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Original Article

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Bone morphogenetic protein-7 delays podocyte injury due to high glucose

Laura De Petris[1,2], Keith A. Hruska[1,3,4], Santina Chiechio[5] and Helen Liapis[2,3]

[1]Department of Pediatrics, [2]Department of Pathology & Immunology, [3]Department of Medicine, [4]Department of Cell Biology and [5]Department of Anesthesiology, Washington University, St Louis, MO, USA

Abstract

Background. The molecular pathogenesis of diabetic glomerulosclerosis remains unknown, but recent studies suggest that podocyte damage may play a role. Bone morphogenetic protein 7 (BMP-7) is physiologically expressed in podocytes and tubular epithelial cells. Our previous studies show that BMP-7 reverses glomerular and tubulointerstitial damage in diabetic rats, but there is little known about possible effects of BMP-7 on podocytes. We postulate that high glucose may injure the podocyte by altering structural proteins such as synaptopodin and podocin. This study investigates the effect of high glucose on mouse podocytes, expression of synaptopodin and podocin under normal and high glucose and the treatment effect of BMP-7 on these molecules. Human diabetic glomeruli are studied in parallel.

Methods. Conditionally immortalized mouse podocytes were exposed to media containing normal (NG) or high (HG) glucose for 2 weeks. Synaptopodin, podocin and BMP-7 gene transcription and protein were assayed with real-time PCR, Western blot or immunohistochemistry, respectively. Synaptopodin and podocin mRNA and protein was evaluated using podocytes incubated in HG for 1 week, in the presence of low (10 ng/ml) and high (300 ng/ml) dose recombinant BMP-7 (rhBMP-7). Human diabetic glomeruli were excised from renal biopsies by laser capture micro-dissection (LCM) and endogenous BMP7 and synaptopodin and podocin were determined by RT-PCR and/or immunohistochemistry.

Results. Culture of podocytes in HG decreases synaptopodin, podocin and BMP-7 transcription and protein synthesis compared to NG. Treatment with rhBMP-7 restores synaptopodin and podocin mRNA and protein. Decreased BMP-7 and synaptopodin is also observed in human diabetic glomeruli both at the transcription and protein level.

Conclusions. BMP-7 may confer resistance to hyperglycaemic injury via synaptopodin and podocin suggesting novel BMP7 therapies for diabetic glomerulosclerosis.

Keywords: BMP-7; high glucose; human; mouse; laser capture microdissection; podocyte

Introduction

Diabetic nephropathy is a serious complication of hyperglycaemia and a frequent cause of end-stage renal disease (ESRD). It is estimated that there are now 150 million people with diabetes worldwide, expected to rise to 300 million by 2025 [1]. In its early stages, diabetic nephropathy is primarily a glomerular disease and recent studies propose that podocyte injury is an important component [2–4]. Podocytes are postmitotic cells composed of a cell body and primary and secondary foot processes. The morphological effects of hyperglycaemia to podocytes are: podocyte foot process effacement and decreased slit diaphragms [2,3]. Structural components of the podocyte and the slit diaphragm such as $\alpha_3\beta_1$ integrin and Nephrin are reduced [2,3]. Sustained damage of podocytes may lead to detachment of their cell body from the glomerular basement membrane (GBM) in some diabetic patients [5]. There is little known about other major podocyte structural molecules such as synaptopodin and podocin the former of which is expressed in foot processes and may have a role in progressive diabetic injury [6]. Synaptopodin is essential for the integrity of the podocyte actin cytoskeleton and controls signal-transduction pathways that influence many aspects of podocyte behaviour, including cytoskeletal dynamics [7]. Podocin is the product of NPHS2 gene and is exclusively localized in podocytes specifically at the point of the slit diaphragm insertion interacting with CD2AP and Nephrin [8]. Recent studies analysed

Correspondence to: Helen Liapis, MD, Department of Pathology & Immunology, Washington University School of Medicine, 660 S. Euclid Avenue, Campus Box 8118, Saint Louis, MO 63110-1093, USA. Email: liapis@path.wustl.edu

podocyte-specific genes in proteinuric diseases and find that among various podocyte genes the only two that had the most varied expression were synaptopodin and podocin [9]. Schmid *et al*. suggested that altered ratio of these two genes may be a useful marker to predict steroid response and thus reversible podocyte damage [9]. Many genes are altered in diabetic kidneys including growth factors and apoptotic regulators [10–12]. One such molecule is bone morphogenetic protein-7 (BMP-7), a developmental morphogen which in the mature kidney localizes to distal tubules/collecting ducts and podocytes [13,14]. Loss of endogenous BMP-7 expression occurs in diabetic rats and is associated with profibrotic effects [15–17]. In the streptozotocin diabetic model BMP-7 is reduced by 50% at 15 weeks and continues to decline further to 10% by 30 weeks [16,17]. A recent study in diabetic animals with targeted (transgenic) expression of BMP-7 in glomerular podocytes further suggests that BMP-7 may play a protective role, but the precise mechanism is unknown. One possibility is that BMP7 may inhibit the TGFβ1 activation signalling pathway in injured podocytes. TGFβ1 is shown to be locally produced by damaged podocytes and is tightly implicated in the pathogenesis of glomerulosclerosis [12,15,18]. In the BMP-7 transgene, BMP-7 prevents podocyte dropout and reduction of nephrin, indicating that endogenous BMP-7 may be a podocyte survival factor [19].

