Original Paper

Activation of COX-2/mPGES-1/PGE2 Cascade via NLRP3 Inflammasome Contributes to Albumin-Induced Proximal Tubule Cell Injury

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Key Words

Albumin • NLRP3 inflammasome • COX-2 • PGE2 • Proximal tubule cells

Abstract

Background/Aims: The activation of NOD-like receptor family, pyrin domain containing3 (NLRP3) inflammasome has been shown to be positively correlated with the severity of proteinuria in chronic kidney disease (CKD) patients. Prostaglandin E2 (PGE2), an important inflammatory mediator, is also involved in various kidney injuries. The aim of the present study was to investigate the involvement of NLRP3 inflammasome and PGE2 synthetic pathway in albumin-induced renal tubular injury. **Methods:** Murine proximal tubular cells (mPCTs) were treated with albumin to induce cell injury. NLRP3 siRNA and specific COX-2 inhibitor NS398 were used to define their roles in mediating albumin-induced mPCT injury or the activation of COX-2/mPGES-1/PGE2 cascade. **Results:** In mPCTs, inhibition of NLRP3 by a small interfering RNA (siRNA) blocked albumin-induced kidney injury molecule 1 (KIM-1) upregulation, inflammatory response, and cell apoptosis. Albumin markedly activated cyclooxygenase-2 (COX-2)/microsomal prostaglandin E synthase-1 (mPGES-1)/PGE2 pathway in this cell line, an effect largely abolished by NLRP3 silencing at both mRNA and protein levels. More interestingly, blockade of COX-2 using a specific COX-2 inhibitor NS398 markedly inhibited the upregulation of KIM-1 and inflammatory cytokines, and attenuated cell apoptosis in line with blunted PGE2 release following albumin treatment. **Conclusions:** The findings suggest that COX-2/mPGES-1/PGE2 axis could be activated by albumin in the proximal tubular cells via a NLRP3 inflammasome-mediated mechanism and could thus contribute to proteinuria-related renal tubular cell injury.

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Introduction

Chronic kidney disease (CKD) is a kind of renal disease with progressive loss of renal function and structural damage caused by various primary and secondary insults over a period of months or years. In the past decades, the prevalence of CKDs is increasing with the rapid growth of hypertension, diabetes and other metabolic disorders [1]. Among the insults resulting in the CKD progression, proteinuria is proven as an independent risk factor [2]. Although a number of studies have been performed to explore the pathogenic mechanisms of proteinuria-mediated kidney injury, we still lack the effective target for the treatment of proteinuria-caused renal injury.

Recently, NOD-like receptor family, pyrin domain containing3 (NLRP3) inflammasome was characterized as a contributor of CKDs [3]. More interestingly, Fang et al. found that the activation of NLRP3 inflammasome was positively correlated with the severity of proteinuria in patients, and the NLRP3 inflammasome was directly activated by albumin in the cultured renal epithelial cells [4]. These findings highly suggested that NLRP3 might serve as an important pathogenic factor in mediating proteinuria-induced renal tubular damage. However, the mechanisms mediating the pathogenesis of NLRP3 inflammasome in proteinuria-induced renal tubular damage remains elusive. Recently, our group reported that COX-2/mPGES-1/PGE2 could be activated by NLRP3 inflammasome in thick ascending limb to downregulate NKCC2 under the proteinuric condition [5], while the role of this cascade in proteinuria-induced renal tubular cell injury was not defined.

