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Podocyte-derived microparticles promote proximal tubule fibrotic signaling via p38 MAPK and CD36

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ABSTRACT

Tubulointerstitial fibrosis is a hallmark of advanced diabetic kidney disease that is linked to a decline in renal function, however the pathogenic mechanisms are poorly understood. Microparticles (MPs) are 100–1000 nm vesicles shed from injured cells that are implicated in intercellular signalling. Our lab recently observed the formation of MPs from podocytes and their release into urine of animal models of type 1 and 2 diabetes and in humans with type 1 diabetes. The purpose of the present study was to examine the role of podocyte MPs in tubular epithelial cell fibrotic responses. MPs were isolated from the media of differentiated, untreated human podocytes (hPODs) and administered to cultured human proximal tubule epithelial cells (PTECs). Treatment with podocyte MPs increased p38 and Smad3 phosphorylation and expression of the extracellular matrix (ECM) proteins fibronectin and collagen type IV. MP-induced responses were attenuated by co-treatment with the p38 inhibitor SB202190. A transforming growth factor beta (TGF-β) receptor inhibitor (LY2109761) blocked MP-induced Smad3 phosphorylation and ECM protein expression but not p38 phosphorylation suggesting that these responses occurred downstream of p38. Finally, blockade of the class B scavenger receptor CD36 completely abrogated MP-mediated p38 phosphorylation, downstream Smad3 activation and fibronectin/collagen type IV induction. Taken together our results suggest that podocyte MPs interact with proximal tubule cells and induce pro-fibrotic responses. Such interactions may contribute to the development of tubular fibrosis in glomerular disease.

Introduction

Diabetic nephropathy (DN) is a frequent complication of diabetes and the leading cause of end stage kidney disease in the developed world [1]. Early DN is typified by glomerular injury including cell loss, basement membrane thickening and mesangial expansion [2]. While all glomerular cells are impacted, podocytes are particularly sensitive to diabetic stress conditions such as hyperglycemia, hydrostatic forces that accompany hyperfiltration and inflammation [2,3]. Podocyte loss is generally irreversible and is associated with increased glomerular permeability and development of albuminuria. While the glomerulus is widely recognized as the primary site of injury in DN, tubular injury is also prominent. Tubular hypertrophy and interstitial inflammation are seen early in the course of DN [4–6] and with disease progression tubular atrophy and interstitial fibrosis develop in concert with declining renal function [5,7]. Cross-talk between podocytes and the tubular epithelium is believed to play an important role in the development of tubulointerstitial fibrosis and renal

functional decline; however, the mechanisms by which this occurs are not fully understood [8–10].

Intercellular communication is a multifaceted process that can involve direct physical contact as well as the secretion of molecular signals (ie cytokines, hormones and neurotransmitters) [11]. In addition, extracellular vesicles including exosomes and microparticles (MPs) are emerging as novel vectors for cell–cell communication [12,13]. MPs are small plasma membrane-derived vesicles with a diameter of 100–1000 nm which carry a variety of proteins, lipids, mRNA and miRNA arising from the cell of origin [13,14]. MPs have been implicated in a host of physiological and pathological processes. In this regard we, and others, showed that endothelial MPs induce pro-inflammatory and pro-oxidative responses in endothelial cells and impair vascular reactivity in isolated vessels [15–19]. The mechanisms by which MPs achieve their effects are not fully understood but may involve transfer of proteins or nucleic acids, immune modulation, release of

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➕ Supplemental data for this article can be accessed here.

free radicals, or cell surface interactions and receptor activation (reviewed in [13,20–23]).

Recently, our lab observed the formation of podocyte MPs in response to diabetic stress conditions [24]. These podocyte MPs are released into the urine with levels increased in experimental and human diabetes [24,25]. However, whether podocyte MPs play a role in podocyte-tubular cross-talk has not yet been examined. In the present study, we investigated the role of podocyte MPs in proximal tubules epithelial cell fibrotic responses and determined molecular mechanisms underlying this process.

Materials and methods

hPOD cell culture

A conditionally immortalized human podocyte (hPOD) cell line was obtained with permission from Moin Saleem (University of Bristol, Bristol, UK) and cultured using the methods described with modification [24,26]. Briefly, cells were grown on collagen I–coated culture plates (0.1 mg/ml; Sigma-Aldrich, St Louis, MO) in RPMI-1640 medium supplemented with vesicle-free 10% FBS (Invitrogen, Carlsbad, CA), and penicillin-streptomycin solution (1:100; Invitrogen). Podocytes were propagated at 33°C in the presence of 10 U/ml recombinant human γ-IFN (Invitrogen). For induction of podocyte differentiation, cells were maintained at 37°C for 14 days in the absence of γ-IFN. Approximately 25 ug of MPs were collected from 1×10^7 podocytes per 24 hours.

Microparticle and exosome isolation

Podocyte MPs were isolated from the media of cultured hPODs as described previously [27]. Media were centrifuged at 2,500 × g for 10 minutes and MPs were isolated from resultant supernatant by centrifugation at 20,000 × g for 20 minutes at 4°C. In some experiments the resultant supernatant was centrifuged at 100,000 x g for 90 minutes at 4°C in order to prepare podocyte-derived exosomes for control experiments. The MP-containing pellet was re-suspended in sterile 1x phosphate buffered saline (PBS, treatment, nanoparticle tracking analysis) or radioimmunoprecipitation assay buffer (RIPA, Western blot analysis) consisting of 50 mM Tris pH 8.0 150 mM NaCl, 0.1% sodium dodecyl sulfate (SDS), 0.5% sodium deoxycholate, 1% Triton X-100 and a 1:10 dilution of protease inhibitor cocktail (Sigma-Aldrich, St Louis, MO, USA). Exosomes were isolated from the 20,000g supernatant by centrifugation at 100,000 x g for 90 minutes at 4°C. The exosome-containing pellet was re-suspended in RIPA buffer and stored at −80°C prior to analysis.

Nanoparticle tracking analysis

Sizing of extracellular vesicles was achieved by nanoparticle tracking analysis (NTA) using the ZetaView PMX110 Multiple Parameter Particle Tracking Analyser (Particle Metrix, Meerbusch, Germany) in size mode as described with modification [25]. Media samples were collected and diluted within the working range of the system in 1X PBS. Approximately 1 ml of sample was loaded into the fluid cell after system calibration with 105 nm and 400 nm polystyrene beads. Videos were acquired with the Zetaview software (version 8.02.28, Meerbusch, Germany) using 11 camera positions, a 2-second video length, and a camera frame rate of 15 fps at 21°C. A minimum of two video recordings were acquired per sample.

Proximal tubule cell culture and treatment

Human proximal tubule epithelial cells (PTECs) were obtained from Sciencell (Carlsbad, CA). Cells were seeded at a density of $2 \times 10^4/cm^2$ and grown on culture plates in Epithelial Cell Medium (Sciencell) supplemented with 2% FBS (Invitrogen), Epithelial cell growth supplement (EpiCGS; 1:100; Sciencell) and penicillin-streptomycin solution (1:100) according to manufacturer's instructions. PTECs were treated with 10 μg/mL of MPs for 30 mins–72 hours. In some experiments PTECs were co-treated with the p38 mitogen-activated protein kinase (MAPK) inhibitor SB203580 (10μM, obtained from Cayman Chemical, Ann Arbour, MI), LY2109761 a transforming growth factor beta (TGF-β) receptor 1 and 2 inhibitor (1μM, Cayman Chemical) or Sulfo-N-Succinimidyl Oleate (SSO) an inhibitor of CD36 (10μM, Sigma-Aldrich, St Louis, MO, USA).