In this study we aim to investigate BMP-7, synaptopodin and podocin expression in an *in vitro* podocyte model exposing podocytes to high glucose. We treated hyperglycaemic podocytes with rhBMP-7 to examine whether BMP-7 treatment has an effect on synaptopodin and podocin. To investigate whether these molecules are altered *in vivo* in humans, we utilized diabetic glomeruli excised by laser capture microdissection (LCM) from renal biopsies.

Subject and methods

In vitro *studies*

Experiments were performed using a thermosensitive SV-40-transfected immortalized mouse podocyte cell line (gift from Peter Mundel, Mount Sinai School of Medicine, New York). Podocytes were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin. Cells were grown at 33°C and treated with 10 U/ml of mouse recombinant γ-interferon (Sigma, St Louis, MO, USA) as previously described [20]. At confluence podocytes were maintained on a bed of type I collagen at 37°C for 14 days deprived of γ-interferon to allow differentiation [20].

Podocytes were exposed to media containing either normal D-glucose concentration (5.5 mmol/l) or a high D-glucose concentration (25 mmol/l) for 14 days. In some experiments, a third group of podocytes was exposed to 5.5 mmol/l D glucose plus 19.5 mmol/l D-mannitol to control for the osmotic effects of high glucose. Exogenous BMP-7 (Curris Inc., Hopkinton, MA), 10 or 300 ng/ml or vehicle was added to podocytes exposed to high glucose for 7 days every other day. BMP7 doses were decided based on our previous work [17]. All experiments were in triplicate; cells were harvested for total RNA and protein extraction.

Human glomeruli excised by LCM

Paraffin-embedded tissue blocks from biopsies of six renal biopsies ($n = 6$) with diabetic glomerulosclerosis were randomly retrieved from the files of the Department of Pathology and Immunology at Washington University in St Louis. Biopsies were retrospectively reviewed and light microscopy, routine immunofluorescence and electron microscopy were evaluated. Controls consisted of histologically normal kidneys obtained from autopsy or patients who had nephrectomy for renal cell carcinoma. The study was approved by the Institutional Review Board (IRB 05-693). Five-micrometer thick sections were deparaffinized in xylene followed by ethanol and H&E stained in RNAase-free media. Laser microdissection was performed using a LCM microscope (PixCell II LCM System, Arcturus Engineering, Mountain View, CA). No less than 15 glomeruli were microdissected for each biopsy. After LCM, the glass slides with the microdissected tissue were coverslipped and examined by light microscopy to determine the accuracy of dissection. Total RNA was extracted immediately.

Real-time PCR

Total RNA was extracted using TRIzol Reagent™ (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. RNA concentration and purity extracted from LCM glomeruli was assed by UV spectroscopy because of the small amounts of RNA available. Average A260/A280 ratio in our LCM samples was 2.01 (1.77–2.08). An aliquot of RNA extracted from cultured podocytes was run on agarose gel. Total RNA was converted into cDNA and amplified by real-time polymerase chain reaction (PCR) in 'one-step' reaction (Qiagen, OneStep RT-PCR, Germantown, MD, USA) as we previously described [21]. The SYBR Green was used as fluorogenic probe system. PCR kinetics and data quantification was performed with 4000^{TM} Multiplex Quantitative PCR System Software (Stratagene, La Jolla, CA). Quantification of the target gene was performed according to the standard curve method in cultured podocytes and according to the $2^{-\Delta\Delta Ct}$ method in laser-captured glomeruli. The mRNA levels were normalized to GAPDH. The primers used for RT-PCR are shown in Table 1. Experiments were in triplicate.