Among five major prostaglandins (PGE2, PGD2, PGI2, PGF2 α , and TXA2) produced in kidney [6], PGE2 has the established roles in mediating the fluid metabolism [7-10], and kidney injury [11-13]. PGE2 is generated through a sequential enzyme cascade of COX/PGE2 synthases (PGES) [14]. By now, two COXs (COX-1 and COX-2) and three PGESs (mPGES-1, mPGES-2, and cPGES) were cloned. COX-1 constitutively expressed in platelets, gastric epithelial cells, and renal collecting ducts and arteriolar endothelial cells, regulates blood pressure (BP) and renal hemodynamics [15, 16]. COX-2 has low basal expression in various organs but is highly inducible by a number of pathological stimuli [15-17]. In kidney, COX-2 distribution has been localized in the macula densa, cortical thick ascending limb of Henle, and interstitial cells [18]. The stimulation of COX-2 in proximal tubules possibly contributes to renal injury [17, 19, 20]. Among three PGESs, mPGES-1 is best identified as a PGE2 synthetic enzyme [14, 21]. In contrast, genetic deletion of mPGES-2 and cPGES had no effect on lowering PGE2 levels in mice [22, 23], which argues their property of PGE2 synthases. Recently, a number of reports demonstrated that COX-2/mPGES-1/PGE2 cascade is of importance in promoting renal injury in CKD and AKI [19, 24]. In the present study, we investigated: 1) whether albumin could activate COX/mPGES-1/PGE2 cascade in proximal tubular cells; 2) whether COX/mPGES-1/PGE2 cascade served as the downstream signaling of NLRP3 inflammasome in this pathological process; and 3) whether COX/mPGES-1/PGE2 cascade contributes to albumin-induced tubular cell injury.

Materials and Methods

Cell culture studies

Immortalized mouse proximal tubular cells (mPTCs) from ScienCell Research Laboratory (Cat #: M4100) were cultured in serum-free keratinocyte medium supplemented with bovine pituitary extract, epidermal growth factor (Wisent, Canada), and penicillin/streptomycin at 37°C with 5% CO₂, and subcultured at 50-80% confluence using 0.25% trypsin-0.02% EDTA (Invitrogen). Delipidated albumin (Sigma, St Louis, MO) was dissolved in the medium with no penicillin/streptomycin to stimulate mPTCs. In brief, albumin was firstly dissolved in culture medium at the concentration of 0.25g/ml which was further diluted to the final concentrations (2.5, 5, 10, and 20 mg/ml) used in the experiments. The control cells were administered with same amount of culture medium. Because the dose-dependent experiment showed that

albumin at 10mg/ml caused striking induction of tubular injury marker KIM-1, 10mg/ml albumin was used in other experiments of the study.

NLRP3 siRNA transfection

mPTCs were cultivated to 50-60% confluence in culture medium with no penicillin or streptomycin. SiNlrp3 and scrambled siRNA were synthesized by the company of GenePharma. Then the cells were transfected with siRNAs using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. In brief, the cells were transfected with 500 nM NLRP3 siRNA or scrambled siRNA 24 h before albumin treatment. siNLRP3: 5'-CGGCCUUACUCAAUCUGUTT-3', 5'-ACAGAUUGAAGUAGCGCGTT-3'; scrambled siRNA: 5'-UUCUCCGAACGUGUCACGUTT-3', 5'-ACGUGACACGUUCGGAGAATT-3'.

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted by using the TRIzol reagent (Invitrogen). Oligonucleotides were designed using Primer3 software (available at <http://frodo.wi.mit.edu/>) and synthesized by Invitrogen company. The sequences of the primer pairs are shown in Table 1. Reverse transcription was performed using a reaction kit (Promega Reverse Transcription System) according to the manufacturer's protocol. Real-time PCR amplification was performed using the ABI 7500 real-time PCR detection system (Foster City, CA, USA) with SYBR Green PCR Master Mix (Applied Biosystems). The cycling conditions were 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The mRNA levels were normalized to GAPDH as a control and calculated using the comparative cycle threshold ($\Delta\Delta C_t$) method.

Western blotting

mPTCs were lysed using a protein lysis buffer containing 50 mM Tris, 150 mM NaCl, 10 mM EDTA, 1% Triton X-100, 200 mM sodium fluoride, and 4 mM sodium orthovanadate as a protease inhibitor (pH 7.5). Immunoblotting was then performed with primary antibodies against NLRP3 (1:500), COX-2 (Cayman Chemical, USA, 1:500), mPGES-1 (Cayman Chemical, USA, 1:500), and β -actin (1:1000), followed by adding HRP-labeled secondary antibodies. The blots were visualized using the Amersham ECL detection system (Amersham, Little Chalfont, UK). Densitometric analysis was performed using Quantity One software (Bio-Rad).