Fluorescent labelling of microparticles

Podocyte MP were labelled with PKH26 as described [28]. Briefly, podocyte MPs were labelled with the red fluorescent dye PKH26 (Sigma) for 5 min at room temperature according to manufacturer's instructions. Labelled MPs were then washed twice by centrifugation (20,000 x g, 20 minutes at 4°C) and re-suspended in PBS before treatment. In concert with the above, PKH26 incubated in the absence of MPs was prepared as a negative control.

PTECs were seeded on glass coverslips and treated with podocyte MPs (10 ug/ml) for 3 hours at 37°C. PTECs were washed three times with cold PBS, fixed for 10 minutes in 4% paraformaldehyde with 0.3% Triton-X100, and washed three times in PBS. Fixed cells were then incubated with

Alexa-Fluor 594 Phalloidin (1:200 dilution, Invitrogen) to stain filamentous actin and Hoechst 33342 (1 μg/ml, Invitrogen) for nuclei labelling. Cover slips were mounted on glass slides using Dako Fluorescent Mounting Medium (Fisher Scientific, Waltham, MA) and images were captured using a Zeiss Axiskop 2 MOT (Carl Zeiss AG, Oberjochen, Germany) equipped with filter set 10 (item # 488010-9901-000, green fluorescence), 2 (488002-9901-000, blue) and 15 (488015-0000-000, red).

Western blot

PTECs and MP/exosome preparations were lysed with RIPA buffer. Protein was quantified with the DC^{TM} Protein Assay Reagent (Bio-Rad, Hercules, CA, USA). All samples were mixed in 1X Laemmli buffer (0.1% 2-mer-captoethanol, 0.0005% bromophenol blue, 10% glycerol, 2% SDS in 63 mM Tris-HCl, pH 6.8) and denatured by heating to 95°C for 5 min, separated in an SDS-polyacrylamide gel and transferred to a nitrocellulose membrane. Membranes were blocked in 5% milk in Tris-buffered-saline with 0.01% Tween (TBS-T) or 5% bovine serum albumin (BSA, Sigma-Aldrich)/TBS-T for 1 hour at room temperature with gentle shaking, incubated overnight at 4°C with primary antibody. The following primary antibodies were used: anti-CD63 (1:1000, System Biosciences, Palo Alto, CA, USA), anti-TSG101 (1:2000, System Biosciences), anti-synaptopodin (1:1000, Santa Cruz, Dallas, TX, USA), anti-fibronectin (1:3000; Sigma-Aldrich) anti-GAPDH (1:2000; Abcam, Cambridge, UK), anti-collagen type IV (1:500; Santa Cruz), both total and phosphorylated anti-p38 (1:1000), anti-ERK1/2 (1:1000), anti-c-JUN and anti-Smad3 (1:1000) (Cell signalling). Following incubation with primary antibody, membranes were washed in TBS-T and incubated with horseradish peroxidase-conjugated secondary antibodies for 1 hour (1:2000, Santa Cruz). Membranes were probed for immunoreactivity by chemiluminescence and quantification of blots was conducted by densitometry (Image J software 1.42q).

RNA isolation and real time PCR

For quantitative PCR (qPCR), RNA was extracted from isolated podocyte MPs and cultured PTECs using the Qiagen RNeasy minikit as per manufacturer's instructions. Extracted RNA was converted to cDNA using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems) with 45 ng starting material per reaction. Samples were treated with DNAse and 9 ng of cDNA was analysed using an ABI Prism 7000 Sequence Detection System with SYBR Advantage qPCR Premix (Clontech) according to manufacturer's instructions. The following primers were purchased from invitrogen: Fibronectin sense (5′- GCAAGCCCAT AGCTGAGAAG –3′), Fibronectin antisense (5′- AGATGCACTGGAGCAGGTTT –3′); GAPDH sense 5′- AGATCCCTCCAAAATCAAGT –3′) and antisense 5′- GGCAGAGATGATGACCCTT –3′). The relative quantity of fibronectin mRNA was normalized to an endogenous gene (GAPDH) and fold changes were calculated with the 2−ΔΔCt method. Melting curves of each amplified products were analysed to ensure uniform amplification of the PCR products.

Statistical analysis

Results are expressed as mean±SEM. Statistical analysis was conducted using GraphPad Prism version 5.0 (GraphPad software, San Diego, California). Parameters were evaluated by One-way ANOVA with Tukey post-test. Values of $p < 0.05$ were considered statistically significant.

Results

Characterization of podocyte MPs and interaction with PTECs

Podocyte MPs were characterized by NTA. As shown in Figure 1, podocyte MP isolates had a mean diameter of 189 ± 5 nm. MP isolates were larger and more heterogeneous than podocyte-derived exosomes isolates (Supplemental Figure 1). Podocyte MPs contained the podocyte marker synaptopodin, but lacked exosomal markers CD63 and TSG101 (Figure 1). In addition, podocyte MPs contained fibronectin mRNA and protein, albeit at much lower levels than that seen in PTEC lysates (Figure 1c,d).

Our previous studies established that podocyte MPs are detectable in urine with levels increased in diabetes [24,25]. Given its location immediately adjacent to the glomerulus, the proximal tubule represents a likely initial site of interaction for podocyte MPs. To assess whether podocyte MPs contribute to podocyte-PTEC cross-talk, we examined whether podocyte derived-MPs physically interact with PTECs. MPs were labelled with the membrane dye PKH26 and incubated in the presence of PTECs. Labelled MPs were visible on the surface of PTECs at 3 hours as 100–1000 nm fluorescent particles suggesting a physical interaction (Figure 2). In rare cases, larger aggregates were also seen. Surface interactions remained detectable up to 72 hours after treatment (not shown).