Table 1. Nucleotide sequences of the primers used for reverse-transcription PCR

Gene	Forward/reverse	Primer sequence 5′ to 3′	Product size (bp)
BMP-7 mouse	Forward	CACTCCCTCCTCAACCCTCGGCA	180
	Reverse	TAGAGGCATCATAGGCCAGGTGCCC	
BMP-7 human	Forward	CCAGGAGCACTTGGGCAG	242
	Reverse	GCCACCATGAAGGGCTGC	
SYNAPTOPODIN mouse	Forward	TGGACTGGTGGACATTGAAA	66
	Reverse	TTTACAACGGTCTGTGGTGA	
SYNAPTOPODIN human	Forward	GGAGGATGATGGGGCAGC	160
	Reverse	GGGTCGGAGCTGGGATAC	
PODOCIN mouse	Forward	GTGTCCAAAGCCATCCAGTT	97
	Reverse	GACCTTTCCTTCTCGTAACG	
GAPDH mouse	Forward	ACTTTGTCAAGCTCATTTCC	170
	Reverse	GTGAGGGGAGGAGTCTCAA	
GAPDH human	Forward	GAGTCCACTGCGTCTTCACCACCA	160
	Reverse	GAGTAAACGTCCCCCCTCGG	

Table 2. Demographic data and pathology in renal biopsies used for LCM

	Patient age/sex	Clinical history and presentation	Glomerular pathological findings
1	34-year-old ♀	History of type-II diabetes presents with nephrotic syndrome	LM: Kimmelstein–Wilson nodules c/w diabetes EM: Foot process effacement, thick GBM = 900 nm (normal 350 ± 50 nm)
2	27-year-old ♀	History of type-II diabetes & sarcoidosis presents with severe proteinuria	LM: Kimmelstein–Wilson nodules EM: Mesangial hypercellularity, foot process fusion, thick GBM = 550 nm
3	47-year-old ♀	History of type-II diabetes presents with severe proteinuria and increased creatinine	LM: Kimmelstein–Wilson nodules EM: Foot process effacement, thick GBM = 890 nm, Mesangial hypercellularity and sclerosis
4	52-year-old ♂	History of type-II diabetes presents with nephrotic syndrome	LM: Kimmelstein–Wilson nodules EM: Foot process effacement, mesangial sclerosis & hypercellularity, thick GBM = 1000 nm
5	60-year-old ♀	History of type-II diabetes presents with kidney mass and proteinuria	LM: Kimmelstein–Wilson nodules (adjacent to renal cell carcinoma) EM: Foot process effacement, mesangial sclerosis, thick GBM+ 780 nm
6	64-year-old ♂	History of type-II diabetes and pulmonary hypertension, presents with severe proteinuria	LM: Kimmelstein-Wilson nodules EM: Foot process effacement, thick GBM = 850 nm

Western blot analysis

Cultured podocytes were harvested at 4°C with 10 mM Tris lysis buffer containing 150 mM NaCl, 2.5 mM $Na_4P_2O_7$, 1 mM EDTA, 1 mM EGTA, 1% Triton-X100, 1 mM phenylmethyl-sulfonylfluoride, 1 g/ml leupeptin and 1 g/ml aprotinin (all from Sigma). After sonication, protein concentration was determined by Bradford method. Thirty micrograms of total protein were loaded on 8% SDS polyacrylamide gel, electrophoresed and transferred to protein-sensitive nitrocellulose membranes (Criterion blotter, Bio-Rad). Membranes were blocked in Odyssey blocker (Li-COR, Bioscience) for 1 h and anti-Synaptopodin primary antibody (Sigma, St Louis, MO; Biodesign, Sabo, ME) or anti-Podocin antibody (Sigma, St Louis, MO) was applied overnight at 4°C (1:1000 dilution in Odyssey blocking buffer (Odyssey, LI-COR Inc.), 0.1% Tween). Goat anti-rabbit antibody labelled with IRD800CW (LI-COR, Bioscience) incubated for 1 h at room temperature (dilution 1:20 000) was detected with Odyssey Infrared Fluorescence Imaging and normalized to β-actin (also to GAPDH).

Immunohistochemistry

A total of 12 renal biopsies were used to perform immunohistochemistry for BMP-7, podocin and synaptopodin expression, including six diabetic and six non-diabetic as controls (two with minimal change disease, two with acute tubular necrosis, one with IgA nephropathy and one normal kidney). All biopsies were retrospectively evaluated by light microscopy, routine immunofluorescence and electron microscopy (IRB 05-693). Demographic data are shown in Table 2. Polyclonal antibody to BMP-7 (a gift from Curris, Inc., Hopkinton, MA) and Synaptopodin (a gift from Jeff Miner at Washington University) and Podocin (purchased from Vector Laboratories, Burlington, CA) were detected by immunohistochemistry as we have described previously [22]. Briefly, BMP7 antibody (diluted to 1:300, synaptopodin 1:300 and podocin 1:50) was applied to paraffin tissue sections at 4°C overnight. Slides were rinsed in PBSX3 and signal detection was achieved using alkaline phosphatase (red colour) or peroxidase (brown colour) as a substrate (Sigma, St Louis).

