Transdominant Negative FGF Receptors Inhibit Proliferation of Cultured Glomerular Mesangial Cells

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ABSTRACT. Following detection of expression of FGF2 and FGF receptors in cultured rat mesangial cells, the inhibiting effects on proliferation of the cultured mesangial cells by transfection of kinase domain-deficient FGFR-1 and FGFR-2 were examined. Cultured rat mesangial cells expressed the FGF2, FGFR-1 and FGFR-2 genes. After 48 hrs of incubation, rh-FGF2 (10 ng/ml) increased the number of Alp-transfected rat mesangial cells to 1.8±0.3 fold that in nonstimulated cells. In contrast, transfection of dominant negative-FGFRs led to a significant inhibition of cell proliferation ($p<0.001$). Our results suggest that the proliferation of mesangial cells is stimulated by FGF2 and reversed by these dominant negative receptors. And FGFR-1 has a much more important role for mesangial cell proliferation than FGFR-2.

Key words: FGF2 — FGF receptors — mesangial cells — dominant negative FGF receptors

Proliferation of glomerular mesangial cells as well as expansion of the mesangial extracellular matrix is one of the major features of numerous experimental and human glomerular diseases.[1-3] Both processes are thought to play an important role in the development of glomerular sclerosis and renal failure. The number of patients with end stage renal failure who will inevitably need hemodialysis or renal transplantation increases every year. Recent studies[1,4] link growth factors and cytokines to the regulation of mesangial cell proliferation and matrix synthesis. Specifically, platelet-derived growth factor (PDGF) and basic fibroblast growth factor (FGF2) are involved in mesangial cell proliferation.[5,6] Based on these findings, several studies have been performed to inhibit mesangial cell proliferation and expansion of the extracellular matrix.[7,8]

The biological effects of FGF2 are mediated through binding to cell surface tyrosine kinase receptors. Four distinct but structurally related receptors, designated FGF receptors (FGFR)-1, -2, -3 and -4, have been identified.[9] Multiple isoforms of the FGFR-1, FGFR-2 and FGFR-3 proteins can arise via alternative splicing of primary transcripts. In some cases, variants

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of a signal receptor type can have distinct ligand-binding specificities.[10,11] FGF2 binds preferentially to the FGFR-1 and FGFR-2 splice variants containing the Ig domain IIIc and FGFR-4.[10–13] The individual FGFR genes have different temporal and spatial expression patterns during development and are also expressed in a tissue-specific manner in adult animals.

We documented the expression of FGF2 and FGF receptors in cultured rat mesangial cells, and we also attempted to analyze the inhibiting effect of cell proliferation on cultured mesangial cells by transfected kinase domain-deficient FGFR-1 and FGFR-2.

MATERIALS AND METHODS

Isolation and culture of mesangial cells

Rat glomerular mesangial cells were isolated and grown in culture as described by Kreisberg *et al*.[14] Glomeruli were isolated from male Wistar strain rats weighing 100-150 g (Clea Japan, Osaka, Japan) by the graded-sieve technique and plated, maintained in 60 mm cell culture dishes (Sumitomo Bakelite, Japan) containing RPMI 1640 tissue culture medium plus 10% fetal calf serum (Gibco, Grand Island, NY), 100 U/ml of penicillin, 100 μg/ml of streptomycin at 37°C in a humidified 5% CO_2 atmosphere. When the cells had grown to confluency, they were washed with phosphate buffered saline (PBS), detached from their plates by trypsin (0.1% in 0.1 mM EDTA) and divided and passaged again. The cells were identified as mesangial cells by standard methods described elsewhere.[14] Cultured cells from the 5th to 15th passages were used for the following experiments.

DNA constructs and expression vectors

Amino-terminal coding sequence of chicken FGFR-1 was generated by digestion of EcoRI and ApaLI and subcloned into pSP64TL,[15] which contains in-frame stop codon and Xenopus globulin 3'-UTR sequence. Amino-terminal coding sequence of chicken FGFR-2 was generated by PCR with forward primer (5'-GGACCAGGAACTTACTCTAA-3') and reverse primer (5'-CTTACACTGTTACCTGTCTGCG-3'), and then subcloned into pCR-Script vector. The reverse primer has one base substitution to generate in-frame stop codon. These dominant negative constructs (DN-FGFR-1, DN-FGFR-2, -primer sequences shown in Table 1) were finally subcloned into pJT4, which has CMV promoter and HTLV-I long terminal repeat R element to express foreign genes in eukaryotic cells and poly (A) signal derived from SV40.[16]