a
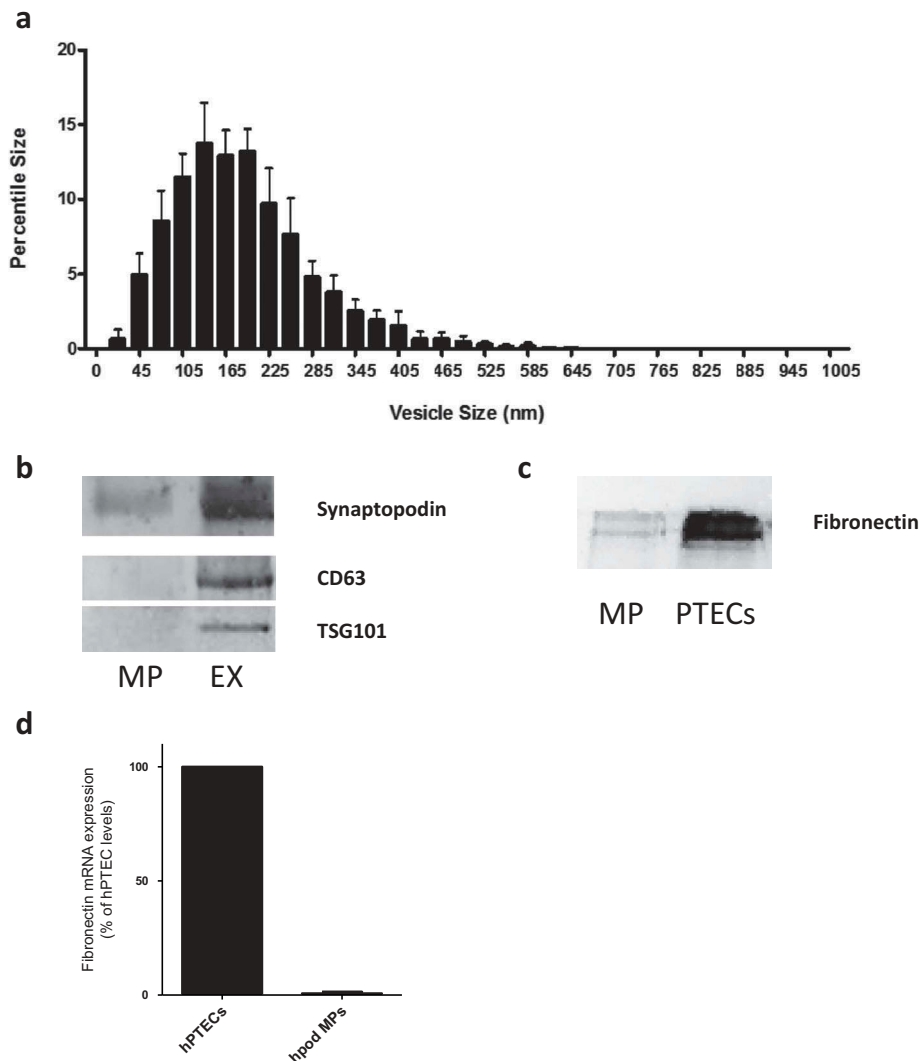

b

Synaptopodin

CD63

TSG101

MP   EX

c

Fibronectin

MP   PTECs

d

Figure 1. Characterization of podocyte MPs. (a) Size distribution of isolated podocytes MPs as determined by nanoparticle tracking analysis. (b) Western blot analysis of podocyte MP and exosome (EX) synaptopodin, CD63 and TSG101 levels. (c) Western blot analysis of fibronectin protein in podocyte MP and proximal tubule epithelial cell (PTEC) lysates. (d) qRT-PCR of fibronectin mRNA expression in podocyte MP and proximal tubule epithelial cells.

Podocyte MPs induce expression of collagen IV and fibronectin in PTECs

To determine whether podocyte MPs play a role in PTEC pro-fibrotic responses, we assessed levels of the ECM fibronectin and collagen type IV in cultured PTECs exposed to podocyte MPs. We exposed primary human PTECs to podocyte MPs (10 μg/mL) and measured fibronectin and collagen type IV expression via Western blot analysis. While no change in fibronectin and collagen type IV expression was observed before 24 hours (not shown), we observed an ~2-fold increase in the expression of both fibronectin (Figure 3(a)) and collagen type IV (Figure 3(b)) after 72 hours. Podocyte-derived exosomes had no effect on fibronectin or collagen Type IV expression (not shown).

Podocyte MPs stimulate p38 and Smad3 activation in PTECs

The activation of MAPK and TGF-β receptor-dependent signalling pathways are associated with tubulointerstitial fibrosis in DN and other renal diseases [29–35]. We therefore examined the effects of podocyte MPs on MAPK and TGF-β signalling cascades in PTECs. As shown in Figure 4, podocyte MPs induced a robust increase in p38 MAPK activation (Figure 4 (a)). By contrast, ERK 1/2 and JNK phosphorylation were not affected by podocyte MP treatment (Figure 4 (b,c) respectively). Western blot analysis also revealed that Smad3, a downstream mediator of TGF-β receptor activation, was phosphorylated in response to podocyte MP stimulation (Figure 4(d)). After 2 hours, there were
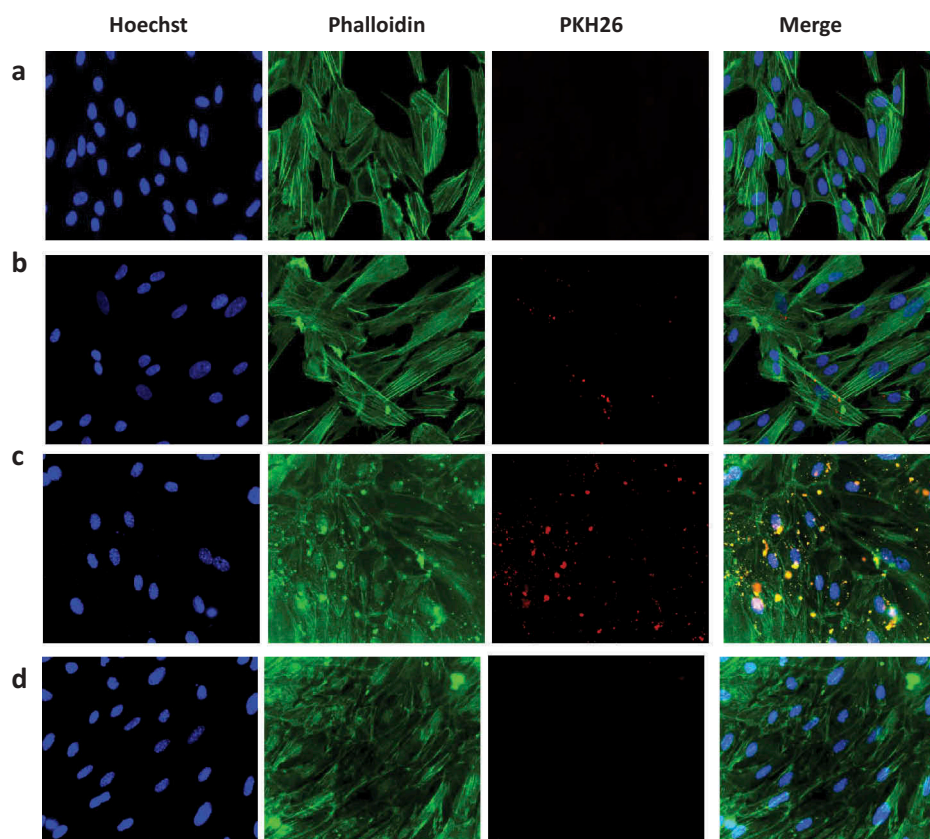
Figure 2. Characterization of podocyte MP interaction with cultured PTECs. (a) Fluorescent micrographs of unlabelled MPs incubated with human proximal tubule epithelial cells for 24 hours. (b) Fluorescent micrographs of PKH26-labelled (red) podocyte MPs incubated with human proximal tubule epithelial cells for 24 hours. (c) Fluorescent micrographs of PKH26-labelled (red) podocyte MPs incubated with human proximal tubule epithelial cells for 72 hours. (d) Cells subjected to the labelling procedure in the absence of MPs. Filamentous actin was stained with phalloidin (green) and nuclei were labelled with Hoechst 33,342 (blue). Note that PKH26, with a broad excitation and emissions spectrum is sometimes visible on both the red and the green fluorescence channel.

no differences in any kinase signalling pathway with respect to controls (not shown).