Phalloidin stain

To determine whether HG induced any changes to podocyte cytoskeleton, subconfluent podocytes cultured on coverslips were fixed in 1% paraformaldehyde, permeabilized with 0.1% Triton-X100 in PBS and stained concurrently with fluorescent phalloidin (Alexa Fluor 488 phalloidin, Molecular Probes, Oregin, USA) and 4′,6-diamidino-2-phenylindole dihydrochloride (Sigma-Aldrich, St Louis, MO, USA).

Statistical analysis

We used Student's *t*-test to compare non-diabetic with diabetic glomeruli in the LCM study. In the *in vitro* study the control and treated podocytes in a time course were compared using a two-way analysis of variance (ANOVA). Results are shown as the mean ± SD. $AP < 0.05$ was considered statistically significant.

Results

Expression of synaptopodin, podocin and BMP-7 was evaluated in two experimental groups and control. Groups 1 and 2 were podocytes treated with high glucose at 1 week and 2 weeks, respectively. Control consisted of podocytes cultured in media containing normal glucose. At 1 week of high glucose exposure transcription of synaptopodin, podocin and BMP7 was down-regulated compared to control ($P < 0.05$). A further decline was observed at 2 weeks of exposure to high glucose (Figure 1A–C). Treatment with D-mannitol did not affect the transcription of these genes (synapotopodin mRNA level: NG 16.26 ± 2.15; mannitol 20.49 ± 4.20, $P > 0.05$; Podocin mRNA level: NG 0.067 ± 0.029, mannitol 0.222 ± 0.36, $P > 0.05$; BMP-7 mRNA level: NG 0.197 ± 0.09, mannitol 0.39 ± 0.20, $P > 0.05$). Exogenous BMP-7 treatment at 1 week was followed by increased synaptopodin and podocin transcription compared to vehicle (Figure 2A and 2C) ($P < 0.05$). High dose rhBMP7 was more effective for the reversal of synaptopodin. A dose difference in podocin reversal maybe due to post-translational regulation. Western blotting results were in agreement with the PCR data and showed increased and restored expression of both proteins by rhBMP-7 (Figure 2B and D). There was no difference when β-actin was used to normalize the values instead of GAPDH [23].

Renal biopsies from diabetic patients were evaluated. Patients' age ranged between 34 and 64 years; four were women and two were men. All patients had type-2 diabetes for >10 years. On light microscopy all showed advanced glomerular lesions with characteristic Kimmelsteil–Wilson nodules. Demographic data and detailed pathological findings are shown in Table 2. Routine immunofluorescence was negative for immune complexes. Electron microscopy showed GBM and mesangial thickening, diffuse foot process

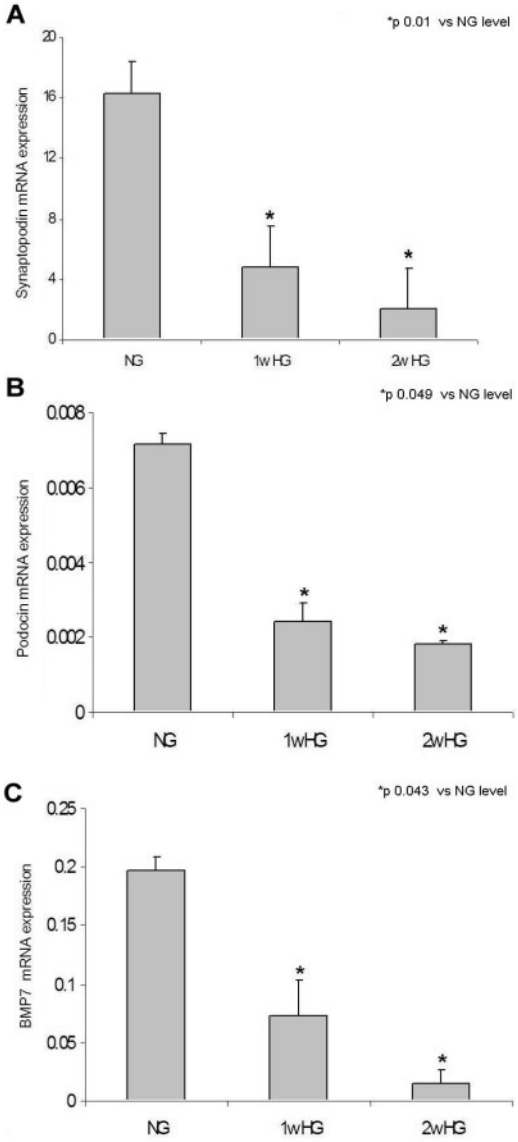

Fig. 1. Time course analysis of synaptopodin, podocin and BMP-7 mRNA expression in podocytes cultured in high glucose (HG) show decreased synaptopodin (**A**) podocin (**B**) and BMP-7 (**C**) at 1 and 2 weeks compared to podocytes cultured in normal glucose (NG) ($P < 0.05$; two-way ANOVA analysis).