EIA assay

The concentration of PGE2 in the medium was examined using the commercial EIA kits purchased from Cayman Chemical.

Apoptosis analysis

Annexin V-fluorescein isothiocyanate conjugated with propidium iodide staining

Following the treatment, mPTCs were collected in suspension by 0.25% trypsin-0.02% EDTA and were washed with PBS. Then the cells were double stained with annexin V-fluorescein isothiocyanate and propidium iodide (Annexin V: FITC Apoptosis Detection Kit, BD Biosciences, San Diego, CA) according to the manufacturer's instructions. Quantification was performed by flow cytometry. There were 6 samples for each group.

TUNEL assay in cultured mPTCs

Apoptotic cell death was determined using TUNEL staining with an In Situ Cell Death Detection Kit (Roche Molecular

Table 1. Primer sequences for qRT-PCR

Gene symbol	Primer sequences
GAPDH	5'- GTCTTCACTACCATGGAGAAGG -3' 5'- TCATGGATGACCTTGGCCAG -3'
NLRP3	5'-GTGGTGACCCTCTGTGAGGT-3' 5'-TCTTCCTGGAGCGCTTCTAA-3'
mPGES1	5'- GGATGCGCTGAAACGTGGA -3' 5'- CAGGAATGAGTACACGAAGCC -3'
mPGES2	5'- CCTCGACTTCCACTCCCTG -3' 5'- TGAGGGCACTAATGATGACAGAG -3'
cPGES	5'- TGTTCGCGAAAAGGAGAATCCG -3' 5'- CCATGTGATCCATCATCTCAGAG -3'
COX1	5'- ATGAGTCGAAGGAGTCTCTCG -3' 5'- GCACGGATAGTAACAACAGGGA -3'
COX2	5'- AACCGTGCGGAATGTATGAG -3' 5'- GCAGGAAGGGGATGTTGTT -3'
IL-1 β	5'- CAAAAATGCCTCGTCTGT -3' 5'- GTGGGTGTGCCGCTCTTC -3'
IL-18	5'- TGGCTGTGACCCTATCTGTG -3' 5'- TCATCTTCCTTTTGCAAGC -3'
IL-6	5'- ATACCACCCACAACAGACCA -3' 5'- CTCCAGAAGACCAGAGCAGA -3'
ICAM-1	5'- GCCTTGGTAGAGGTGACTGAG -3' 5'- GACCGAGCTGAAAAGTTGTA -3'
KIM-1	5'- ATGAATCAGATTCAAGTCTTC -3' 5'- TCTGGTTTGTGAGTCCATGTG -3'

Biochemicals, Mannheim, Germany) following the manufacturer's protocol. mPTCs undergoing apoptosis were detected by counterstaining with Hoechst 33258. The slides were examined by confocal microscopy. There were 6 samples for each group and 6 fields were randomly taken in each slide.

Statistical analysis

All data are presented as means $\pm$ standard deviation (SD). The statistical analysis was performed using ANOVA followed by Bonferroni's test or unpaired Student's t-test with SPSS 13 statistical software. $P < 0.05$ was considered significant.

Results

Inhibition of NLRP3 by siRNA approach blunted albumin-induced cell injury in mPTCs

Application of NLRP3 siRNA significantly reduced protein and mRNA expressions of NLRP3 in mPTCs (Fig. 1A-C). Interestingly, the tubular injury marker of KIM-1 was remarkably induced by albumin in dose- and time-dependent manners (Fig. 2A&B), and

Fig. 1. Transfection of NLRP3 siRNA. (A) Western blots of NLRP3 after transfection of siNLRP3. Cells were transfected with siNLRP3 or scramble (Vehi) for 48 h, and untreated cells were used as the control. (B) Desitometric analysis of NLRP3. (C) qRT-PCR analysis of NLRP3 mRNA expression. Cells were transfected with siNLRP3 or scrambled siRNA for 24 h, and untreated cells were used as the control. The values represent means $\pm$ SD (n=6 per group). * $P < 0.01$ vs. scrambled siRNA group. Cntl: treatment with transfection reagent alone.