Inhibition of p38 MAPK attenuates podocyte MP-mediated induction PTEC fibrotic responses

Using an inhibitor of p38 MAPK (SB203580), we assessed the role of p38 MAPK in MP-induced PTEC responses. While SB203580 treatment alone had no effect on cultured PTECs, co-treatment with podocyte MPs and SB203580 blocked MP-mediated increases in Smad3 phosphorylation (Figure 5(a)), fibronectin expression (Figure 5(b)) and collagen type IV expression (Figure 5(c)).

Inhibition of TGF-β receptor blocks podocyte MP-mediated induction of Smad3 and ECM proteins but not p38 MAPK

To test the role of the TGF-β receptor in the effects of podocyte MPs on PTECs, we inhibited the TGF-β receptor (types 1 and 2) using LY2109761. As shown in Figure 6, LY2109761 reduced podocyte MP-mediated increases in Smad3 phosphorylation (Figure 6(a)), fibronectin expression (Figure 6(b)), and collagen type IV expression (Figure 6(c)). Notably, the physical interaction between podocyte MPs and PTECs was not altered by co-treatment with LY2109761 (results not shown) and LY2109761 did not block podocyte MP-mediated induction of p38 MAPK, suggesting that podocyte MPs do not directly activate the TGF-β receptor and that TGF-β receptor signalling is downstream of p38 MAPK.

Inhibition of CD36 blocks podocyte MP-mediated PTECs responses

CD36 is a cell surface class B scavenger receptor, expressed in PTECs and recently identified as a putative receptor for MPs in platelets and endothelial cells [36–38]. To determine whether CD36 plays a role in podocyte MP-mediated induction of PTEC fibrotic responses we inhibited CD36 using the irreversible
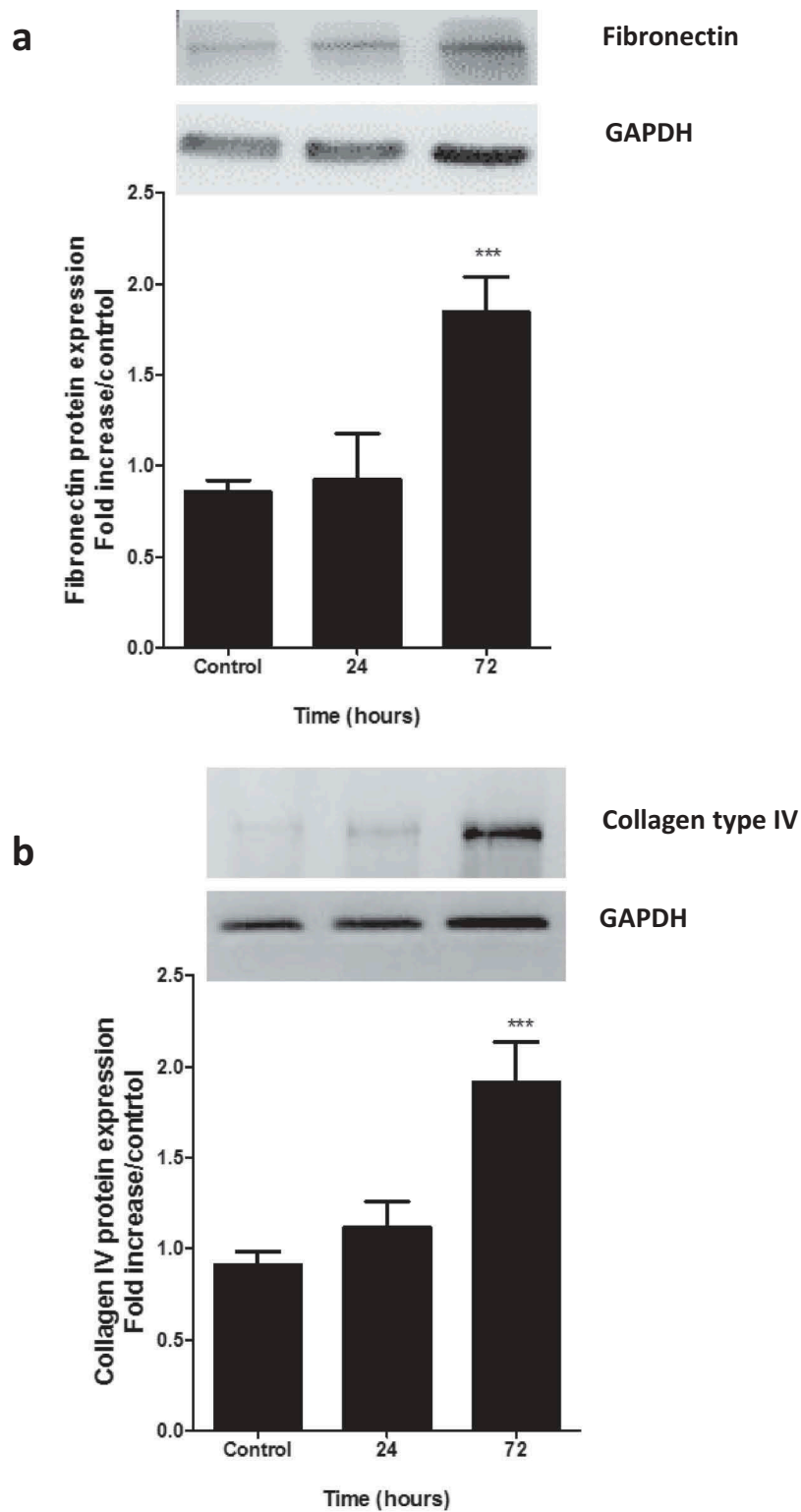
Figure 3. Effect of podocyte MPs on fibronectin (a) and collagen type-IV (b) expression in cultured PTECs. Cells were exposed to podocyte MPs (10 µg/ml) for up to 72 hours and fibronectin and collagen type-IV expression was examined by Western blot analysis. ***$P < 0.001$ vs Control, n = 6.
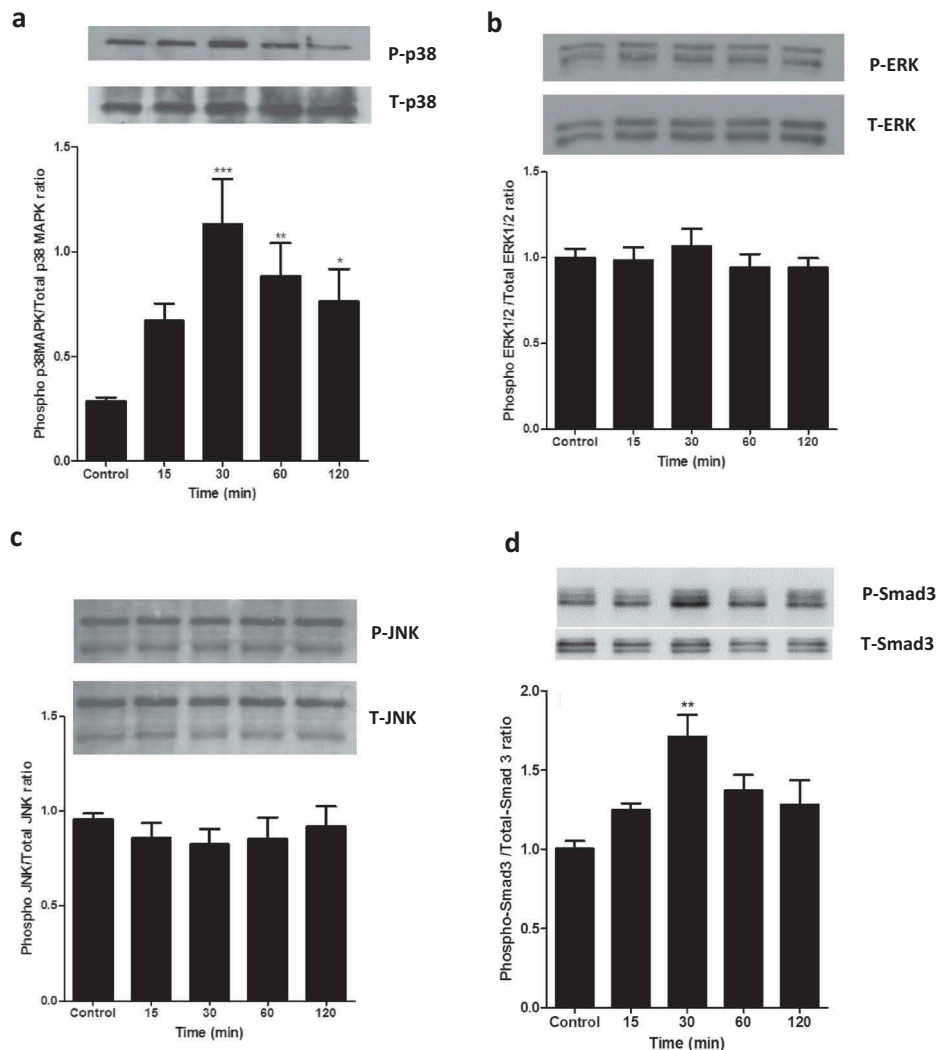
Figure 4. Effects of podocyte microparticles on p38 MAPK (a), Extracellular Signal-regulated kinase (ERK)1/2 (b), c-Jun N-terminal kinase (JNK) (c) and SMAD3 (d) in cultured human proximal tubule cells. Cells were exposed to podocyte microparticles (10ug/ml) and phosphorylation of protein kinases were examined by Western blot analysis. *P < 0.05 vs no treatment time matched control (Control), n = 4–6.