effacement and degeneration of the podocyte cytoplasm (representative sample is shown in Figure 3A). Successful dissection of glomeruli by LCM was assessed by light microscopy (shown in Figure 3B and C). Diabetic glomeruli showed statistically significant decrease in synaptopodin and BMP-7 mRNA compared to normal glomeruli ($P < 0.02$) (Figure 4A and B). There was insufficient tissue to test for podocin mRNA but we performed immunohistochemistry instead. In normal kidney BMP-7 was strongly positive in cytoplasm of non-diabetic visceral epithelial cells (podocytes) and tubular epithelial cells (Figure 5A, arrows), but was absent in diabetic glomeruli with Kimmelsteil–Wilson nodules; minimal staining is noted

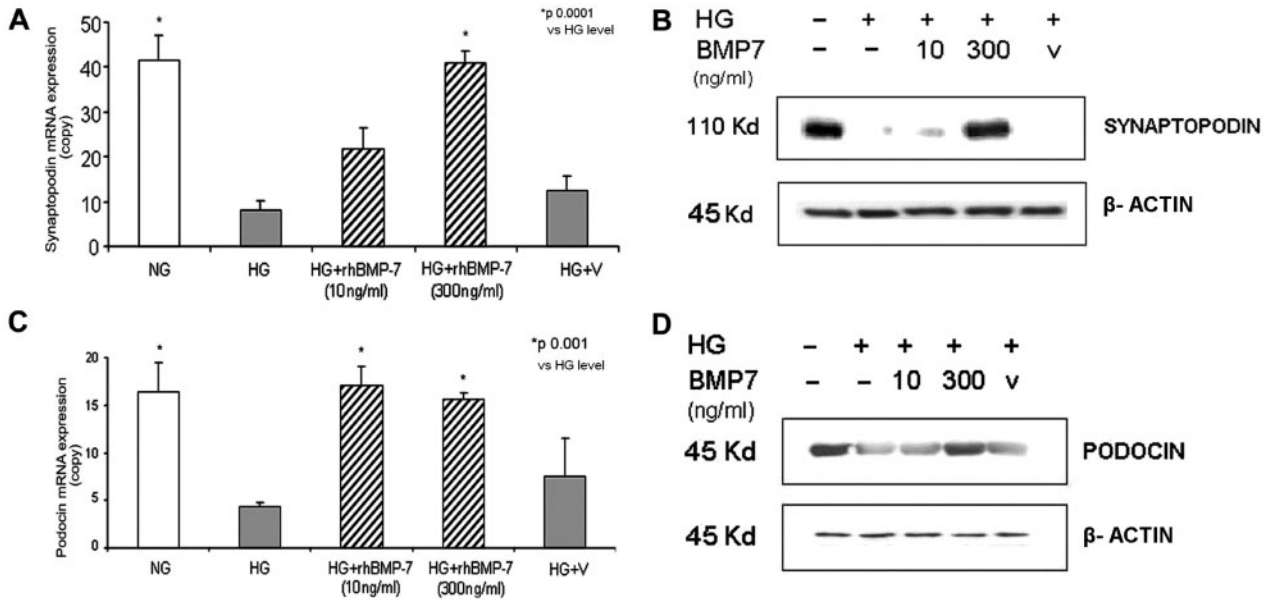

Fig. 2. Effects of recombinant human BMP-7 (rhBMP-7) on podocytes cultured in high glucose media. Podocytes cultured in HG were treated with low (10 ng/ml), high (300 ng/ml) rhBMP-7 dose or vehicle for 1 week. mRNA levels were normalized to GAPDH in the RT-PCR experiments and to β-actin in the Western blot. BMP-7 restored synaptopodin mRNA (**A**) and protein (**B**) in a dose-dependent manner ($P = 0.0001$; two-way ANOVA analysis). Podocin mRNA (**C**) and protein level (**D**) is restored with low rhBMP-7 dose ($P = 0.001$; two-way ANOVA analysis).