Fig. 2. NLRP3 siRNA blunted albumin-induced KIM-1 expression. (A) qRT-PCR analysis of KIM-1 mRNA expression in mPTCs after treatment by different doses of albumin (0-20mg/ml). (B) qRT-PCR analysis of KIM-1 mRNA expression in mPTCs following 10mg/ml albumin treatment in a time course study. (C) qRT-PCR analysis of KIM-1 mRNA expression in mPTCs following 10mg/ml albumin treatment with or without NLRP3 silencing. Confluent mPTCs were transfected with siNlrp3 or scrambled siRNA for 48 h and then incubated with albumin (10 mg/ml) for additional 24 h. The values represent means $\pm$ SD (n=6 per group). * $P < 0.01$ vs. scrambled siRNA group. # $P < 0.01$ vs. Albumin + Scrambled siRNA group.

Fig. 3. NLRP3 silencing blunted albumin-induced over-expressions of inflammatory cytokines in mPCTs. mPCTs were transfected with siNlrp3 for 48 h and then incubated with albumin (10 mg/ml) for additional 24 h. (A) qRT-PCR analysis of IL-1 β . (B) qRT-PCR analysis of IL-18. (C) qRT-PCR analysis of IL-6. (D) qRT-PCR analysis of ICAM-1. The values are means $\pm$ SD (n=6 per group). * P < 0.01 vs. Scrambled siRNA group. # P < 0.01 vs. Albumin + Scrambled siRNA group. Cntl: treatment with transfection reagent alone.

Fig. 4. NLRP3 silencing attenuated albumin-induced apoptosis in mPCTs. mPCTs were transfected with siNlrp3 for 48 h and then incubated with albumin (10 mg/ml) for additional 24 h. (A) Quantification of apoptotic cells by flow cytometry. (B) Representative photographs of double-fluorescence labelling of Hoechst nuclear staining (blue) and TUNEL (green). mPCTs were incubated with albumin at 10 mg/ml for 24 h. The values represent means $\pm$ SD (n=6 in each group). * P < 0.01 vs. Scrambled siRNA group. # P < 0.01 vs. Albumin + Scrambled siRNA group. Cntl: treatment with transfection reagent alone.

such an induction was significantly blunted by NLRP3 siRNA treatment (Fig. 2C). Meanwhile, the elevation of inflammatory cytokines of IL-1 β , IL-18, IL-6, and ICAM-1 following albumin treatment was markedly blocked by NLRP3 siRNA, as determined by qRT-PCR (Fig. 3A-D). In line with the inhibition of KIM-1 and inflammatory cytokines, albumin-induced cell apoptosis was also attenuated by siNLRP3 (Fig. 4).

Fig. 5. NLRP3 silencing inhibited albumin-mediated upregulation of COX-2 and mPGES-1 mRNA expressions in mPTCs. mPTCs were transfected with siNlrp3 for 48 h and then incubated with albumin (10 mg/ml) for additional 24 h. (A) qRT-PCR analysis of mPGES-1. (B) qRT-PCR analysis of mPGES-2. (C) qRT-PCR analysis of cPGES. (D) qRT-PCR analysis of COX-1. (E) qRT-PCR analysis of COX-2. The values represent means $\pm$ SD (n=6 per group). * P < 0.01 vs. Scrambled siRNA group. # P < 0.01 vs. Albumin + Scrambled siRNA group. Cntl: treatment with transfection reagent alone.

Fig. 6. NLRP3 silencing inhibited albumin-induced upregulation of COX-2 and mPGES-1 proteins and PGE₂ secretion in mPTCs. mPTCs were transfected with siNlrp3 for 48 h and then incubated with albumin (10 mg/ml) for additional 48 h. (A) Western blots of mPGES-1 and COX-2. (B) Densitometric analysis of mPGES-1. (C) Densitometric analysis of COX-2. (D) PGE₂ secretion in cell culture medium. The values are means $\pm$ SD (n=6 per group). * P < 0.01 vs. Scrambled siRNA group. # P < 0.01 vs. Albumin + Scrambled siRNA group. Cntl: treatment with transfection reagent alone.

