Low Insulin-Like Growth Factor Binding Protein-2 Expression Is Responsible for Increased Insulin Receptor Substrate-1 Phosphorylation in Mesangial Cells from Mice Susceptible to Glomerulosclerosis

Alessia Fornoni, Steven A. Rosenzweig, Oliver Lenz, Ana Rivera, Gary E. Striker, and Sharon J. Elliot

Division of Nephrology and Hypertension (A.F., O.L., S.J.E.) and Vascular Biology Institute (O.L., A.R., G.E.S., S.J.E.), University of Miami Miller School of Medicine, Miami, Florida 33136; and Department of Cell and Molecular Pharmacology and Experimental Therapeutics and Hollings Cancer Center (S.A.R.), Medical University of South Carolina, Charleston, South Carolina 29425

Mesangial cells (MC) isolated from glomerulosclerosis-prone ragged, olygosyndactilism, pintail (ROP) mice retain a stable phenotype after exposure to elevated glucose concentrations, whereas MC from glomerulosclerosis-resistant C57BL/6 (C) mice do not. In NOD and db/db mice, the stable phenotype induced by diabetes consists of autocrine activation of the IGF-I signaling pathway. We hypothesized that high ambient glucose activates the IGF-I pathway in ROP but not in C MC. MC were propagated in either 6 or 25 mM glucose. Isolated murine glomeruli were used to confirm in vitro experiments.

25 mM glucose induced increased insulin receptor substrate (IRS)-1 phosphorylation in ROP but not C MC. However, IGF-I, IGF-I receptor, and IRS-1 protein levels were induced by exposure to 25 mM glucose in both cell lines. This occurred without a change in IGF-I binding sites, suggesting a role for IGF binding protein (IGFBP). ROP MC and glomeruli expressed less IGFBP-2 than C MC and glomeruli. Addition of exogenous IGFBP-2 partially blunted the effect of 25 mM glucose on IRS-1 phosphorylation in ROP MC. Renal biopsies from patients with diabetic nephropathy also showed markedly decreased IGFBP-2 expression when compared with patients without nephropathy. In summary, glucose induces IRS-1 phosphorylation in MC isolated from ROP mice susceptible to glomerulosclerosis. IGFBP-2 expression was low in ROP MC and glomeruli from patients with diabetic nephropathy, suggesting that this may represent a new marker of susceptibility to diabetic nephropathy. Finally, addition of exogenous IGFBP-2 in ROP MC partially blunted the effect of high glucose on IRS-1 phosphorylation and might have a protective role. (Endocrinology 147: 3547–3554, 2006)

DIABETIC NEPHROPATHY, a major complication of both type 1 and type 2 diabetes mellitus, is the most common single cause of end stage renal disease in the United States (1). Among patients with diabetes, only 20–30% develop progressive nephropathy, and the severity and the rate of progression of the lesions varies widely. We have identified mouse models with different propensities to develop glomerulosclerosis: a sclerosis-prone mouse that develops rapidly progressing glomerulosclerosis [ragged, olygosyndactilism, pintail (ROP) mice] and sclerosis-resistant mice [C57BL/6 (C) mice and B6SJL] (2–4). We have shown that the genetic background is a major determinant of the severity of diabetic glomerulosclerosis in these models (2). In addition, we have shown that mesangial cells (MC) isolated from ROP mice retain a stable phenotype in vitro after exposure to high ambient glucose (5). MC have been postulated to be a major contributor to the sclerotic lesion in glomeruli of diabetic patients with nephropathy (6). Using NOD mice, a model of type 1 diabetes with nephropathy, we have shown that isolated MC undergo stable phenotypic changes after the onset of diabetes, and that these changes mimic the alterations in extracellular matrix turnover found in vivo (7–9). The changes include modifications in IGF-I synthesis (8) and IGF-I pathway activation (9). Similarly, MC isolated from a mouse model of type 2 diabetes (db/db), exhibit higher IGF-I receptor expression and activation than MC from their control littermates (10, 11), suggesting that activation of the IGF-I pathway in MC also occurs in type 2 diabetes. Autocrine activation of the IGF-I system has been reported in other microvascular complications such as diabetic retinopathy and choroidal neovascularization (12–14). The IGF-I system is a complex system regulated by the interaction of IGF-I with several IGF binding proteins (IGFBPs) (15–18). The net result of this complex interaction between IGF-I, IGFBP, and IGF-I receptor results in increased or decreased insulin receptor substrate (IRS)-1 phosphorylation. IRS-1 is the main IRS activated by IGF-I and responsible for most of the IGF-I intracellular signaling (18–20). The principal IGFBP produced by MC is IGFBP-2 (21). IGFBP-2 binds IGF-I (22) through a specific binding domain (23), and it is involved in glucose homeostasis (24). Data from several in vivo and in vitro models suggest that IGFBP-2 primarily inhibits IGF actions and that local overexpression of IGFBP-2 in the kidney might prevent IGF-I-induced lesions (25).