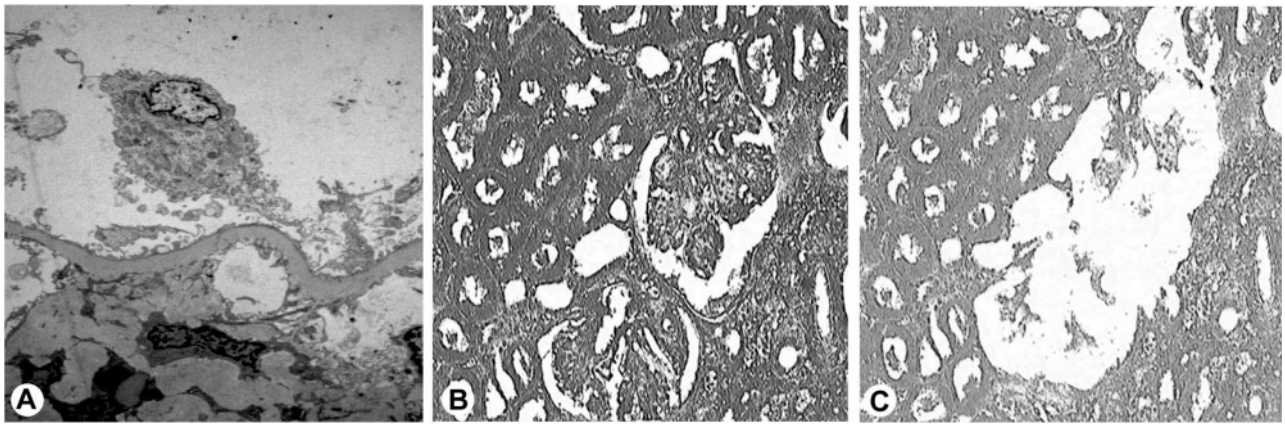

Fig. 3. RT-PCR for synaptopodin and BMP-7 in laser-captured (LCM) human glomeruli. Representative diabetic glomerulus by electron microscopy shows diffuse foot process effacement, cytoplasmic degeneration with lifting of the podocyte cytoplasm away from the GBM. GBM and mesangium are sclerotic and thickened characteristic of diabetic glomerulosclerosis (electron microscopy ×6000) (**A**). Representative sections of diabetic glomeruli used for LCM photographed before (**B**) and after microdissection (**C**). Original magnification: ×20.

in tubular epithelial cells (Figure 5B). BPM7 was mildly decreased in glomeruli with minimal change and IgA nephropathy (data not shown). Immunohistochemistry results in human diabetic glomeruli are in agreement with the RT-PCR data of LCM glomeruli and further suggest that endogenous BMP-7 may not only participate in glomerular but also in tubular epithelial injury in diabetes. Podocin and synaptopodin staining revealed diffuse linear capillary loop staining in normal glomeruli (Figure 5C and E). Podocin is markedly decreased in diabetic glomeruli (Figure 5D) and synaptopodin was entirely absent compared to normal (Figure 5E).

Phalloidin staining in podocytes cultured with media containing high glucose (HG) for 2 weeks maintained normal cytoskeleton compared to control (data not shown). The results are in agreement with previous studies that show preservation of the actin cytoskeleton in other cell types (mesangial cells) exposed to high glucose *in vitro* [24].

Discussion

Podocyte injury is an important aspect of progressive glomerulosclerosis in diabetic nephropathy but the

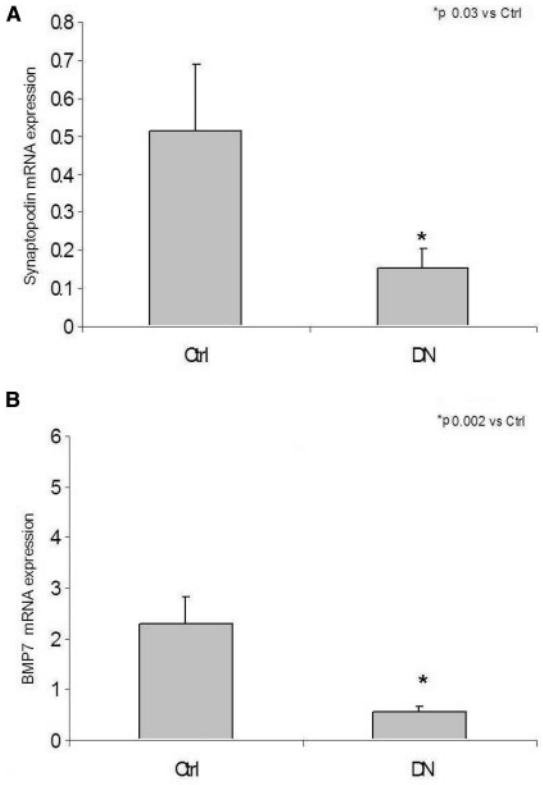

Fig. 4. RT-PCR performed from RNA extracted from LCM captured glomeruli. Synaptopodin (**A**) and BMP-7 (**B**) are down-regulated in diabetic glomeruli (DN) compared to control (Ctrl) ($P < 0.05$; Student's *t*-test).