Fig. 7. Inhibition of COX-2 activity by NS398 reversed albumin-induced over-expressions of inflammatory cytokines in mPCTs. mPCTs were treated with NS398 for 30 min and then incubated with albumin (10 mg/ml) for additional 24 h. (A) qRT-PCR analysis of IL-1 β . (B) qRT-PCR analysis of IL-18. (C) qRT-PCR analysis of IL-6. (D) qRT-PCR analysis of ICAM-1. The values are means $\pm$ SD (n=6 per group). * P < 0.01 vs. control. # P < 0.01 vs. albumin-treated mPCTs.

Inhibition of NLRP3 by siRNA approach blunted albumin-induced activation of COX-2/mPGES-1/PGE2 cascade in mPCTs

After treating the mPCTs with albumin at the dose of 10mg/ml for 24h, the mRNA expressions of COX-2 and mPGES-1, but not COX-1, mPGES-2, and cPGES, were selectively up-regulated as determined by qRT-PCR (Fig. 5A-E). By application of NLRP3 siRNA, albumin-induced upregulation of COX-2 and mPGES-1 mRNA expressions were significantly blunted (Fig. 5A&E) without affecting COX-1 and other PGE2 synthases (Fig. 5B-D). By Western blotting, we further confirmed the induction of COX-2 and mPGES-1 at protein levels (Fig. 6A-C), which was remarkably blocked by NLRP3 silencing. (Fig. 6A-C). In line with the activation of COX-2/mPGES1 pathway, the PGE2 release in the cell culture medium was also significantly elevated in response to the albumin treatment for 24 h (Fig. 6D). Such an increase of PGE2 was completely abolished by silencing NLRP3 (Fig. 6D).

Inhibition of COX-2 abolished albumin-stimulated inflammatory response and cell injury in mPCTs

Following the activation of NLRP3 inflammasome by albumin, the mRNA levels of IL-1 β , IL-18, IL-6, and ICAM-1 were all stimulated in mPCTs (Fig. 7A-D). Inhibition of COX-2 by a specific COX-2 inhibitor of NS398 robustly abolished the induction of these inflammatory cytokines (Fig. 7A-D). At the same time, albumin-induced KIM-1 expression and cell apoptosis were also diminished by NS398 in parallel with reduced PGE2 production (Fig. 8A-D). These novel findings highly suggested that COX-2/mPGES-1/PGE2 cascade served as a downstream signaling of NLRP3 inflammasome in albumin-induced renal tubular cell injury.

Discussion

Proteinuria is not only a common phenomenon in CKD patients but also a direct insult promoting the renal injury [2, 25, 26]. By now, there is no effective target for treating proteinuria-associated kidney injury possibly due to the incomplete understanding of

Fig. 8. Effects of COX-2 inhibition on albumin-induced cell injury and PGE₂ production in mPCTs. mPCTs were treated with NS398 for 30 min and then incubated with albumin (10 mg/ml) for additional 24 h. (A) qRT-PCR analysis of KIM-1 mRNA expression. (B) Quantification of apoptotic cells by flow cytometry. (C) Representative photographs of double-fluorescence labelling of Hoechst nuclear staining (blue) and TUNEL (green). (D) EIA assay of medium PGE₂ concentration. Confluent mPCTs were incubated with albumin 24 h following NS398 treatment and the medium was collected to detect PGE₂ by EIA. The values are means $\pm$ SD (n=6 per group). * P < 0.01 vs. control. # P < 0.01 vs. albumin-treated mPCTs.

the mechanisms mediating the proteinuria-induced tubular damage. Inflammation is a common feature of various CKDs and is highly involved in the development and progression of CKD. NLRP3 inflammasome is one of the best characterized inflammasomes [27-30]. Recently, accumulating evidence demonstrated that NLRP3 inflammasome contributes to the CKDs [3, 31,32] and can be activated by the albumin in human proximal tubule cells [4]. In present study, by blocking NLRP3 via a siRNA strategy, the tubular injury marker of KIM-1, cell apoptosis and inflammatory cytokines were all suppressed, suggesting a critical role of NLRP3 in mediating albumin-caused tubular cell injury possibly via augmenting the inflammatory response.