Transient expression in mesangial cells

In this study, a human placental alkaline phosphatase (Alp) gene was used as the reporter gene in pJT4. Mesangial cell aliquots containing 1×10^6 cells were transfected with 20 μg of Alp, DN-FGFR-1, and DN-FGFR-2 expression vectors by electroporation (Gene Pulser, Invitorgen, USA) at 4.0 kV/cm, with a capacitance of 71 μF. Alp-transfected cells were seeded at 10^5 cells per 30 mm diameter plate. After 48 hours, these cells were fixed with 2% paraformaldehyde and examined by a histochemical staining method using Alp substrate as described.[17] The transfection efficiency was higher than approximately 35% (Fig 1).

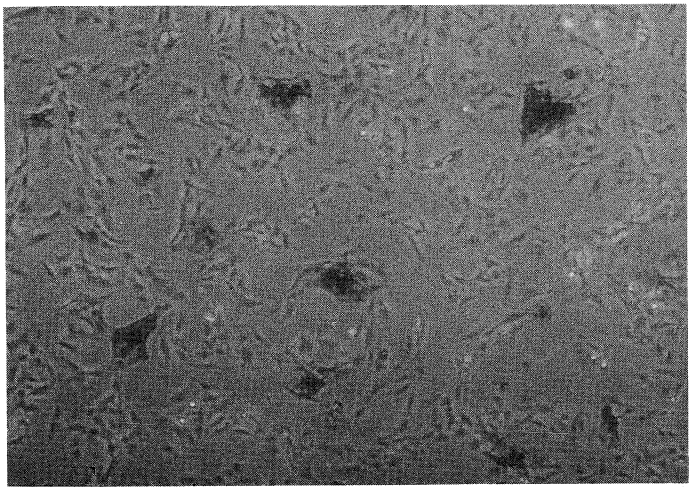

Fig 1. Light micrographs showing the expression of a human placental alkaline phosphatase (Alp) gene in a cultured mesangial cell. The cytoplasm of transfected cells is stained purple. Original magnification ×400.

RNA isolation from cultured mesangial cells and with or without transfection

Total RNA from cultured mesangial cells and from mesangial cells 48 hours after transfection was prepared as described.[18] The RNA concentration was calculated from the absorbance at 260 nm. All RNA samples contained intact rRNA and lacked genomic DNA as evaluated by ethidium bromide staining of agarose-formaldehyde gels.

Reverse transcription-polymerase chain reaction (RT-PCR) analysis

We performed RT-PCR to detect endogenous FGF2, FGFR-1 and FGFR-2, using rat sequence specific primers. And we also performed RT-PCR to detect transfected DN-FGFRs using chicken sequence specific primers.

Ten μg of total RNA was applied to reverse-transcription using an oligo dT primer (24 mer) and an RNA PCR Kit (Perkin-Elmer, USA) in a total volume of 20 μl. The reaction mixture was incubated at 65°C for 60 min. No-RT mixture was omitted by reverse-transcriptase.

For PCR amplification, 1 μl of cDNA or no-RT mixture was added to 5 μl of PCR buffer (50 mM KCl, 10 mM Tris-Cl, pH 8.3, 1.5 mM MgCl2, and 0.001% gelatin) containing 200 μM of 2'-deoxynucleoside 5'-triphosphates, 0.2 μM of forward primer, 0.2 μM of reverse primer, and 1.25 U/μl of Taq DNA polymerase in a total volume of 50 μl. (Perkin-Elmer, USA). The FGFR-1, FGFR-2, DN-FGFR-1 and DN-FGFR-2 primers were designed to amplify selectively the messages transcribed from the introduced genes (Table 1).[19,20] The PCR was carried out at an annealing temperatures from 63°C to 53°C decreasing by 0.5°C per cycle for 20 cycles, followed by an additional 15 cycles at an annealing temperature of 53°C. Denaturation was affected at 94°C for 30 sec, annealing period was 30 sec and extension was at 72°C for 90 sec. After reaction, additional incubation at 72°C was carried out for 5 min. All PCR