Here, we investigated whether the different response to
high ambient glucose of ROP and C MC in vitro could be related to an altered autocrine activation of the IGF-I pathway reflected by increased IRS-1 phosphorylation in ROP MC. In particular, we studied baseline IGF-I expression, IGF-I receptor expression and activation, and IRS-1 production and phosphorylation in both low and high ambient glucose. IGFBP-2 expression was tested both in vitro in ROP and C MC and in vivo in microdissected glomeruli and renal biopsy from patients with and without diabetic nephropathy. Finally, we treated MC with exogenous IGFBP-2 to study whether IRS-1 phosphorylation could be regulated.

Materials and Methods

Cell culture and characterization

MC were isolated and characterized from microdissected glomeruli of C and ROP/Lee-+ EsIb/EsIa (ROP) mice (26). Two cell lines for each strain, isolated from different mice, were propagated and used to perform experiments. Cells were maintained in DMEM/F12 (3:1) medium (Invitrogen Corp., Carlsbad, CA) supplemented with 20% fetal bovine serum (FBS; Invitrogen Corp.), 1 mg/ml glucose (Biosource, Camarillo, CA), 0.075% Na2HCO3 (Invitrogen Corp.), 100 μg/ml penicillin-streptomycin (100 U/ml) (Biosource), and trace elements (Biosource). The culture medium glucose concentration was 6.6 mM. MC were propagated in fibronectin-coated Nunc flasks. For experiments with high ambient glucose, MC were grown for 5 wk in 6 or 25 mM glucose, MC were plated into fibronectin-coated six-well plates. MC mRNA was isolated and characterization from microdissected glomeruli and renal biopsy from patients with and without diabetic nephropathy. Preliminary experiment with a mannitol control showed that osmolarity did not affect IGF-I, IGFBP-2, or IGF-I receptor protein level.

Induction of diabetes

Female C and ROP mice were obtained from The Jackson Laboratory (Bar Harbor, ME), and diabetes was induced at 8 wk of age using streptozocin as previously described (2). After 12 wk of follow-up without insulin treatment, groups of four to six mice were killed (according to the guidelines approved by the University of Miami School of Medicine and by the Institution Animal Care and Use Committee) and 100 glomeruli microdissected for RT-PCR.

RT-PCR and competitive PCR

MC were plated into fibronectin-coated six-well plates. MC mRNA was extracted with Tri-Reagent (MRC, Inc.). For in vitro experiments, RNA extracted from 100 microdissected glomeruli was used. RT and standard PCR were performed as previously described. The following set of primers have been used: 5′-ATGGGAGATGGTGGAGAGGTA-3′ and 5′-CAGTCTATGGTGGAGAGGTA-3′ for IGFBP-2; 5′-AAGCAT- TACGAGCTGAAG-3′ and 5′-CAGTCTATGGTGAGACTGTA-3′ for IGF-I receptor (a gift from Dr. Yingcai Wang, Molecular and Cellular Pharmacology, University of Miami Miller School of Medicine, Miami, FL). Primers for IGF-I and GAPDH were previously published (26, 27).

Transient transfection with an IGF-I receptor promoter construct

MC at a cell density of 40,000 cells per square centimeter in 24-well plates were transfected using TransFast Transfection Reagent (Promega Corp., Madison, WI) with 0.25 μg of an IGF-I receptor promoter/luciferase construct (gift from Dr. H. Werner, Department of Clinical Biochemistry, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel) (28) and 0.25 μg of a PRSV β-galactosidase reporter construct (gift from Dr. J. Segars, United States Uniformed Health Services, Bethesda, MD) as previously described (11). Briefly, MC were washed with PBS and the media was replaced with 200 μl of DMEM/F12 (3:1) with TransFast Transfection reagent and the constructs. After incubation for 1 h at 37 C, sufficient media supplemented with 0.1% FBS and either 6 or 25 mM glucose was added to result in a total volume of 1 ml. MC were harvested at 24 h with 100 μl of cell culture lysis reagent (Promega) and assayed for luciferase activity using the luciferase assay system (Promega) as described by the manufacturer.