antagonist SSO. Co-treatment with SSO completely blocked MP-induced p38 MAPK phosphorylation (Figure 7(a)), Smad3 phosphorylation (Figure 7(b)) and fibronectin expression (Figure 7(c)).

Discussion

The purpose of the present study was to examine the effects of podocyte MPs on PTECs with a focus on induction of pro-fibrotic responses. The major finding is that podocyte MPs induce pro-fibrotic responses in PTECs characterised by up-regulation of fibronectin and collagen type IV expression. Podocyte MP-mediated responses were dependent upon p38 MAPK-dependent activation

of the TGF-β receptor, a process which was abrogated by antagonism of the scavenger receptor CD36. Taken together these results suggest that podocyte MPs act on PTEC CD36 to induce p38 MAPK/TGF-β receptor-dependent fibrotic responses.

Extracellular vesicles, including MPs, are emerging as important vectors for intercellular communication in a host of biological systems [13,39]. Despite this, comparatively little is known about vesicle-based signalling pathways in the kidney. One report by Eyre and colleagues showed that endothelial and monocyte-derived MPs induce pro-inflammatory responses in cultured podocytes [40]. Similarly, endothelial MPs have been reported to stimulate hypoxia inducible factor-α expression in HK-2 cells [41]. Both of these
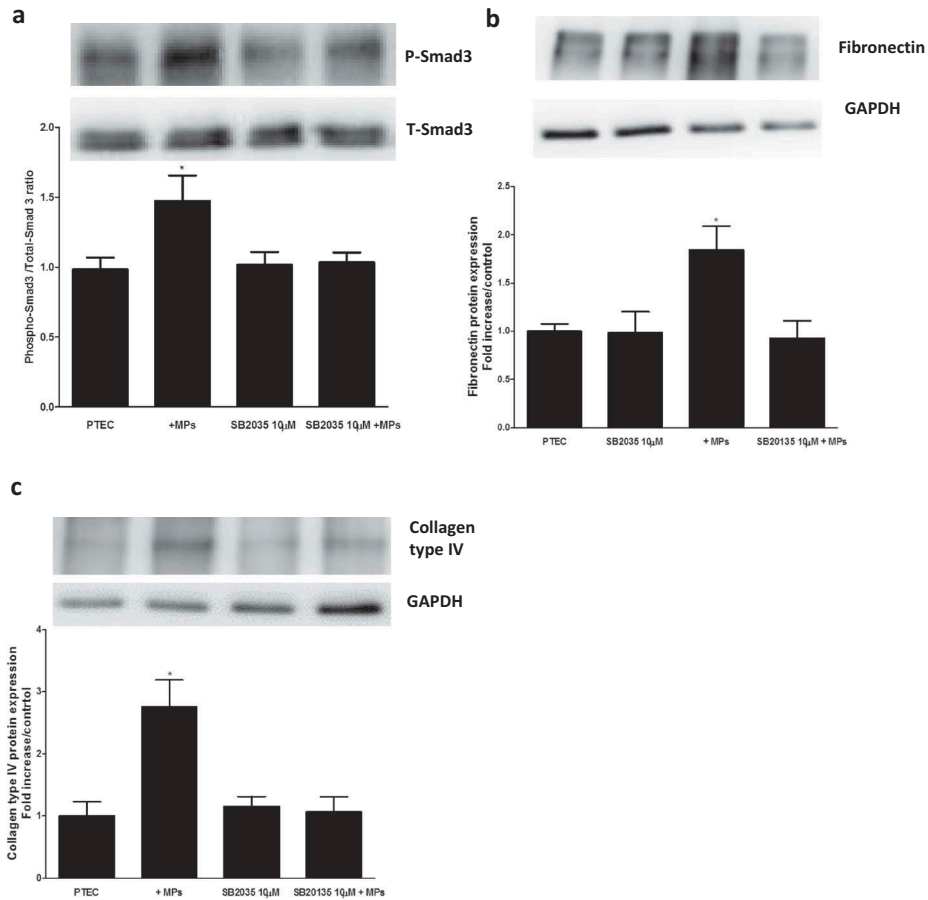
Figure 5. Role of p38 in podocyte MP-induced fibrotic responses in PTECs. PTECs were treated with podocyte MPs (10 µg/ml) for 30 minutes (Smad3 phosphorylation, A) or 72 hours (Fibronectin and Collagen type IV expression, B and C respectively) in the presence and absence of the p38 inhibitor SB203580 (SB2035, 10µM). **P < 0.001 vs Control, n = 6.

reports involved the stimulation of renal cells by extra-renal MPs. Extending from this, we report a novel mechanism by which vesicles from one renal cell population (ie podocytes) stimulated a response in a separate renal cell population (ie PTECs). The close proximity of podocytes and PTECs within the nephron suggests that this intercellular communication may be important in vivo.