TABLE 1. Designs of RT-PCR primers

Gene	Forward	Reverse	PCR product size(bp)
FGF2	GCCGGCAGCATCACTTCGCT	CTCTTAGCAGACATTGGAAG	433
FGFR-1	CCGTGATGACCTCACCTCTG	GCTCATATTCAGAGACGCCA	294
FGFR-2	CTGTGCCGAATGAAGAACACGACC	CCCAAAGTCTGCTATCTTCATCAC	743
DN-FGFR-1	ATGTTTACCTGGAGGTGCCT	CTACAGACAGAGGGGGGGACAT	905
DN-FGFR-2	GGACCAGGAACTTACACTAA	TTACAGGTAGTCTGGAGATG	1161

FGF2, FGFR-1 and FGFR-2 are rat specific sequences
DN-FGFR-1 and DN-FGFR-2 are chicken specific sequences

products were visible after agarose gel electrophoresis when stained with ethidium bromide. Each FGF receptor's dominant negative cDNAs were used as positive controls. No product was amplified in any no-RT controls.

Analysis of mesangial cell proliferation

Alp, DN-FGFR-1 or DN-FGFR-2 transfected mesangial cells were plated on 12-well plates coated with collagen type IV. After 48 hours, RPMI 1640 tissue culture medium plus 10% fetal calf serum, 100 U/ml of penicillin, 100 μg/ml of streptomycin containing 10 ng/ml FGF2 was added to the wells. Cell counting was done after 48 hours.

Statistical analysis

All values were expressed as the mean $\pm$SD. Comparisons between groups were made using the Student's t-test.

RESULTS

Cultured rat mesangial cells express FGF2 and FGFRs

We examined cultured rat mesangial cells for expression of endogenous FGF2, FGFR-1 and FGFR-2 using the RT-PCR with rat sequence specific primers. Cultured rat mesangial cells expressed the FGF2, FGFR-1 and FGFR-2 genes. All lanes have a single band, conforming to molecular weight expectation for each product (Fig 2).

Transient gene expression

Transcription of dominant negative FGFR-1 and FGFR-2 was confirmed by RT-PCR analysis of cultured mesangial cells. We made chicken sequence specific primer sets for detection of transfected DN-FGFR-1 and DN-FGFR-2. We previously confirmed these primers did not amplify RT-PCR products of RNA from rat mesangial cells (Data not shown). Therefore PCR amplification of mesangial cell cDNA with these primers can exactly distinguish the transcribed transfect DNAs. RT-PCR products of DN-FGFR-transfected rat mesangial cells and chicken cDNA show the expected molecular weight (Fig 3).

Effects of dominant negative FGFR-1 and FGFR-2 on mesangial cell proliferation

DN-FGFRs which have no kinase activity construct proteins truncated at

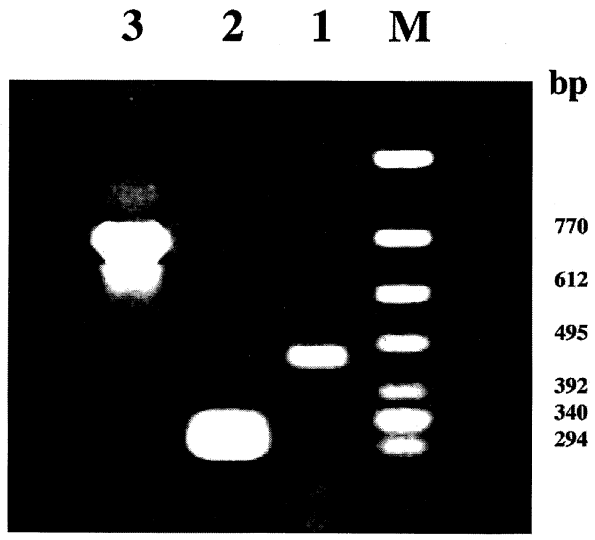

Fig 2. Detection of mRNA for FGF2, FGFR-1 and FGFR-2 in cultured mesangial cells with the RT-PCR.

RT-PCR was performed to detect endogenous gene expression. A single band is seen in all lanes (1: FGF2, 2: FGFR-1, 3: FGFR-2), which shows the expected molecular weights of the respective genes produced by the primers specific to the mRNA.