molecular changes are poorly understood [2–8,25,26]. Cytoplasmic changes manifested by foot process effacement, hypertrophy/degeneration and subsequent detachment of the cell body from the GBM were described both in animal and human studies of diabetes as well as of other glomerular diseases now linked to podocyte malfunction, for example focal segmental glomerulosclerosis (FSGS) [27–29]. Terminally differentiated podocytes have intact foot processes and express synaptopodin and podocin [7,20]. Our *in vivo* results suggest that decreased synaptopodin may be involved in foot process effacement in early diabetic injury. Based on the results from human diabetic glomeruli it appears that there is decreased synaptopodin and podocin in late stages of diabetic glomerulosclerosis as well. However, since all renal biopsies in this study had advanced disease the results at this stage of the disease may reflect podocyte detachment and loss and/or decreased global gene transcription due to the severity of glomerulosclerosis. If the total number of podocytes declines relative to the total number of glomerular cells as suggested by studies in Pima Indians [30], decreased expression of podocyte proteins such as synaptopodin may decrease with progressive disease and may be irrespective of any changes in gene expression at the cellular level. These are limitations of this study and further work is required to address the effect of glomerulosclerosis to podocyte gene and protein expression. Synaptopodin is thought of as a differentiation podocyte marker and altered expression has been described in other glomerulopathies such as collapsing FSGS [31]. The term de-differentiation is applied to denote altered podocyte phenotype in response to injury that may precipitate capillary loop collapse and subsequent sclerosis [31–33]. Phenotypic podocyte changes are a current hot topic in podocyte disease because of potential reversibility of induced injury early in disease process.

Podocin is a major protein linked to the slit diaphragm apparatus. Recent studies demonstrate that podocin is required for efficient Nephrin signalling suggesting an important role in maintaining the integrity of the glomerular filter [34]. Podocin mutations cause proteinuria in congenital, hereditary and sporadic nephrotic syndrome [31]. However, nephrotic syndrome is not a usual manifestation of diabetes. Further studies are required to define the precise role of podocin in diabetic injury.

In our study we observed no changes in the actin cytoskeleton following HG exposure. The results are similar to previous studies by Dai *et al*. in which high-glucose-treated mesangial cells induced p38MAPK signalling. The authors suggest that this response may be an adaptive processes leading to cytoskeletal stabilization [24]. However, in other models such as the puromycin–aminoglycoside model, podocyte injury is followed by measurable cytoskeletal changes [35].

The role of BMP7 in podocytes remains unresolved, but high physiological expression in adult kidney podocytes is documented. We show here dramatic decrease of BMP7 in diabetic podocytes. BMP-7 belongs to the TGF-β superfamily, members of which bind to type I and II receptors. In contrast to TGF-β that activates Smad 2 and 3, BMP-7 activates Smad 1, 5 and 8. These activated Smads interact with the common Smad 4 and this complex translocates to the nucleus to regulate gene transcription [15]. We hypothesize that BMP-7 may restore podocyte damage due to high glucose by activating or inhibiting (perhaps TGF-β) alternative gene transcription processes. Beyond the molecules that we investigate in this study, it is likely, that hyperglycaemic injury to podocytes also involves other molecules. In fact, in addition to podocin and synaptopodin in preliminary GeneChip experiments of mouse podocytes exposed to high glucose, we find that as many as 16 000 podocyte genes have varied expression [36]. Of these 39 are statistically significantly up-regulated and an almost equal number is down-regulated to include genes not previously thought as podocyte-specific. For example, endothelial lipase (EL) was increased in hyperglycaemic podocytes. EL is known to protect cells from anti-oxidant stress by modulating lipoprotein metabolism and to promote monocyte adhesion [37]. EL in the kidney appears to be specific and a particular regulatory flanking region is responsible for kidney-specific EL expression [38]. Makino *et al*. recently reported hypoxia-inducible factor 1 alpha (HIF-1alpha)