Among the inflammatory mediators, PGE₂ has a known role in mediating the inflammatory response in various kidney injuries [11, 12, 33, 34]. Blockade of PGE₂ production by inhibiting its synthetic enzymes of COX-2 or mPGES-1 could improve many kidney diseases in animals [19, 24, 35-37], suggesting a pathogenic role of COX-2/mPGES-1/PGE₂ pathway in kidney injury. In the present study, we found that albumin significantly up-regulated COX-2/mPGES-1/PGE₂ cascade in mPCTs. By blocking NLRP3 via a siRNA approach, the induction of this prostaglandin cascade was largely normalized, indicating that NLRP3 inflammasome could be responsible for mediating the albumin effect on activating COX-2/mPGES-1/PGE₂ cascade.

To evaluate whether activation of COX-2/mPGES-1/PGE₂ signaling contributes albumin-induced cell injury, a specific COX-2 inhibitor of NS398 was applied. Strikingly, inhibition of COX-2 reversed KIM-1 induction and cell apoptosis caused by albumin challenge. Meantime, the PGE₂ release was also blunted. These results highly supported that COX-2/PGE₂ cascade served as a detrimental factor in albumin-induced tubular cell injury.

Among three PGE2 synthases, only mPGES-1 has been proved to have the ability in producing PGE2 *in vivo* [14, 21], and is thought as the most promising target of next generation of anti-inflammatory drugs [38, 39]. As a downstream enzyme of COX, it is possible that the mPGES-1 inhibitors may preserve less adverse effects than the COX inhibitors in treating the inflammatory diseases. Proteinuria is known as an insult of renal tubulointerstitial inflammation resulting in the subsequent renal lesions in morphology and function [25]. In agreement with this notion, we observed an obvious elevation of mPGES-1 at mRNA and protein levels in this experimental setting, suggesting that mPGES-1-derived PGE2 might be a pathogenic contributor of proteinuria-associated renal injury.

In summary, the present study demonstrated a critical role of NLRP3 in mediating the albumin effect on activating COX-2/mPGES-1/PGE2 cascade in renal tubular cells. COX-2/mPGES-1/PGE2 cascade activation could contribute to albumin-induced tubular cell injury. By now, there are no effective clinical strategies in treating proteinuria-related kidney injury. These findings could shed new light on the understanding and treatment of proteinuria-caused renal damage.

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Disclosure Statement

There is no conflict of interests to disclose.

References

- 1 Kazancioglu R: Risk factors for chronic kidney disease: an update. *Kidney Int Suppl* (2011) 2013;3:368-371.
- 2 Burton C, Harris KP: The role of proteinuria in the progression of chronic renal failure. *Am J Kidney Dis* 1996;27:765-775.
- 3 Vilaysane A, Chun J, Seamone ME, Wang W, Chin R, Hirota S, Li Y, Clark SA, Tschopp J, Trpkov K, Hemmelgarn BR, Beck PL, Muruve DA: The NLRP3 inflammasome promotes renal inflammation and contributes to CKD. *J Am Soc Nephrol* 2010;21:1732-1744.
- 4 Fang L, Xie D, Wu X, Cao H, Su W, Yang J: Involvement of endoplasmic reticulum stress in albuminuria induced inflammasome activation in renal proximal tubular cells. *PLoS One* 2013;8:e72344.
- 5 Zhuang Y, Jia Z, Hu C, Ding G, Zhang X, Zhang Y, Yang G, Rohatgi R, Huang S, He JC, Zhang A: Albuminuria confers renal resistance to loop diuretics via the stimulation of NLRP3 inflammasome/prostaglandin signaling in thick ascending limb. *Oncotarget* DOI:10.18632/oncotarget.10257.
- 6 Nasrallah R, Clark J, Hebert RL: Prostaglandins in the kidney: developments since Y2K. *Clin Sci (Lond)* 2007;113:297-311.
- 7 Stokes JB, Kokko JP: Inhibition of sodium transport by prostaglandin E2 across the isolated, perfused rabbit collecting tubule. *J Clin Invest* 1977;59:1099-1104.
- 8 Stokes JB: Effect of prostaglandin E2 on chloride transport across the rabbit thick ascending limb of Henle. Selective inhibitions of the medullary portion. *J Clin Invest* 1979;64:495-502.
- 9 Stokes JB: Integrated actions of renal medullary prostaglandins in the control of water excretion. *Am J Physiol* 1981;240:F471-480.

