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The receptor for advanced glycation endproducts mediates podocyte heparanase expression through NF-κB signaling pathway

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ABSTRACT

Heparanase degrades heparan sulfate in glomerular basement membrane (GBM) and plays an important role in diabetic nephropathy (DN). However, its regulating mechanisms remain to be deciphered. Our present study showed that the major advanced glycation endproducts (AGEs), CML-BSA, significantly increased heparanase expression in cultured podocytes and the effect was blocked by the receptor for advanced glycation endproducts (RAGE) knockdown, antibody and antagonist. In addition, NF-κB p65 phosphorylation was elevated and the increased heparanase expression and secretion upon CML-BSA could be attenuated by NF-κB inhibitor PDTC. Mechanistically, CML-BSA activated heparanase promoter through p65 directly binding to its promoter. Furthermore, the *in vivo* study showed that serum and renal cortex AGEs levels, glomerular p65 phosphorylation and heparanase expression were significantly increased in DN mice. Taken together, our data suggest that AGEs and RAGE interaction increases podocyte heparanase expression by activating NF-κB signal pathway, which is involved in GBM damages of DN.

1. Introduction

As one of the life-threatening diabetic complications, DN is a clinical syndrome characterized by persistent albuminuria and progressive renal function decline (Gross et al., 2005; Gnudi et al., 2016). Albuminuria is regarded as the major feature of DN and an independent risk factor for renal failure (Wada et al., 2012; Oh et al., 2012). Several studies showed that glomerular basement membrane (GBM) dysfunction and damage is the initial glomerular alteration and a major contributor to appearance of albuminuria in DN (Suh and Miner, 2013; Lewis and Xu, 2008). Heparan sulfate (HS) proteoglycans are widely distributed in GBM and act as the major determinants for the charge dependent permeability of the GBM (Morita et al., 2005; Raats et al., 2000). The decreased HS content in GBM causes decreased perm-selectivity to negatively charged macromolecules and allow albumin in plasma to leak into the urinary space (Raats et al., 2000; Zhang and Huang, 2012). Notably, glomerular HS loss is associated with the development of proteinuria in DN patients and experimental DN animals (Lewis and Xu, 2008; Wijnhoven et al., 2008).

Heparanase is an endo-β (1–4)-D-glucuronidase that can specifically degrade HS structural component in extracellular matrix (van den Hoven et al., 2007). The decreased content of HS in the GBM was associated with an abnormally increased glomerular heparanase expression in DN patients, which is critical to development of proteinuria (Wijnhoven et al., 2008; van den Hoven et al., 2006). The recent studies have suggested that heparanase plays a crucial role in pathogenesis in DN (Gil et al., 2012). Renal

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heparanase expression was mainly confined to podocytes and glomerular endothelial cells (van den Hoven et al., 2007). Glomerular heparanase expression is mainly up-regulated in podocytes in DN and to a much lesser extent in glomerular endothelial cells (Maxhimer et al., 2005; Garsen et al., 2014; Garsen et al., 2016). It is reported that heparanase expression can be induced by high glucose, angiotensin II, aldosterone and radical oxidative species (ROS) (Maxhimer et al., 2005; van den Hoven et al., 2009).

The metabolic abnormality of DN is characterized by high glucose and advanced glycation end-products (AGEs) accumulation (Daroux et al., 2010; Busch et al., 2010). AGEs are products of non-enzymatic glycation and oxidation of proteins and lipids and it is induced and increased in situations with hyperglycemia and oxidative stress especially in diabetes (Busch et al., 2010; Wendt et al., 2003). As the signal transduction receptor for AGEs, RAGE is closely linked to diabetic vascular injury (Manigrasso et al., 2014; Sourris et al., 2010). AGEs and RAGE interaction has been primarily implicated in the pathophysiology of DN (Ramasamy et al., 2011; Reiniger et al., 2010; Tesch et al., 2014). RAGE is majorly distributed in podocytes in kidney and its expression is abnormally increased in DN (Busch et al., 2010; Wendt et al., 2003; D'Agati et al., 2010; Tanji et al., 2000).

RAGE-ligand interaction in kidney results in the activation of down-stream signal transductions and then exerts various pathophysiological functions in DN such as cell apoptosis, oxidative stress and inflammation (Ramasamy et al., 2011; Gu et al., 2006; Tan et al., 2007). AGEs was found to significantly increase heparanase expression in macrophages through RAGE and phosphoinositide-3-kinase (PI3K) and AKT signaling pathway (Qin et al., 2013). AGEs could also induce heparanase expression in human micro-vascular endothelial cells by RAGE (An et al., 2011). In kidney, RAGE and heparanase are majorly distributed in podocytes and they are closely involved in pathogenesis of DN (van den Hoven et al., 2007; Busch et al., 2010). We deduced that RAGE might be involved in up-regulation of heparanase in podocytes while there is no direct and precise experimental evidence for RAGE and heparanase regulation in podocytes till now. Therefore, the aim of the present study was to evaluate the role of RAGE on heparanase expression in cultured mouse podocytes and the detailed molecular mechanisms were further unraveled.

