynski P (1993) A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. Biotechniques 15:532–534
5. Cox KH, DeLeon DV, Angerer LM, Angerer RC (1984) Detection of mRNAs in sea urchin embryos by in situ hybridization using asymmetric RNA probes. Dev Biol 101:485–502
6. Floege J, Johnson RJ, Gordon K, Iida H, Pritzl P, Yoshimura A, Campbell C, Alpers CE, Couser WG (1991) Increased synthesis of extracellular matrix in mesangial proliferative nephritis. Kidney Int 40:477–488
7. Floege J, Eng E, Lindner V, Alpers CE, Young BA, Reidy MA, Johnson RJ (1992) Rat glomerular mesangial cells synthesize basic fibroblast growth factor. Release, upregulated synthesis, and mitogenicity in mesangial proliferative glomerulonephritis. J Clin Invest 90:2362–2369
8. Floege J, Eng E, Young BA, Alpers CE, Barrett TB, Bowen PD, Johnson RJ (1993) Infusion of platelet-derived growth factor or basic fibroblast growth factor induces selective glomerular mesangial cell proliferation and matrix accumulation in rats. J Clin Invest 92:2952–2962
9. Ford MD, Cauchi J, Greferath U, Bertram JF (1997) Expression of fibroblast growth factors and their receptors in rat glomeruli. Kidney Int 51:1729–1738
10. Gospodarowicz D (1974) Localization of a fibroblast growth factor and its effect alone and with hydrocortisone on 3T3 cell growth. Nature 249:123–127
11. Gospodarowicz D (1975) Purification of a fibroblast growth factor and receptor families: complexity, functional diversity, and implications for future cardiovascular research. Cardiovasc Res 27:1199–1203
12. Hanneken A, Baird A (1992) Immunolocalization of basic fibroblast growth factor: dependence on antibody type and tissue fixation (letter). Exp Eye Res 54:1011–1014
13. Hsu S-M, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques. J Histochem Cytochem 29:577–580

14. Hughes SE, Hall PA (1993) Overview of the fibroblast growth factor and receptor families: complexity, functional diversity, and implications for future cardiovascular research. Cardiovasc Res 27:1199–1203
15. Iida H, Seifert R, Alpers CE, Gronwald RG, Phillips PE, Pritzl P, Gordon K, Gown AM, Ross R, Bowen PD, et al. (1991) Platelet-derived growth factor (PDGF) and PDGF receptor are induced in mesangial proliferative nephritis in the rat. Proc Natl Acad Sci U S A 88:6560–6564
16. Johnson DE, Williams LT (1993) Structural and functional diversity in the FGF receptor multigene family. Adv Cancer Res 60:1–41
17. Johnson RJ, Pritzl P, Iida H, Alpers CE (1991) Platelet-complement interactions in mesangial proliferative nephritis in the rat. Am J Pathol 138:313–321
18. Klagsbrun M, Edelman ER (1989) Biological and biochemical properties of fibroblast growth factors. Implications for the pathogenesis of atherosclerosis. Arteriosclerosis 9:269–278
19. Miyamoto M, Naruo K, Seko C, Matsumoto S, Kondo T, Kurokawa T (1993) Molecular cloning of a novel cytokine cDNA encoding the ninth member of the fibroblast growth factor family, which has a unique secretion property. Mol Cell Biol 13:4251–4259
20. Roy CP, Wu B, McDonald S, Haites NE, Simpson JG, Power DA (1995) Phenotypic analysis of the glomerular and periglomerular mononuclear cell infiltrates in the Thy 1.1 model of glomerulonephritis. Lab Invest 72:524–531
21. Sasaki T, Hatta H, Kawakami Y, Nohno T, Osawa G (1996) The transdominant negative FGF receptor inhibits proliferation of glomerular mesangial cells. J Am Soc Nephrol 7:1665
22. Shimasaki S, Emoto N, Koba A, Mercado M, Shibata F, Cooksey K, Baird A, Ling N (1988) Complementary DNA cloning and sequencing of rat ovarian basic fibroblast growth factor and tissue distribution study of its mRNA. Biochem Biophys Res Commun 157:256–263
23. Shimizu A, Kitamura H, Masuda Y, Ishizaki M, Sugisaki Y, Yamanaka N (1995) Apoptosis in the repair process of experimental proliferative glomerulonephritis. Kidney Int 47:114–121
24. Silver BJ, Jaffer FE, Abboud HE (1989) Platelet-derived growth factor synthesis in mesangial cells: induction by multiple peptide mitogens. Proc Natl Acad Sci U S A 86:1056–1060
25. Striker GE, Striker LJ (1985) Glomerular cell culture. Lab Invest 53:122–131
26. Takeuchi A, Yoshizawa N, Yamamoto M, Sawasaki Y, Oda T, Senoo A, Niwa H, Fuse Y (1992) Basic fibroblast growth factor promotes proliferation of rat glomerular visceral epithelial cells in vitro. Am J Pathol 141:107–116
27. Tanaka A, Miyamoto K, Minamino N, Takeda M, Sato B, Matsuo H, Matsumoto K (1992) Cloning and characterization of an androgen-induced growth factor essential for the androgen-dependent growth of mouse mammary carcinoma cells. Proc Natl Acad Sci U S A 89:8928–8932
28. Winkles JA, Alberts GF, Peifley KA, Nomoto K, Liau G, Majesky MW (1996) Postnatal regulation of fibroblast growth factor ligand and receptor gene expression in rat thoracic aorta. Am J Pathol 149:2119–2131
29. Yamamoto T, Wilson CB (1987) Complement dependence of antibody-induced mesangial cell injury in the rat. J Immunol 138:3758–3765
30. Yazaki N, Fujita H, Ohta M, Kawasaki T, Itoh N (1993) The structure and expression of the FGF receptor-1 mRNA isoforms in rat tissues. Biochim Biophys Acta 1172:37–42
31. Yazaki N, Hosoi Y, Kawabata K, Miyake A, Minami M, Satoh M, Ohta M, Kawasaki T, Itoh N (1994) Differential expression patterns of mRNAs for members of the fibroblast growth factor receptor family, FGFR-1-FGFR-4, in rat brain. J Neurosci Res 37:445–452
32. Yoshimura A, Gordon K, Alpers CE, Floege J, Pritzl P, Ross R, Couser WG, Bowen PD, Johnson RJ (1991) Demonstration of PDGF B-chain mRNA in glomeruli in mesangial proliferative nephritis by in situ hybridization. Kidney Int 40:470–476