Western blots and ligand blots

Proteins were purified from MC plated into fibronectin-coated six-well plates as previously described (9). Total protein extracted from equal amount of cells showed that MC exposed to 25 mM glucose produced 3- to 4-fold more protein than MC exposed to 6 mM glucose, as previously described (29). Because glucose increases translation of several genes (30), we loaded gels with an amount of protein normalized for cell number rather than for total protein. Staining for actin using a polyclonal rabbit antimiouse antibody (Abcam, Cambridge, MA) was used to control for loading. Ligand blots for IGFBP-2 were performed as previously described (17). Briefly, supernatants from ROP and C MC cells were subjected to trichloroacetic acid precipitation, and electrophoresed on 12% sodium dodecyl sulfate nonreducing polyacrylamide gels. After transfer of protein and washes, the blots were exposed to biotinylated IGF-I and developed with chemiluminescence as described for Western blots. For immunoprecipitation studies, cell lysates were also immunoprecipitated with an antibody to IRS-1 and Western blots exposed to an antibody to a phosphorylated tyrosine (Py-20) as previously described (11).

IGF-I enzyme immunoassay

Cells were plated in 75-cm² flasks. After 4 d of culture in 20% FBS, the media was replaced with media containing 0.1% BSA for a washout period of 24 h, then replaced with fresh 0.1% BSA media. Supernatants were collected after 48 h and processed as previously described (27). Supernatants were acidified to dissociate IGF-I from binding proteins and concentrated by manual reverse-phase chromatography onto Sep-Pak C18 cartridges (Millipore, Milford, MA). Acetic acid solution followed by Sep Pak separation removes binding proteins from MC medium as previously described (27). Supernatants were subsequently analyzed for the presence of IGF-I by competitive binding enzyme immunoassay according to the manufacturer suggestions (Diagnostic Systems Laboratories, Inc., Webster, TX).

IGF-I binding studies

Cells were plated in 24-well plates, and binding experiments on intact cells were performed at a cell density of 80% as previously described (31). Briefly, cell monolayers were washed twice with HEPES binding buffer (HBB) and incubated with HBB containing labeled [125I]-IGF-I and unlabelled IGF-I. At the end of the overnight incubation time at 4 °C, the test medium was removed, monolayers were washed three times with ice cold HBB, solubilized in 0.1% sodium dodecyl sulfate, and the cell-associated radioactivity was determined.

Immunofluorescence microscopy for IGFBP-2

Human kidney biopsies from five patients with diabetic nephropathy and five patients without diabetic nephropathy who underwent nephrectomy for renal cell carcinoma were studied. After standard deparaffinization and rehydration, antigen retrieval was performed with citrate buffer (pH 6) in a pressure boiler for 15 min. After blocking for 30 min at room temperature with 10% goat serum, samples were incubated overnight with primary goat anti-IGFBP-2 at the concentration of 1:100 in 1% BSA (Santa Cruz Biotechnology, Santa Cruz, CA). After three washes in PBS, the slides were incubated with donkey-antigoat IgG (FITC) (Serotec, Raleigh, NC) at 1:500 for 1 h. Incubation with an irrelevant antibody instead of primary antibody was used as negative control. Semiquantitative analysis was performed with Metamorph imaging software, and a fluorescence intensity score between 0 and 5 was assigned by a blinded investigator to each glomerulus per section. The average score for each section was calculated.

Data analysis

Results represent the mean of three to five independent experiments performed in duplicate or triplicate. Results are expressed as mean ± sd.
Results were compared using unpaired t test or ANOVA. When ANOVA showed a statistically significant difference, a group-by-group comparison was performed using a t test with a Tukey’s correction for multiple comparisons. Statistical significance was set at \( P < 0.05 \). Binding kinetics and Scatchard analysis were calculated with the use of Prism Software; the number of binding sites per cells and equilibrium dissociation constant (K_d) were calculated with the LIGAND software.