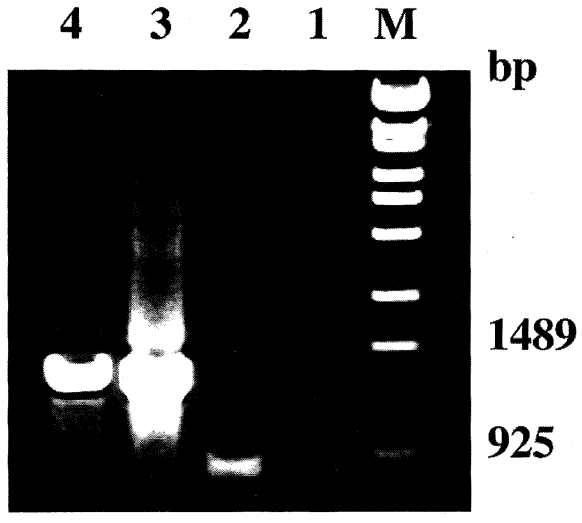

Fig 3. Detection of mRNA for DN-FGFR-1 and DN-FGFR-2 with the RT-PCR.

RT-PCR was performed to detect transfected gene expression. A single band is seen in all lanes: Lane 1=Chicken cDNA of DN-FGFR-1, Lane 2=DN-FGFR-1 transfected rat mesangial cells, Lane 3=Chicken cDNA of DN-FGFR-2, Lane 4=DN-FGFR-2 transfected rat mesangial cells. Each lane shows the expected molecular weight of the respective genes produced by the primers specific to the mRNA for dominant negative FGF receptors.

the trans membrane domain. And these DN-FGFRs also compete with the regular FGFRs, producing a dominant negative effect not on transfected cells but also on non-transfected cells. After a 48-hour incubation, rh-FGF2 (10 ng/ml) increased the number of Alp transfected rat mesangial cells to 1.8±0.3 fold of that in nonstimulated cells. In contrast, transfection of DN-FGFRs led to a significant inhibition of cell proliferation ($P<0.001$). DN-FGFR-1 transfected cells showed a significant suppression of mesangial cell proliferation, but DN-FGFR-2 transfected cells did not show any significant suppression (Fig 4).

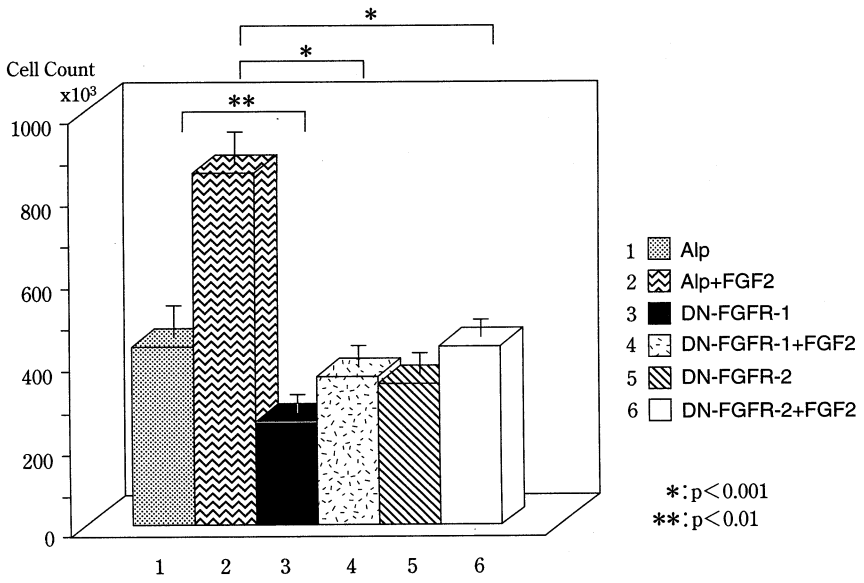

Fig 4. Mesangial cell proliferation was inhibited by DN-FGFR-1 and DN-FGFR-2.
Transfected mesangial cells were plated on 12-well plates. After 48 hours, RPMI 1640 10% FCS containing 10 ng/ml FGF2 or the same volume vehicle was added to the wells.
After FGF2 was added, Alp transfected cells show a 1.8±0.3-fold cell proliferation. DN-FGFR-transfected cells do not show cell proliferation after FGF2 was added. DN-FGFR-1 transfected cells suppress cell proliferation significantly, but DN-FGFR-2 did not.