We observed a pro-fibrotic response by PTECs when treated with podocyte MPs, but not podocyte exosomes. This is consistent with a previous report by Zhou that showed that vesicles released by injured rat proximal tubules (NRK cells) induced mesenchymal transition of recipient NRK cells [42]. The results of Zhou differ from ours in that they identified a novel autocrine signalling pathway (rather than the paracrine signalling pathway reported here) and that the vesicles isolated were the result of sediment after 100,000 g centrifugation (likely a heterogeneous mixture of exosomes and MPs). Pro-fibrotic effects of extracellular vesicles have also been reported in the liver where hepatocyte-derived extracellular vesicles (obtained from 100,000 g sediment) induced

up-regulation of pro-fibrotic genes in hepatic stellate cells by miR-128-3p [43]. Our results suggest that podocyte MPs are capable of inducing PTEC fibrosis. Our MP isolates appear to be largely free of exosomes and other small (<100 nm) particles since we did not observe the presence of exosomal markers TSG101 or CD63 and the majority of particles were of 100–1000 nm in size. Based on several lines of evidence we postulate that these responses are a result of receptor activation rather than nucleic acid transfer. First, podocyte MPs induced a rapid increase in phosphorylation of p38 MAPK and Smad3 with responses as early as 30 minutes and had returned to baseline levels by 2 hours. Such a rapid response is more likely associated with receptor stimulation rather than alterations in nucleic acid processing. Consistent with this, the changes in fibronectin/collagen type IV expression were not observed until 72 hours, which is more consistent with an induction of expression than a direct transfer of protein from the MPs. Finally, antagonism of the cell surface scavenger receptor CD36 blocked all podocyte MP-induced responses in PTECs. Nevertheless, one cannot rule out the possibility of
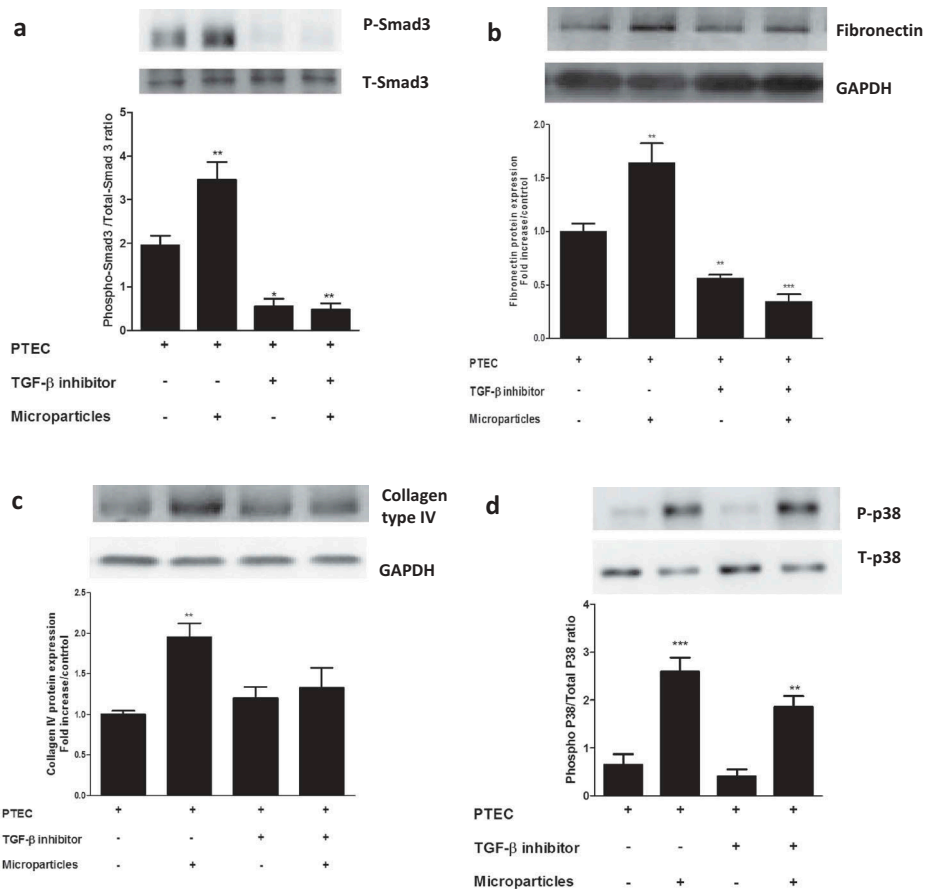
Figure 6. Role of TGF-β receptor in podocyte MP-induced fibrotic responses in PTECs. PTECs were treated with podocyte MPs (10 μg/ml) for 30 minutes (p38, Smad3 phosphorylation, A) or 72 hours (Fibronectin, Collagen type IV expression, B and C respectively) in the presence and absence of the TGF-β receptor inhibitor LY2109761 (TGF-ßI, 1μM). **P < 0.001 vs Control, n = 6.

other mechanisms of action such as a fusion and transfer of protein or nucleic acids.

CD36 is a class B scavenger receptor expressed in a variety of tissues [36,37,44]. In the kidney, CD36 is expressed in the proximal tubule, collecting duct and loop of Henle with expression in the proximal tubule increased in diabetes [44]. Previous studies in cultured proximal tubule epithelial cells (PTECs) showed that CD36 activation leads to oxidative stress, apoptosis, and pro-fibrotic signalling (fibronectin expression, TGF-β release) [44–46]. Similarly, mice deficient in CD36 are resistant to renal fibrosis and oxidative stress in unilateral ureteral obstruction [46,47]. Our data also support a role for CD36 in PTEC fibrogenesis through the stimulation of p38 MAPK and TGF-β receptor-mediated activation of Smad3, collagen type IV and fibronectin. This, in turn, could contribute to the development of renal fibrosis in vivo leading to impaired function. The MP-associated ligands responsible for CD36 activation are not clear at this time. Indeed, identification of the ligands responsible is likely to be challenging due to the wide spectrum of bioactive compounds known to activate this scavenger receptor including thrombospondin, lipoproteins, and glycated or oxidized proteins and lipids [36,37,44].

Activation of the TGF-β receptor appears to be indirect and ligand independent since downstream Smad3 activation is rapid and p38 dependent. Accordingly, we hypothesize that p38 MAPK transactivates the TGF-β receptor resulting in downstream pro-fibrotic signalling. Indeed, transactivation of the TGF-β receptor by other mechanisms has been reported in cultured proximal tubule epithelial cells and vascular smooth muscle cells [48,49].