- 10 Guan Y, Zhang Y, Breyer RM, Fowler B, Davis L, Hebert RL, Breyer MD: Prostaglandin E2 inhibits renal collecting duct Na⁺ absorption by activating the EP1 receptor. *J Clin Invest* 1998;102:194-201.
- 11 Mohamed R, Jayakumar C, Ranganathan PV, Ganapathy V, Ramesh G: Kidney proximal tubular epithelial-specific overexpression of netrin-1 suppresses inflammation and albuminuria through suppression of COX-2-mediated PGE2 production in streptozotocin-induced diabetic mice. *Am J Pathol* 2012;181:1991-2002.
- 12 Ranganathan PV, Jayakumar C, Mohamed R, Dong Z, Ramesh G: Netrin-1 regulates the inflammatory response of neutrophils and macrophages, and suppresses ischemic acute kidney injury by inhibiting COX-2-mediated PGE2 production. *Kidney Int* 2013;83:1087-1098.
- 13 Ren Y, D'Ambrosio MA, Garvin JL, Wang H, Carretero OA: Prostaglandin E2 mediates connecting tubule glomerular feedback. *Hypertension* 2013;62:1123-1128.
- 14 Murakami M, Naraba H, Tanioka T, Semmyo N, Nakatani Y, Kojima F, Ikeda T, Fueki M, Ueno A, Oh S, Kudo I: Regulation of prostaglandin E2 biosynthesis by inducible membrane-associated prostaglandin E2 synthase that acts in concert with cyclooxygenase-2. *J Biol Chem* 2000;275:32783-32792.
- 15 Crofford LJ: COX-1 and COX-2 tissue expression: implications and predictions. *J Rheumatol Suppl* 1997;49:15-19.
- 16 Smith WL, Garavito RM, DeWitt DL: Prostaglandin endoperoxide H synthases (cyclooxygenases)-1 and -2. *J Biol Chem* 1996;271:33157-33160.
- 17 Breyer MD, Harris RC: Cyclooxygenase 2 and the kidney. *Curr Opin Nephrol Hypertens* 2001;10:89-98.
- 18 Norregaard R, Kwon TH, Frokiaer J: Physiology and pathophysiology of cyclooxygenase-2 and prostaglandin E2 in the kidney. *Kidney Res Clin Pract* 2015;34:194-200.
- 19 Jia Z, Wang N, Aoyagi T, Wang H, Liu H, Yang T: Amelioration of cisplatin nephrotoxicity by genetic or pharmacologic blockade of prostaglandin synthesis. *Kidney Int* 2011;79:77-88.
- 20 Jia Z, Zhang Y, Ding G, Heiney KM, Huang S, Zhang A: Role of COX-2/mPGES-1/prostaglandin E2 cascade in kidney injury. *Mediators Inflamm* 2015;2015:147894.
- 21 Inada M, Matsumoto C, Uematsu S, Akira S, Miyaura C: Membrane-bound prostaglandin E synthase-1-mediated prostaglandin E2 production by osteoblast plays a critical role in lipopolysaccharide-induced bone loss associated with inflammation. *J Immunol* 2006;177:1879-1885.
- 22 Jania LA, Chandrasekharan S, Backlund MG, Foley NA, Snouwaert J, Wang IM, Clark P, Audoly LP, Koller BH: Microsomal prostaglandin E synthase-2 is not essential for in vivo prostaglandin E2 biosynthesis. *Prostaglandins Other Lipid Mediat* 2009;88:73-81.
- 23 Lovgren AK, Kovarova M, Koller BH: cPGES/p23 is required for glucocorticoid receptor function and embryonic growth but not prostaglandin E2 synthesis. *Mol Cell Biol* 2007;27:4416-4430.
- 24 Jia Z, Wang H, Yang T: Microsomal prostaglandin E synthase 1 deletion retards renal disease progression but exacerbates anemia in mice with renal mass reduction. *Hypertension* 2012;59:122-128.
- 25 Eddy AA: Proteinuria and interstitial injury. *Nephrol Dial Transplant* 2004;19:277-281.
- 26 Hu J, Zhu Q, Li PL, Wang W, Yi F, Li N: Stem cell conditioned culture media attenuated albumin-induced epithelial-mesenchymal transition in renal tubular cells. *Cell Physiol Biochem* 2015;35:1719-1728.
- 27 Huang Z, Zhuang X, Xie C, Hu X, Dong X, Guo Y, Li S, Liao X: Exogenous Hydrogen Sulfide Attenuates High Glucose-Induced Cardiotoxicity by Inhibiting NLRP3 Inflammasome Activation by Suppressing TLR4/NF-κB Pathway in H9c2 Cells. *Cell Physiol Biochem* 2016;40:1578-1590.
- 28 Liu W, Zhang X, Zhao M, Zhang X, Chi J, Liu Y, Lin F, Fu Y, Ma D, Yin X: Activation in M1 but not M2 Macrophages Contributes to Cardiac Remodeling after Myocardial Infarction in Rats: a Critical Role of the Calcium Sensing Receptor/NLRP3 Inflammasome. *Cell Physiol Biochem* 2015;35:2483-2500.
- 29 Wang P, Ren D, Chen Y, Jiang M, Wang R, Wang YG: Effect of sodium alginate addition to resveratrol on acute gouty arthritis. *Cell Physiol Biochem* 2015;36:201-207.
- 30 Schroder K, Tschopp J: The inflammasomes. *Cell* 2010;140:821-832.
- 31 Bakker PJ, Butter LM, Kors L, Teske GJ, Aten J, Sutterwala FS, Florquin S, Leemans JC: Nlrp3 is a key modulator of diet-induced nephropathy and renal cholesterol accumulation. *Kidney Int* 2014;5:1112-1122.
- 32 Zhao J, Wang H, Dai C, Zhang H, Huang Y, Wang S, Gaskin F, Yang N, Fu SM: P2X7 blockade attenuates murine lupus nephritis by inhibiting activation of the NLRP3/ASC/caspase 1 pathway. *Arthritis Rheum* 2013;65:3176-3185.