DISCUSSION

In agreement with previous findings by Silver *et al*,[6] who showed that exposure of cultured mesangial cells to FGF2 (10 ng/ml) induced a 4-5-fold increase in their [^{3}H] thymidine incorporation rate, the present study confirmed that cultured mesangial cells proliferate in response to exogenous FGF2. Both studies thus indicate the presence of FGF2 receptors on rat mesangial cells. Our present study showed that cultured rat mesangial cells expressed FGF2, FGFR-1 and FGFR-2 mRNAs. In addition to high affinity FGF2 receptors, heparan sulfate proteoglycans, especially sydecan, are essential requirements for the binding of FGF2 to its cellular receptors.[21,22] Also of relevance is the fact that cultured mesangial cells have been shown to produce heparan sulfate proteoglycans.[23] Furthermore, this study clearly demonstrated that the

mesangial cells that expressed kinase domain-deficient FGF receptors had their proliferation inhibited by FGF2 stimulation.

FGF is a member of a family of polypeptide growth factors (of which nine have been identified to date), which in turn are capable of being bound to any of the components of the tyrosine kinase receptors (four of which have been identified to data).[24] One of FGFs, FGF2, has received particular attention as a potential mediator of glomerular injury. The role of FGF2 in *in vivo* models of wound healing, angiogenesis, and cardiovascular development has been demonstrated. In *in vitro* studies, a number of glomerular cell types — mesangial cells,[25] endothelial cells[26] and visceral epithelial cells[27] — can be stimulated to proliferate in response to exposure to FGF2. This implies that each of these cell types bears specific FGF receptors. In our previous studies, FGF2 protein was found in normal rat glomeruli, but its mRNA was not detected. However, FGF2 protein and FGFR1-4 mRNAs were present in the collecting ducts and distal tubules.[28,29] It has been demonstrated further that cultured rat mesangial cells synthesize both FGF2 mRNA and protein, and that FGF2 is released into the culture medium from these cells following exposure to injurious stimuli.[30] In *in vivo* studies, demonstration of the role of FGF2 in glomerular disease has been widely examined. Floege *et al* first demonstrated the presence of FGF2 in uninjured rat mesangial cells *in vivo*. Then, a loss of FGF2 expression was demonstrated concomitantly with acute mesangiolytic injury that occurred in an anti-Thy 1 nephritis model.[31] Rat mesangial cells are injured by subnephritogenic doses of anti-Thy 1 antibody, but normal rat glomeruli demonstrate dramatic proliferation, which indicates the capacity for sublethally injured mesangial cells to be responsive to the stimulatory effects of locally released FGF2. Therefore, the proliferative action of FGF2 may be an important mechanism after mesangial cell injuries.

FGF2 mediates the biological response of mesangial cells by binding itself to and activating specific cell surface receptors. The cDNA for the chicken FGF2 receptor was isolated first (FGFR-1). The chicken FGF2 receptor is a transmembrane protein that contains three extracellular immunoglobulin-like domains and two intracellular tyrosine kinase domains.[32] The cDNAs for mouse, rat, and human homologs (flg) have also been isolated,[33] FGFR-1 binds itself to and mediates biological responsiveness to both FGF1 and FGF2. FGFR-1 has therefore been considered to be a common receptor for FGF1 and FGF2. Furthermore, the cDNAs for three more members (FGFR-2, FGFR-3 and FGFR-4) have been isolated.[34] These receptors also contain three extracellular tyrosine kinase domains as well as FGFR-1, and share 55 to 70% amino acid identity. FGFR-2 and FGFR-3 bind themselves to, or mediate biological responsiveness to both FGF1 and FGF2,[35] whereas FGFR-4 binds to FGF1 but not to FGF2.[36] Therefore, we attempted to produce cDNAs which encode kinase-domain deficient FGFR-1 and FGFR-2, and to examine the inhibition of mesangial cell proliferation by stimulated FGF2 by using mutant receptors. This study demonstrated that transfection of dominant negative FGFR-1 and FGFR-2 led to a significant inhibition of mesangial cell proliferation. Furthermore FGFR-1 is thought to play a much more important role in mesangial cell proliferation than FGFR-2.

We found consistent expression of FGFR-1 and FGFR-2 mRNA in cultured rat mesangial cells. Several investigations have explored the use of

toxins conjugated to FGFs as a strategy to prevent excessive proliferation of mesangial cells after injury. Our data suggested that the proliferation of mesangial cells stimulated by FGF2 could be controlled by these dominant negative receptors.