Our results suggest that podocyte MPs induce a fibrogenic response in PTECs; however, the functional significance of this is currently unclear. As MP formation is in response to cell stress/injury [50,51], it is likely that podocyte MP-mediated cross-talk is seen under conditions of glomerular injury. Indeed, our previous studies suggest that levels of podocyte-derived MPs in urine are minimal in the absence of disease, but increased in diabetes [24,25]. One limitation of the present study is that we studied podocyte MPs from unstimulated podocytes rather than
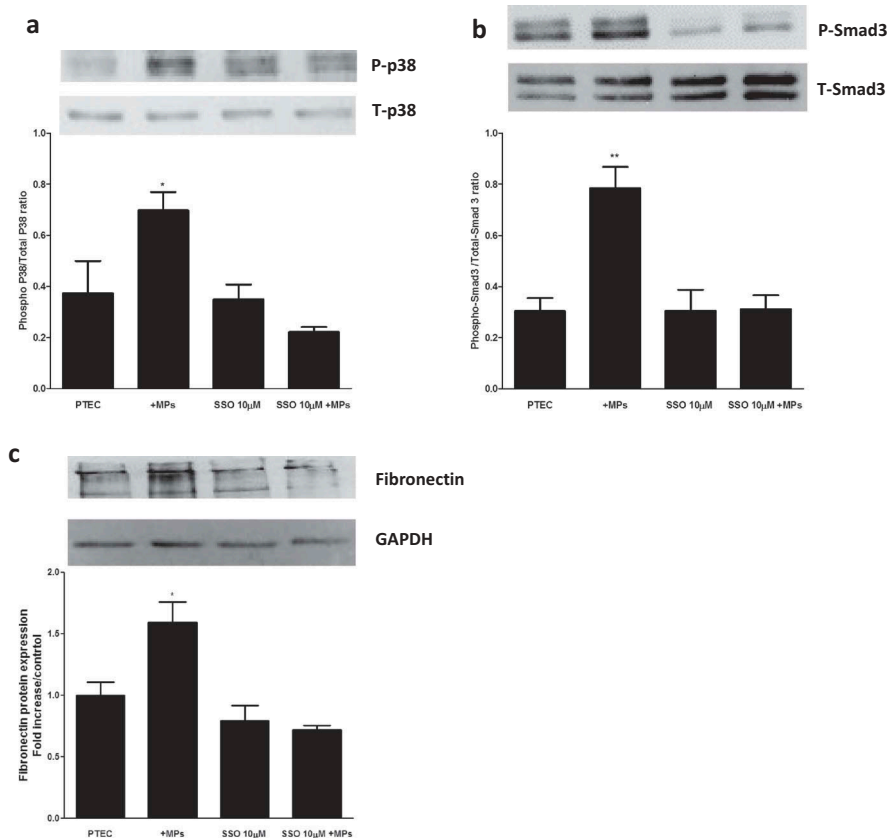
Figure 7. Role of CD36 in podocyte MP-induced fibrotic responses in PTECs. PTECs were treated with podocyte MPs (10 µg/ml) for 30 minutes (p38, Smad3 phosphorylation, A) or 72 hours (Fibronectin, Collagen type IV expression, B and C respectively) in the presence and absence of the inhibitor of CD36 Sulfo-N-succinimidyl oleate (SSO, 10 µM). *P < 0.05, **P < 0.01 vs Control, n = 6.

those formed following external stress. It is possible that podocyte MPs may differ in their bioactivity depending on the stimulus, as we and others have reported in endothelial MPs [52,53]. Pathogenically, diabetic kidney disease typically involves podocyte/glomerular injury followed by development of tubulointerstitial fibrosis which more closely associates with declining renal function [54,55]. Putative mechanisms linking glomerular injury to tubular dysfunction include increases in ultrafiltered albumin and/or cytokines [9,10,56]. Based on the results from the present study we speculate that the early release of MPs from podocytes may represent an alternative mechanism linking glomerular injury to tubular fibrosis in diabetic nephropathy. Indeed, it is possible that this mechanism may extend to other glomerular diseases affecting podocytes, such as IgA nephropathy or minimal change disease.

In summary, this study provides, for the first time, evidence that podocyte MPs induce pro-fibrotic responses in PTECs. Podocyte MP-mediated responses were dependent upon activation of CD36-dependent transactivation of the TGF-β receptor. This novel pathway for podocyte-PTEC cross-talk may contribute to the development of tubulointerstitial fibrosis and renal decline in glomerular disease.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] Tuttle KR, Bakris GL, Bilous RW, et al. Diabetic kidney disease: a report from an ADA Consensus Conference. Am J Kidney Dis. 2014;64(4):510–533.

[2] Pavenstädt H, Kriz W, Kretzler M. Cell biology of the glomerular podocyte. Physiol Rev. 2003;83(1):253–307.

[3] Stitt-Cavanagh E, MacLeod L, Kennedy C. The podocyte in diabetic kidney disease. ScientificWorldJournal. 2009;9:1127–1139.

[4] Adler S. Diabetic nephropathy: linking histology, cell biology, and genetics. Kidney Int. 2004;66(5):2095–2106.

[5] Maezawa Y, Takemoto M, Yokote K. Cell biology of diabetic nephropathy: roles of endothelial cells, tubulointerstitial cells and podocytes. J Diabetes Investig. 2015;6(1):3–15.

[6] Najafian B, Alpers CE, Fogo AB. Pathology of human diabetic nephropathy. Contrib Nephrol. 2011;170:36–47.

[7] Najafian B, Kim Y, Crosson JT, et al. Atubular glomeruli and glomerulotubular junction abnormalities in diabetic nephropathy. J Am Soc Nephrol. 2003;14 (4):908–917.

[8] Gilbert RE, Cooper ME. The tubulointerstitium in progressive diabetic kidney disease: more than an aftermath of glomerular injury? Kidney Int. 1999;56(5):1627–1637.

[9] Qian Y, Feldman E, Pennathur S, et al. From fibrosis to sclerosis: mechanisms of glomerulosclerosis in diabetic nephropathy. Diabetes. 2008;57(6):1439–1445.

[10] Gorriz JL, Martinez-Castelao A. Proteinuria: detection and role in native renal disease progression. Transplant Rev (Orlando). 2012;26(1):3–13.

[11] Keener J, Sneyd J. Intercellular communication. In: Keener J, Sneyd J, editors. Mathematical physiology: I: cellular physiology. New York, NY: Springer New York; 2009. p. 347–384.

[12] Erdbrugger U, Le TH. Extracellular vesicles in renal diseases: more than novel biomarkers? J Am Soc Nephrol. 2015;27(1):12-26.

[13] Mause SF, Weber C. Microparticles: protagonists of a novel communication network for intercellular information exchange. Circ Res. 2010;107(9):1047–1057.

[14] Hugel B, Martínez MC, Kunzelmann C, et al. Membrane microparticles: two sides of the coin. Physiology (Bethesda). 2005;20:22–27.

[15] Burger D, Montezano AC, Nishigaki N, et al. Endothelial microparticle formation by angiotensin II is mediated via Ang II receptor type I/NADPH oxidase/ Rho kinase pathways targeted to lipid rafts. Arterioscler Thromb Vasc Biol. 2011;31(8):1898–1907.

[16] Brodsky SV, Zhang F, Nasjletti A, et al. Endothelium-derived microparticles impair endothelial function in vitro. Am J Physiol Heart Circ Physiol. 2004;286(5): H1910–H1915.

[17] Burger D, Kwart DG, Montezano AC, et al. Microparticles induce cell cycle arrest through redox-sensitive processes in endothelial cells: implications in vascular senescence. J Am Heart Assoc. 2012;1(3): e001842.

[18] Burger D, Turner M, Munkonda MN, et al. Endothelial microparticle-derived reactive oxygen species: role in endothelial signaling and vascular function. Oxid Med Cell Longev. 2016;2016:5047954.

[19] Niessen A, Heyder P, Krienke S, et al. Apoptotic-cell-derived membrane microparticles and IFN-alpha induce an inflammatory immune response. J Cell Sci. 2015;128 (14):2443–2453.

[20] Burger D, Schock S, Thompson CS, et al. Microparticles: biomarkers and beyond. Clin Sci (Lond). 2013;124 (7):423–441.

[21] Gyorgy B, Szabo TG, Pasztoi M, et al. Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles. Cell Mol Life Sci. 2011;68(16):2667–2688.

[22] Morel O, Toti F, Bakouboula B, et al. Procoagulant microparticles: 'criminal partners' in atherothrombosis and deleterious cellular exchanges. Pathophysiol Haemost Thromb. 2006;35(1–2):15–22.

[23] Wang Y, Chen L-M, Liu M-L. Microvesicles and diabetic complications [mdash] novel mediators, potential biomarkers and therapeutic targets. Acta Pharmacol Sin. 2014;35(4):433–443.