**Results**

Glucose increases IRS-1 phosphorylation in ROP but not C MC

Exposure to 25 mM glucose induces IRS-1 phosphorylation in ROP MC but not in C MC (Fig. 1, A and C). This may be a partial consequence of a greater increase in IRS-1 protein level in ROP MC when compared with C MC (Fig. 1, A and B).

**ROP MC have higher IGF-I mRNA expression and protein level than C MC**

ROP MC have 2- to 3-fold higher IGF-I mRNA expression and protein level than C MC. The ratio of IGF-I to GAPDH was 3.74 ± 0.36 in ROP cells and 1.15 ± 0.14 in C cells grown in 6 mM glucose (R6 and C6, \( P < 0.01 \); Fig. 2A). In addition, the IGF-I peptide detected in the supernatant of ROP MC was 1.47 ± 0.62, whereas, in the supernatant of C MC, it was 0.56 ± 0.20 ng per million cells (\( P < 0.01 \); Fig. 2). The induction of the IGF-I peptide by glucose was similar in ROP MC and C MC; the IGF-I concentration in ROP MC cultured in 25 mM glucose was higher than the one observed in C MC (\( P < 0.05 \); Fig. 2B).

**ROP MC have less IGF-I receptor mRNA expression and peptide level than C MC**

ROP MC had lower IGF-I receptor mRNA expression and protein levels than C MC when cultured in 6 mM glucose (Fig. 3, A and B).

**Glucose increases IGF-I receptor protein but not mRNA expression in both ROP and C MC**

Glucose increased IGF-I receptor protein levels in both cell lines, but to a greater extent in ROP MC (Fig. 3B). The increase in IGF-I receptor protein level by glucose is a post-transcriptional event, consistent with the observation that glucose did not stimulate IGF-I receptor mRNA expression in either ROP or C MC (Fig. 3A). Similarly, glucose did not activate an IGF-I receptor luciferase reporter construct when transiently transfected in ROP or C MC grown in 25 mM glucose when compared with 6 mM glucose (38450 ± 7530 vs. 26875 ± 3480 Luc/β-gal activity in ROP MC, 24872 ± 6486 vs. 31358 ± 2846 Luc/β-gal activity in C MC, \( P > 0.05 \)). As a net result, IGF-I receptor expression was lower in ROP MC than C MC grown in 25 mM glucose but higher when cultured in 25 mM glucose.

The number of binding sites for IGF-I does not differ between ROP and C MC

There were a similar number of binding sites for IGF-I when ROP MC were compared with C MC. Specifically, ROP MC had 213.9 ± 21.50 sites per cell, whereas C MC had 235.6 ± 28.12 (\( P > 0.05 \); Fig. 4). The IGF-I K_d was identical in the two cell lines, with a mean K_d of 6.8 × 10^{-10} in C cells and 7.1 × 10^{-10} in ROP cells. The equal number of binding sites but different expression of IGF-I receptor suggested a role for IGFBPs in competing with IGF-I receptors for IGF-I peptide.

ROP MC and glomeruli are characterized by very low IGFBP-2 mRNA expression

We analyzed IGFBP-2, the most abundant BP produced by MC. ROP glomeruli express less IGFBP-2 mRNA than glomeruli. Fig. 1. Glucose increases IRS-1 phosphorylation in ROP but not C MC. A, Representative Western blot of IRS-1 and phosphorylated IRS-1 after immunoprecipitation with an antibody against a phosphorylated tyrosine (Py-20) in ROP (R) and C MC (C) grown in either 6 or 25 mM glucose (R6 and B6 for ROP and B6 MC grown in 6 mM glucose, R25 and B25 for ROP and B6 MC grown in 25 mM glucose). Actin was used as control for the amount of protein loaded. IRS-1 phosphorylation occurs solely in ROP MC after exposure to 25 mM glucose. B, Bar graph of IRS-1 protein expression from three independent experiments in ROP (black bars) and C (white bars) MC after exposure to 6 and 25 mM glucose. C, Bar graph from three independent experiments of IRS-1 phosphorylation in ROP (black bars) and C (white bars) MC after exposure to 6 and 25 mM glucose. *, \( P < 0.05 \); **, \( P < 0.01 \).
MERULI FROM C MICE (FIG. 5A). SIMILARLY, ROP MC ARE CHARACTERIZED BY LESS IGFBP-2 mRNA EXPRESSION AND PROTEIN LEVEL THAN C MC (FIG. 5, B AND C). THERE WAS NO DIFFERENCE IN IGFBP-2 mRNA EXPRESSION BETWEEN ROP AND C MICE WHEN mRNA FROM THE WHOLE KIDNEY OR LIVER WERE ANALYZED (DATA NOT SHOWN). HIGH AMBIENT GLUCOSE INCREASED IGFBP-2 PRODUCTION IN BOTH ROP AND C MC (FIG. 5, B AND C). HOWEVER, SYNTHESIS OF IGFBP-2 BY ROP MC EXPOSED TO 25 mM GLUCOSE NEVER REACHED THAT OF C MC EXPOSED TO THE SAME GLUCOSE CONCENTRATIONS. THUS, ROP MC ARE CHARACTERIZED BY A RELATIVE IGFBP-2 DEFICIENCY WHEN COMPARED WITH C MC.