- 33 Nakagawa N, Yuhki K, Kawabe J, Fujino T, Takahata O, Kabara M, Abe K, Kojima F, Kashiwagi H, Hasebe N, Kikuchi K, Sugimoto Y, Narumiya S, Ushikubi F: The intrinsic prostaglandin E2-EP4 system of the renal tubular epithelium limits the development of tubulointerstitial fibrosis in mice. *Kidney Int* 2012;82:158-171.
- 34 Kvirkvelia N, McMenamin M, Chaudhary K, Bartoli M, Madaio MP: Prostaglandin E2 promotes cellular recovery from established nephrotoxic serum nephritis in mice, prosurvival, and regenerative effects on glomerular cells. *Am J Physiol Renal Physiol* 2013;304:F463-470.
- 35 Jia Z, Sun Y, Liu S, Liu Y, Yang T: COX-2 but Not mPGES-1 Contributes to Renal PGE2 Induction and Diabetic Proteinuria in Mice with Type-1 Diabetes. *PLoS One* 2014;9:e93182.
- 36 Cheng H, Wang S, Jo YI, Hao CM, Zhang M, Fan X, Kennedy C, Breyer MD, Moeckel GW, Harris RC: Overexpression of cyclooxygenase-2 predisposes to podocyte injury. *J Am Soc Nephrol* 2007;18:551-559.
- 37 Murakami M, Kudo I: Prostaglandin E synthase: a novel drug target for inflammation and cancer. *Curr Pharm Des* 2006;12:943-954.
- 38 Dallaporta M, Pecchi E, Thirion S, Jean A, Troadec JD: Toward the management of inflammation: recent developments of mPGES-1 inhibitors. *Recent Pat CNS Drug Discov* 2010;5:70-80.
- 39 Chang HH, Meuillet EJ: Identification and development of mPGES-1 inhibitors: where we are at? *Future Med Chem* 2011;3:1909-1934.