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REFERENCES

1) Striker LJ, Peten EP, Elliot SJ, Doi T, Striker GE: Mesangial cell turnover: effect of heparin and peptide growth factors. Lab Invest **64**: 446-456, 1991
2) Alpers CE, Hudkins KL, Gown AM, Johnson RJ: Enhanced expression of "muscle-specific" actin in glomerulonephritis. Kidney Int **41**: 1134-1142, 1992
3) Klahr S, Schreniner G, Ichikawa I: The progression of renal disease. N Eng J Med **318**: 1657-1666, 1988
4) Floege J, Eng E, Young BA, Johnson RJ: Factors involved in the regulation of glomerular mesangial cell proliferation in vitro and in vivo. Kidney Int **43**: S47 - S54, 1993
5) Barnes JL, Hevey KA: Glomerular mesangial cell migration in response to platelet-derived growth factor. Lab Invest **62**: 379-382, 1990
6) Silver BJ, Jaffer FE, Abboud HE: Platelet-derived growth factor synthesis in mesangial cells: induction by multiple peptide mitogens. Proc Natl Acad Sci USA **86**: 1056-1060, 1988
7) Johnson RJ, Raines E, Floege J, Yoshimura A, Pritzl P, Alpers CE, Ross R: Inhibition of mesangial cell proliferation and matrix expansion in glomerulonephritis in the rat by antibody to platelet derived growth factor. J Exp Med **175**: 1413-1416, 1992
8) Isaka Y, Fujiwara Y, Ueda N, Kaneda Y, Kamada T, Immai E: Glomerulosclerosis induced by in vivo transfection on transforming growth factor-beta or platelet-derived growth factor gene into the rat kidney. J Clin Invest **92**: 2597-2601, 1993
9) Johnson DE, Williams LT: Structural and functional diversity in the FGF receptor multigene family. Adv Cancer Res **60**: 1-40, 1993
10) Miki T, Bottaro DP, Fleming TP, Smith CL, Burgess WH, Chan AML, Aaronson SA: Determination of ligand-binding specifcity by alternative splicing: two distinct growth factor receptors encoded by a single gene. Proc Natl Acad Sci USA **89**: 246-250, 1992
11) Chellaiah AT, McEwen DG, Werner S, Xu J, Ornitz DM: Fibroblast growth factor receptor (FGFR) 3: alternative splicing in immunoglobulin-like domain III creates a receptor highly specific for acidic FGF/FGF-1. J Biol Chem **269**: 11620-11627, 1994
12) Dionne CA, Crumley G, Bellot F, Kaplow JM, Searfoss G, Ruta M, Burgess WH, Jaye M, Schlessinger J: Cloning and expression of two distinct high-affinity receptors cross-reacting with acdic and basic fibroblast growth factors. EMBO J **9**: 2685-2692, 1990
13) Ornitz DM, Leder P: Ligand specificity and heparin dependence of fibroblast growth factor receptor-1 and receptor-3. J Biol Chem **267**: 16305-16311, 1992
14) Kreisberg J, Karnovsky MJ: Glomerular cells in culture. Kidney Int **23**: 439-447, 1983
15) Ishikawa T, Yoshioka H, Ohuchi H, Noji S, Nohno T: Truncated type II receptor for BMP - 4 induces secondary axial structures in xenopus embryos. Biochem Biophys Res Commun **216**: 26-33, 1995
16) Koening BB, Cook JS, Wolsing DH, Ting J, Tiesman JP, Correa P, Olson CA, Pecquet AL, Ventura F, Grant RA, Chen GX, Wrana JL, Massague J, Rosenbaum JS: Characterization and cloning of a receptor for BMP-2 and BMP-4 from NIH 3T3 cells. Mol Cell Biol **14**: 5961-5974, 1994