2. Materials and methods

2.1. Reagents

N^{ε}-(carboxymethyl) lysine (CML)-BSA (Cell Biolabs, San Diego, CA, USA), mouse HMBG-1 protein (Thermo Scientific, Shanghai, China) and mouse S100B protein (Creative Biomart, Shirley, NY, USA) were diluted in PBS at different concentration. TPP488, PDTC, SB203580 and LY294002 used for cell experiment were from Sigma Corporation (Hong Kong, China) and dissolved in 0.1% DMSO.

2.2. Cell culture

MPC5 conditionally immortalized mouse podocytes (passage 7–8) were kindly provided by Dr Peter Mundel (Albert Einstein College of Medicine, Bronx, NY) and cultured as previously described (Yuan et al., 2012; Yuan et al., 2015). Briefly, podocytes were cultivated in RPMI 1640 cell medium containing 10% fetal bovine serum, 100 U/ml penicillin, 100 mg/ml streptomycin, and 2 mM L-glutamine (Krtil et al., 2007). To permit immortalized growth, the culture medium was supplemented with 10U/ml of recombinant mouse γ-interferon to induce the expression of T antigen and cells were cultured at 33 °C (permissive conditions). To induce differentiation, cells were cultured on collagen IV under non-permissive condition (37 °C) without γ-interferon for 10–14 days (Saleem et al., 2002). The differentiated podocytes were used in our cell experiment. Primary mouse mesangial cells were isolated and cultured as previously described (Cortes-Hernandez et al., 2002; Mene and Stoppacciaro, 2009).

2.3. Diabetic nephropathy model

Type 1 diabetes was induced by streptozotocin (STZ) injection according to the previous study (Brosius et al., 2009) and all experimental protocol was approved by the Animal Ethics Committee of Fudan University. Seven to eight weeks old female C57BL6 mice were injected intra-peritoneally with STZ (Sigma, 40 mg/kg body weight) dissolved in 10 mM sodium citrate buffer (pH = 4.6) for 5 consecutive days (after an overnight fast). Blood glucose levels were measured every 3 days and the mice with blood glucose between 13.9 and 22.2 mmol/L were regarded as mice with diabetes mellitus (DM). Those received the same volume of vehicle served as normal mice (the control group). Each group had 10 mice. All animals were fed with standard laboratory diet and provided with water ad libitum. Blood glucose levels of DM mice were monitored in tail vein blood every week and urine was tested for ketone bodies. When necessary, DM mice were given supportive insulin treatment (1 IU/kg body weight, Ultratard, Novo Nordisk, Denmark) twice a week to prevent apparent exhaustion and ketosis. The ratio of urinary micro-albumin to creatinine (urinary mAlb/Cr) was assayed every two weeks according to our previous study. Those with elevated urinary mAlb/Cr over than 30 mg/g were regarded as DN mice. When DN mice model was established 16 weeks after STZ treatment, fresh kidney cortices were excised right after animals were sacrificed and blood samples were collected and centrifuged to obtain serum. All samples were stored at −80 °C until further study.

2.4. Enzyme-linked immunosorbent assay (ELISA)

The serum and renal cortex CML and HMBG-1 levels were measured by ELISA kits (Beyotime Biotechnology, Shanghai, China) according to the manufacture. The heparanase protein levels in incubation medium were also measured by commercial kits (Advanced BioChemicals, Lawrenceville, GA, USA) according to the operating instructions. The intra-assay and the inter-assay co-efficient of variation was 4.2–3.6%, respectively (range 78.1–5000 pg/ml).

2.5. Real time RT-PCR

Total RNA was extracted from cultured podocytes using RNeasy mini kit (Qiagen, MD, USA). Total 1 μg RNA was reverse transcribed into cDNA using the Revert-Aid First Strand cDNA Synthesis Kit (Thermo Scientific, MA, USA). Heparanase expression was quantified by the delta-delta CT method using SYBRs Green Supermix and the ABI system (Bio-Rad, Berkeley, CA, USA). Mouse heparanase primers: (Forward) 5′-GGAGCAAACTCCGAGTGTATC-3′ and (Reverse) 5′-CAGAATTTGACCGTTCAGTTGG-3’. GAPDH was used as the housekeeping gene. Mouse GAPDH primers: (Forward) 5′-AGAAACCTGCCAAGTATGATGAC-3′ and (Reverse) 5′-TCATTGTCATACCAGGAAATGAG-3’. Three independent experiments were performed.

2.6. Western blot

The cultured podocytes were homogenized in radioimmunoprecipitation assay (RIPA) buffer at 4 °C. After

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