[24] Burger D, Thibodeau J-F, Holterman CE, et al. Urinary podocyte microparticles identify prealbuminuric diabetic glomerular injury. J Am Soc Nephrol. 2014;25 (7):1401–1407.

[25] Lytvyn Y, Xiao F, Kennedy CR, et al. Assessment of urinary microparticles in normotensive patients with type 1 diabetes. Diabetologia. 2017;60(3):581–584.

[26] Saleem MA, O'Hare MJ, Reiser J, et al. A conditionally immortalized human podocyte cell line demonstrating nephrin and podocin expression. J Am Soc Nephrol. 2002;13(3):630–638.

[27] Burger D, Oleynik P. Isolation and characterization of circulating microparticles by flow cytometry. Methods Mol Biol. 2017;1527:271–281.

[28] Viñas JL, Burger D, Zimpelmann J, et al. Transfer of microRNA-486-5p from human endothelial colony forming cell-derived exosomes reduces ischemic kidney injury. Kidney Int. 2016;90(6):1238–1250.

[29] Biernacka A, Dobaczewski M, Frangogiannis NG. TGF-β signaling in fibrosis. Growth Factors. 2011;29(5):196–202.

[30] Pohlers D, Brenmoehl J, Löffler I, et al. TGF-beta and fibrosis in different organs - molecular pathway imprints. Biochim Biophys Acta. 2009;1792(8):746–756.

[31] Samarakoon R, Overstreet JM, Higgins SP, et al. TGF-beta1 → SMAD/p53/USF2 → PAI-1 transcriptional axis in ureteral obstruction-induced renal fibrosis. Cell Tissue Res. 2012;347(1):117–128.

[32] Vallon V. The proximal tubule in the pathophysiology of the diabetic kidney. Am J Physiol Regul Integr Comp Physiol. 2011;300(5):R1009–R1022.

[33] Adhikary L, Chow F, Nikolic-Paterson DJ, et al. Abnormal p38 mitogen-activated protein kinase signalling in human and experimental diabetic nephropathy. Diabetologia. 2004;47(7):1210–1222.

[34] Nishida M, Okumura Y, Sato H, et al. Delayed inhibition of p38 mitogen-activated protein kinase ameliorates renal fibrosis in obstructive nephropathy. Nephrol Dial Transplant. 2008;23(8):2520–2524.

[35] Stambe C, Atkins RC, Hill PA, et al. Activation and cellular localization of the p38 and JNK MAPK pathways in rat crescentic glomerulonephritis. Kidney Int. 2003;64(6):2121–2132.

[36] Nicholson AC, Han J, Febbraio M, et al. Role of CD36, the macrophage class B scavenger receptor, in atherosclerosis. Ann N Y Acad Sci. 2001;947:224–228.

[37] Park YM. CD36, a scavenger receptor implicated in atherosclerosis. Exp Mol Med. 2014;46:e99.

[38] Lopez-Vilchez I, Diaz-Ricart M, Galan AM, et al. Internalization of tissue factor-rich microvesicles by platelets occurs independently of GPIIb-IIIa, and involves CD36 receptor, serotonin transporter and cytoskeletal assembly. J Cell Biochem. 2016;117(2):448–457.

[39] Ranghino A, Dimuccio V, Papadimitriou E, et al. Extracellular vesicles in the urine: markers and mediators of tissue damage and regeneration. Clin Kidney J. 2015;8(1):23–30.

[40] Eyre J, Burton JO, Saleem MA, et al. Monocyte- and endothelial-derived microparticles induce an inflammatory phenotype in human podocytes. Nephron Exp Nephrol. 2011;119(3):e58–e66.

[41] Fernandez-Martínez AB, Torija AV, Carracedo J, et al. Microparticles released by vascular endothelial cells increase hypoxia inducible factor expression in human proximal tubular HK-2 cells. Int J Biochem Cell Biol. 2014;53:334–342.

[42] Zhou Y, Xiong M, Fang L, et al. miR-21-containing microvesicles from injured tubular epithelial cells promote tubular phenotype transition by targeting PTEN protein. Am J Pathol. 2013;183(4):1183–1196.

[43] Povero D, Panera N, Eguchi A, et al. Lipid-induced hepatocyte-derived extracellular vesicles regulate hepatic stellate cells via microRNA targeting peroxisome proliferator-activated receptor-γ. Cell Mol Gastroenterol Hepatol. 2015;1(6):646–663.e644.

[44] Susztak K, Ciccone E, McCue P, et al. Multiple metabolic hits converge on CD36 as novel mediator of tubular epithelial apoptosis in diabetic nephropathy. PLoS Med. 2005;2(2):e45.

[45] Yang Y-L, Lin S-H, Chuang L-Y, et al. CD36 is a novel and potential anti-fibrogenic target in albumin-induced renal proximal tubule fibrosis. J Cell Biochem. 2007;101(3):735–744.

[46] Okamura DM, Pennathur S, Pasichnyk K. López-Guisa JM, Collins S, Febbraio M, Heinecke J, Eddy AA. CD36 Regulates Oxidative Stress and Inflammation in Hypercholesterolemic CKD. J Am Soc Nephrol: JASN. 2009;20(3):495–505.

[47] Souza AC, Bocharov AV, Baranova IN, et al. Antagonism of scavenger receptor CD36 by 5A peptide prevents chronic kidney disease progression in mice independent of blood pressure regulation. Kidney Int. 2016;89(4):809–822.

[48] Burch ML, Ballinger ML, Yang SNY, et al. Thrombin stimulation of proteoglycan synthesis in vascular smooth muscle is mediated by protease-activated receptor-1 transactivation of the transforming growth factor beta type I receptor. J Biol Chem. 2010;285(35):26798–26805.

[49] Chung H, Ramachandran R, Hollenberg MD, et al. Proteinase-activated receptor-2 transactivation of epidermal growth factor receptor and transforming growth factor-beta receptor signaling pathways contributes to renal fibrosis. J Biol Chem. 2013;288(52):37319–37331.

[50] Barteneva NS, Fasler-Kan E, Bernimoulin M, et al. Circulating microparticles: square the circle. BMC Cell Biol. 2013;14:23.

[51] Morel O, Jesel L, Freyssinet J-M, et al. Cellular mechanisms underlying the formation of circulating microparticles. Arterioscler Thromb Vasc Biol. 2011;31(1):15–26.

[52] Burger D, Turner M, Xiao F, et al. High glucose increases the formation and pro-oxidative activity of endothelial microparticles. Diabetologia. 2017;60:1791–1800.

[53] Jansen F, Yang X, Franklin BS, et al. High glucose condition increases NADPH oxidase activity in endothelial microparticles that promote vascular inflammation. Cardiovasc Res. 2013;98(1):94–106.

[54] Taft JL, Nolan CJ, Yeung SP, et al. Clinical and histological correlations of decline in renal function in diabetic patients with proteinuria. Diabetes. 1994;43(8):1046–1051.

[55] Bohle A, Christ H, Grund KE, et al. The role of the interstitium of the renal cortex in renal disease. Contrib Nephrol. 1979;16:109–114.

[56] Brosius FC 3rd. New insights into the mechanisms of fibrosis and sclerosis in diabetic nephropathy. Rev Endocr Metab Disord. 2008;9(4):245–254.