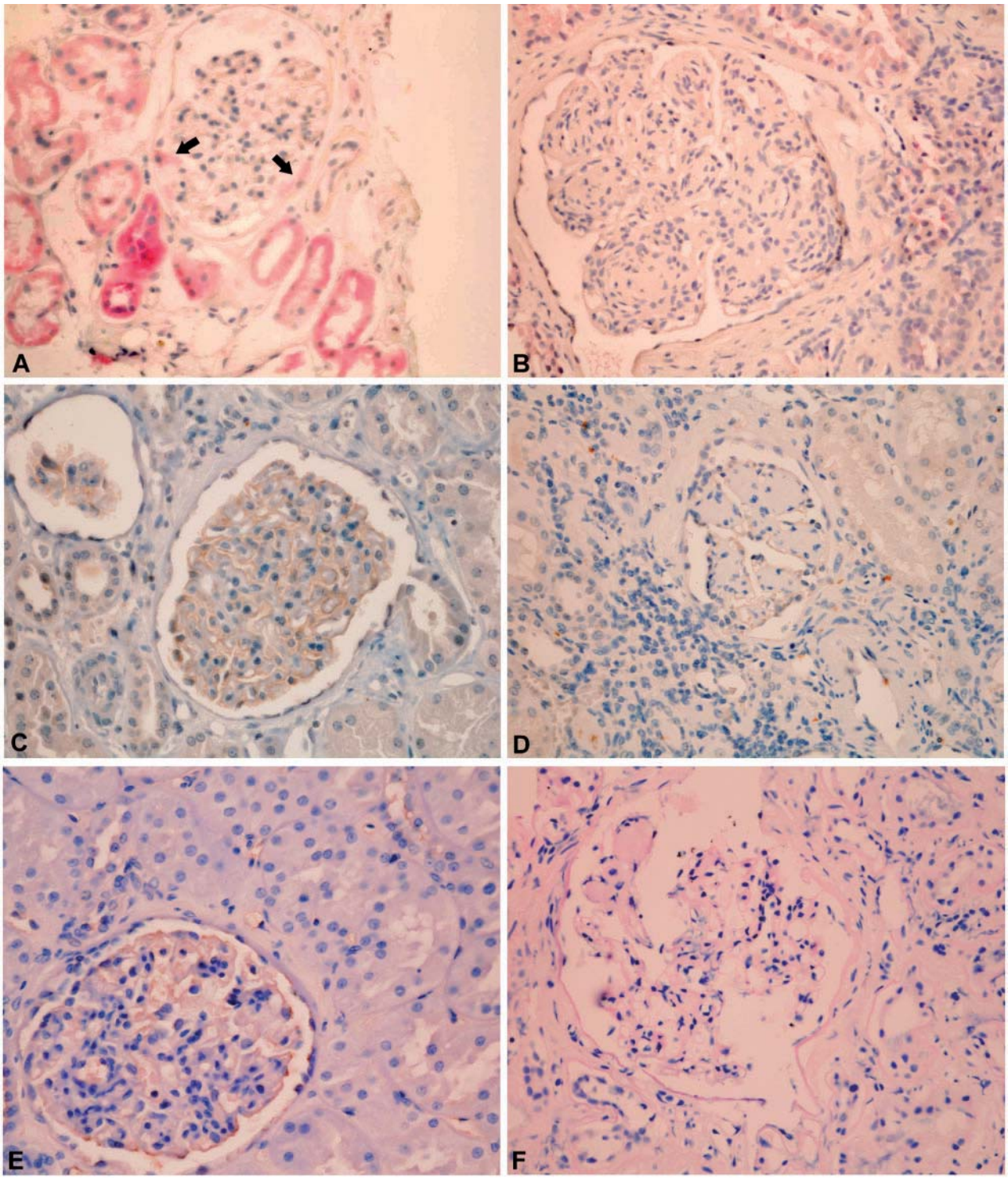

Fig. 5. Immunohistochemistry for BMP-7, Podocin and Synaptopodin in human glomeruli. BMP-7 staining is strong in podocyte cytoplasm (**A**, arrow) and tubular epithelial cells of normal kidney. In diabetic glomeruli with Kimmelsteil–Wilson nodules BMP7 is absent (**B**). Podocin is greatly reduced (**D**) and synaptopodin is absent (**F**) compared to control (**C** and **E**, respectively) (×200).

gene up-regulation in diabetic db/db mice In addition, podocyte structural genes such as dystroglycan 1 and actinin 4-alpha are up-regulated in these mice [39]. Therefore it is likely that there are more genes than those we describe in this study, involved in diabetic podocyte injury. We focused on BMP-7 guided by results in our previous studies in diabetic rats that show BMP-7 to be a novel modulator of diabetic injury that halts the pro-fibrotic effects of hyperglycaemia [15,17,40] and results by others who reported similar findings [19,41,42]. For example, the recent findings by Wang *et al.* demonstrate that maintenance of podocyte

BMP-7 levels reduces early accumulation of collagen and fibronectin in transgenic mice [19].

In conclusion, we demonstrate that high glucose decreases BMP-7 expression in podocytes and treatment of podocytes with rhBMP-7 restores podocin and synaptopodin. Our results suggest that BMP-7 may function as an autocrine podocyte differentiation factor perhaps restoring structural proteins of the foot processes such as synaptopodin and podocin. Our study suggests that these molecules may be important in human diabetic glomerulopathy, even though the sample size of human tissues is small and we only studied advanced diabetic glomerar disease. Based on this and our previous studies with BMP-7 we propose that BMP-7 may be useful in delaying diabetic glomerulosclerosis and or reversing early podocyte injury.

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Conflict of interest statement. None declared.

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