17) Fields-Berry SC, Halliday AL, Cepko CL: A recombinant retrovirus encoding alkaline phosphatase confirms clonal boundary assignment in lineage analysis of murine retina. Proc Natl Acad Sci USA **89**: 693-697, 1992
18) Winkles JA, Gray CG: Serum, phorbol ester, and polypeptide mitogens increase class 1 and 2 heparin binding (acidic and basic fibroblast growth factor) gene expression in human vascular smooth muscle cells. Cell Growth Differ **2**: 531-540, 1991
19) Shimasaki S, Emoto N, Koba A, Mercado M, Shibata F, Cooksey K, Baird A, Ling N: Complementary DNA cloning and sequencing of rat ovarian basic fibroblast growth factor and tissue distribution study of its mRNA. Biochem Biophys Res Commun **30**: 256-263, 1988
20) Brongi E, Winkles JA, Underwood R, Clinton SK, Alberts GF, Libby P: Distinct patterns of expression of fibroblast growth factors and their receptors in human atheroma and nonatherosclerotic arteries. J Clin Invert **92**: 2408-2418, 1993
21) Yayon A, Klagsbrun M, Esko JD, Leder P, Ornitz DM: Cell surface heparin-like molecules are required for binding of basic fibroblast growth factor to its high affinity receptor. Cell **64**: 841-848, 1991
22) Rapraeger AC, Krufka A, Olwin BB: Requirement of heparin sulfate for bFGF-mediated fibroblast growth factor and myoblast differentiation. Science **252**: 1705-1708, 1991
23) Klein DJ, Brown DM, Kim Y, Oegema TR: Proteoglycans synthesized by human glomerular mesangial cells in culture. J Biol Chem **265**: 9533-9543, 1990
24) Hughes SE, Hall PA: Overview of the fibroblast growth factor and receptor families: complexity, functional diversity, and implications for future cardiovascular research. Cardiovasc Res **27**: 1199-1203, 1993
25) Issandou M, Darbon JM: Basic fibroblast growth factor stimulates glomerular mesangial cell proliferation through a protein kinase C-independent pathway. Growth Factors **5**: 255-264, 1991
26) Ballermann BJ: Regulation of bovine glomerular endothelial cell growth in vitro. Am J Physiol **256**: C182-C189, 1989
27) Takeuchi A, Yoshizawa N, Yamamoto M, Sawasaki Y, Oda T, Senoo A, Niwa H, Fuse Y: Basic fibroblast growth factor promotes proliferation on rat glomerular visceral epithelial cells in vitro. Am J Pathol **141**: 107-116, 1992
28) Jyo Y, Sasaki T, Tamai H, Nohno T, Itoh N, Osawa G: Demonstration of basic fibroblast growth factor receptor mRNA in glomeruli in mesangial proliferative nephritis by in situ hybridization. J Am Soc Nephrol **5**: 789, 1994
29) Sasaki T, Jyo Y, Nohno T, Osawa G: A role of basic fibroblast growth factor in mesangial cell injury. *In* Current Topics of Mesangial Cells, ed by Uchiyama M. Niigata, Kohnkodo. 1994, pp 85-93
30) Floege J, Eng JE, Lindner V, Alpers CE, Young BA, Reidy MA, Johnson RJ: Rat glomerular mesangial cell synthesize basic FGF: release, upregulated synthesis, and mitogenicity in mesangial proliferative glomerulonephritis. J Clin Invest **90**: 2362-2369, 1992
31) Floege J, Eng E, Young BA, Alpers CE, Barrett TB, Bowen-Pope DF, Johnson RJ: 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, 1993
32) Lee PL, Johnson DE, Cousens LS, Fried VA, Williams LT: Purification and complementary cDNA cloning of a receptor basic fibroblast growth factor. Science **245**: 57-60, 1989
33) Isacchi A, Bergonzonli L, Sarmientos P: Complete sequence of a human receptor for acidic and basic fibroblast growth factors. Nucleic Acid Res **18**: 1906, 1990
34) Hattori Y, Odagiri H, Nakatani H, Miyagawa K, Naito K, Sakamoto H, Katih O, Yoshida T, Sugimura T, Terada M: K-sam, an amplified gene in stomach cancer, is a member of the heparin-binding growth factor genes. Proc Natl Acad Sci USA **87**: 5986-5987, 1990
35) Keegan K, Johnson DE, Williams LT, Hayman MJ: Isolation of an additional member of the fibroblast growth factor receptor family, FGFR-3. Proc Natl Acad Sci USA **88**: 1095-1099, 1991
36) Partanen J, Makela TP, Eerola E, Korhonen J, Hirvonen H, Lena CW, Alitalo K: FGFR-4, a novel acidic fibroblast growth factor receptor with a distinct expression pattern. EMBO J **10**: 1347-1354, 1991