IGFBP-2 PROTEIN LEVEL IS MARKEDLY REDUCED IN GLomeruli FROM DIABETIC PATIENTS WITH NEPHROPATHY

We performed immunofluorescence staining for IGFBP-2 on paraffin-embedded kidney biopsies from five patients with diabetic nephropathy and five patients without diabetic nephropathy who underwent nephrectomy for renal cell carcinoma. IGFBP-2 staining was predominantly localized in glomeruli as well as afferent and efferent arterioles. We found a marked down-regulation of IGFBP-2 staining in glomeruli from patients with nephropathy when compared with patients without nephropathy (Fig. 6, mean fluorescence intensity score 1.57 ± 0.24 vs. 3.56 ± 0.22, respectively).

ADMINISTRATION OF EXOGENOUS IGFBP-2 IN ROP MC DECREASES IRS-1 PHOSPHORYLATION

Because ROP cells expressed less IGFBP-2 than C MC, we hypothesized that the decreased IGFBP-2 expression could account for increased IRS-1 phosphorylation. To test this hypothesis, we treated ROP cells with exogenous IGFBP-2 (10 nm) and found that the effect of 25 mM glucose on IRS-1 phosphorylation in ROP cells was partially blunted (Fig. 7).

DISCUSSION

Progressive diabetic nephropathy occurs in 20–30% of patients with diabetes (1). Because the positive predictive value of microalbuminuria is limited (32, 33), there is a need for the identification of other markers of progression. For the identification of such markers, research on pathways that are thought to contribute to pathogenetic mechanisms for disease progression may provide leads enabling one to predict progression and understand the pathogenesis of the disease. ROP and C6 strains respond differently to streptozocin-induced diabetes in vivo (2) and to glucose in vitro (5). In
addition, the IGF-I signaling pathway is up-regulated in the NOD model of type I diabetes both in vivo (34, 35) and in vitro (8), as well as in type 2 diabetes (11). Therefore, we hypothesized that glucose and diabetes activate the IGF-I pathway in MC and glomeruli of susceptible ROP mice, but not resistant C mice.

We found that high glucose concentrations (25 mM) induced IRS-1 phosphorylation in ROP MC but not in C MC, suggesting that the IGF-I pathway is activated by glucose only in MC from sclerosis-prone ROP mice. Given the fact that the IGF-I pathway is activated in other models of type 1 and type 2 diabetes (8, 34, 35), our data on ROP MC exposed to glucose suggests a role for hyperglycemia in the autocrine activation of the IGF-I system observed in diabetes. We analyzed the expression of IGF-I, IGF-I receptor, and IGFBP-2 in both MC types to determine the contribution of these molecules to this phenomenon. In particular, we hypothesized that IGFBP-2 deficiency was responsible for increased IRS-1 phosphorylation in ROP MC.

We observed that ROP MC express 2- to 3-fold higher IGF-I mRNA and protein levels than C MC after chronic exposure to elevated glucose concentrations. Preliminary experiments failed to identify an effect on IGF-I protein and mRNA expression after short-term (48 h) exposure to 25 mM glucose (data not shown). Glucose increased IGF-I protein levels in both cell types to a similar extent, resulting in a significantly higher production of IGF-I by ROP cells than C MC. Because IGF-I down-regulates IGF-I receptor expression in vascular smooth muscle cells (36), we were not surprised to find that C MC expressed more IGF-I receptor mRNA and protein than ROP MC. However, when cultured in 25 mM glucose, it appears that there was a disruption in the ability of IGF-I to down-regulate its receptor because both IGF-I and IGF-I receptor protein levels were increased.

The effect of glucose on IGF-I receptor was likely a post-transcriptional event, because glucose did not alter IGF-I receptor mRNA expression or IGF-I receptor promoter activation under the same experimental conditions. Our findings are in accordance with previous data showing that glucose can activate protein translation as a general phenomenon in yeast (29) or increase the translational efficiency of specific molecules such as macrophage CD36 via ribosomal reinitiation (30). Our data on increased IGF-I production by elevated glucose concentrations may seem contradictory to the current clinical studies showing that treat-
ment with recombinant IGF-I may improve insulin resistance and neuropathy (37, 38). Thus, the endocrine and autocrine actions of IGF-I may have to be considered separately.

Competition studies between recombinant IGF-I and radiolabeled IGF-I peptide have been used as an indirect measurement of the number of IGF-I receptors (31). However, we found that IGF-I binding sites were identical in ROP and C MC despite a difference in IGF-I receptor protein and mRNA expression. Therefore we hypothesized that the equal number of binding sites in our model was the result of IGFBPs binding to soluble IGF-I, as previously described (39). To investigate this possibility, we measured IGFBP-2, the most abundant IGFBP expressed by MC (21) and found that ROP MC and glomeruli express less IGFBP-2 than C MC and glomeruli. Thus, the combination of less IGFBP-2 and more IGF-I in ROP MC may account for the apparent equal number of binding sites on the cell surface despite higher IGF-I receptor expression in C mice. We found both membrane-bound (40) and extracellular matrix-bound (15) IGFBP-2 in our model (data not shown), consistent with IGFBP-2 binding to IGF-I in solution preventing IGF-I from binding to the IGF-I receptor. However, based on immunoblot analysis, we have not observed an increase in IGFBP-2 proteolysis in either MC type (data not shown), although this has been suggested by others (41).

Exposure to 25 mM glucose led to a higher increase in IGF-I receptor than IGFBP-2 protein levels in ROP MC. Thus, we hypothesized that an imbalance between IGFBP-2 and IGF-I receptor in favor of the IGF-I receptor facilitates IGF-I downstream activation in ROP MC. Consequently, we expected that glucose would increase the activation of downstream events to a larger extent in ROP MC than in C MC. Indeed we found that, after exposure to 25 mM glucose, IRS-1 phosphorylation occurred only in ROP MC. This difference may be responsible for the different response to high ambient glucose of ROP and C MC, as we previously described (5). With the goal to add clinical relevance to our findings, we tested IGFBP-2 expression by immunostaining using kidney biopsies from patients with and without diabetic nephropathy. We found a profound down-regulation of IGFBP-2 immunostaining in glomeruli from patients with nephropathy when compared with unaffected glomeruli. A recent study suggests that modulation of IGFBP-2 expression may occur through modulation of AKT phosphorylation (42), which correlates with preliminary findings from our laboratory (data not shown).

Finally, to determine whether the low expression of IGFBP-2 in ROP MC could account for increased IRS-1 phosphorylation, we treated ROP MC with exogenous IGFBP-2.
and we found that IRS-1 phosphorylation was reduced. Although studies by others have shown that IGFBP-2 can directly affect cellular function independent of IGF binding (43), the effects of IGFBP-2 on MC physiology we have observed to date have all been the result of an attenuation of the IGF-1 interaction with the IGF-1 receptor. Thus, the effect observed in our model is most likely IGF dependent. Future studies using IGFBP mutant proteins may shed further light on the mechanism of action of IGFBP-2 in MC (44).

In conclusion, we found that glucose activated the IGF-1 pathway only in MC isolated from ROP mice susceptible to glomerulosclerosis. Our data suggest that the low expression of IGFBP-2 in ROP MC and glomeruli as well as glomeruli of patients with diabetic nephropathy may be partially responsible for the autocrine IGF-1 activation observed in susceptible individuals. Thus, IGFBP-2 expression might have a protective role in diabetic nephropathy and low IGFBP-2 expression may represent a new marker of susceptibility to diabetic nephropathy.

Acknowledgments

We thank Dr. Mariana Berho for providing kidney biopsy specimens.

References


22. Duan C, Ding J, Li Q, Tsai W, Pozios K 1999 Insulin-like growth factor binding protein 2 is a growth inhibitory protein conserved in zebrafish. Proc Natl Acad Sci USA 96:15274–15279


Endocrinology is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.