II-induced fibronectin accumulation in MCs and adhesion molecule expression in ECs. We propose that HG and Ang II promote eNOS uncoupling in the glomerular mesangium and endothelium in diabetes, thereby resulting not only in the elimination of the protective effect of eNOS, but also converting the enzyme to a phlogistic mediator that further enhances ROS generation and renal cell injury. Understanding the mechanisms of eNOS uncoupling is essential if the above observations are to be translated to clinical therapeutic regimens aimed at recoupling of eNOS and restoring NO production.

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F-PO1340

Lack of Heparan Sulfate in Perlecan Accelerates Diabetic Nephropathy Li-Jun Ma,[1] Jun Zhou,[1] Sebastian A. Potthoff,[1] Haichun Yang,[1] Dorin-Bogdan Borza,[1] Zhongjun Zhou,[3] Kevin McCarthy,[2] Agnes B. Fogo.[1] *[1]Pathology and Medicine, Vanderbilt Univ. Med. Ctr., Nashville, TN; [2]Pathology, LSU Health Sciences Ctr., Shreveport, LA; [3]Biochemistry, Univ. of Hong Kong, Hong Kong.*

Perlecan is a heparan sulfate (HS) proteoglycan in the glomerular basement membrane (GBM) and mesangial matrix. We assessed whether lack of HS in perlecan modulates diabetic nephropathy.

Male 8 wk old mice, with perlecan lacking HS attachment sites in N-terminal domain I (Hspg2Δ3/Δ3, n=8, C57Bl/6 background), and littermate WT (n=8) were given STZ. Metabolic parameters and urinary albumin/creatinine ratio (ACR) were examined. Glomerular mesangial expansion (0-4+) and GBM thickness were assessed at 20 wks. GBM anionic sites were detected by polyethyleneimine staining.

Blood glucose and body weight were similar at baseline in both groups. Hyperglycemia was more severe in Hspg2Δ3/Δ3 vs WT (wk 20: 480±24 vs 317±80 mg/dl, $p<0.05$). ACR was markedly higher in Hspg2Δ3/Δ3 vs WT at 4 wks after STZ (wk 0: 50±7 vs 46±6; wk 4: 118±26 vs 41±5, $p<0.05$ at wk 4; wk 20: 62±5 vs 63±8 µg/mg, pNS). Systolic blood pressure was comparable in diabetic Hspg2Δ3/Δ3 vs diabetic WT at 20 wks. Non-diabetic Hspg2Δ3/Δ3 mice at baseline had mild mesangial expansion and arteriolar hyalinization. At 20 wks post STZ, Hspg2Δ3/Δ3 mice developed moderate mesangial expansion (1.4±0.1) and more tubular dilation vs WT (1.0±0.1, $p<0.01$). Mesangial laminin alpha 2 expression was increased in diabetic Hspg2Δ3/Δ3 vs diabetic WT. GBM was thicker in diabetic Hspg2Δ3/Δ3 vs diabetic WT (0.24±0.01 vs 0.19±0.01 µm, $p<0.05$). Charge density of the GBM was not altered.

We conclude that lack of perlecan-HS accelerates the onset and progression of diabetic nephropathy, and transient increase in albuminuria is not due to GBM charge density. We speculate that effects of heparan sulfate of perlecan on glomerular cells (including mesangial cells) may contribute to the severe kidney phenotype seen in diabetic Hspg2Δ3/Δ3 mice.

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F-PO1341

Accelerated Diabetic Renal Injury in Catalase Null Mice Inah Hwang,[1] H. Bahl Lee,[2] Hunjoo Ha.[1] *[1]College of Pharmacy and Division of Life & Pharmaceutical Sciences, Ewha Womans University, Seoul, Korea; [2]Kim's Clinic and Dialysis Unit, Miryang, Korea.*

Backgrounds: Reactive oxygen species (ROS) play an important role in the development and progression of diabetic renal injury. Catalase is a major antioxidant enzyme that decomposes hydrogen peroxide, thereby preventing the generation of hydroxyl radical by the Fenton reaction. The antioxidant activity of catalase is dependent on the type of tissue and pathologic condition. Catalase overexpression was recently shown to protect against diabetic renal injury, but the effect of catalase deficiency on diabetic nephropathy has not been reported.

Methods: Six-week-old male homozygous catalase knock-out (*Cat*$^{-/-}$) mice and wild-type (WT) mice were divided into two subgroups (8 mice per group): control and streptozotocin (STZ)-induced diabetic group. Experimental diabetes was induced by intraperitoneal injection of STZ 50 mg/kg for 5 days. At 4 weeks after the induction of diabetes, blood glucose, plasma creatinine, and urinary albumin excretion rate were measured. Mice were then sacrificed and kidney weight was obtained. Whole kidneys were homogenized and E-cadherin, α-smooth muscle actin (α-SMA), bone morphogenic protein-7 (BMP-7), and fibronectin protein and mRNA were measured.

Results: STZ induced hyperglycemia in both WT and *Cat*$^{-/-}$ diabetic mice. Urinary albumin excretion was significantly increased only in *Cat*$^{-/-}$ diabetic mice among experimental groups. Renal expression of E-cadherin and BMP-7 decreased in both diabetic mice groups compared to respective control mice. Changes in E-cadherin and BMP-7 expression in *Cat*$^{-/-}$ diabetic kidneys tended to be higher compared to WT diabetic kidneys, although the statistical significance has not been reached. Fibronectin and α-SMA expression was significantly increased in *Cat*$^{-/-}$ diabetic mice compared to WT control and WT diabetic mice.

Conclusion: Catalase deficiency appears to render diabetic animals more susceptible to renal injury, supporting the importance of catalase against oxidative renal injury in diabetes. Further studies are required for long-term effect of catalase deficiency in the progression of diabetic nephropathy.

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F-PO1342

IRS-2 Signalling during Kidney Fibrosis in Diabetic Nephropathy Rosemarie M. Carew, Derek P. Brazil. *Biomolecular and Biomedical Science, Conway Institute UCD, Dublin, Ireland.*

Dysregulation of insulin signalling and secretion results in the development of diabetes and its associated complications. Insulin requires a family of adaptor molecules, called insulin receptor substrate (IRS) proteins, to transmit its signal within cells. Mice lacking IRS-2 develop type 2 diabetes and defects in organs such as the brain and retina.

Our hypothesis is that mice lacking IRS-2 will display signalling defects in the kidney that contribute to diabetic nephropathy in vivo.

In vitro experiments to investigate the role of IRS-2 in TGF- β-mediated fibrosis in diabetic kidney disease were carried out. TGF- β stimulation causes cells to undergo epithelial-mesenchymal like changes leading to tubular dysfunction. IRS-2 is expressed in multiple kidney epithelial cells. siRNA was utilised to knockdown IRS-2 expression and the effect of TGF- β-induced EMT was examined. TGF- β causes serine phosphorylation of IRS-2 and retards its mobility in HK-2 cells. The FOXO family and FOXO regulated genes e.g. MnSOD and Bim, which are downstream of TGF- β and IRS-2 have been analysed. Stimulation of HK-2 cells by TGF- β caused a significant decrease in the expression of MnSOD and Bim at 24hr, 48hr and 72hr.

Downregulation in IRS-2 mRNA was detected in the kidneys of a streptozotocin-induced type 1 diabetic mouse. Our in vivo strategy involved analysing pre and post diabetic time points for both male and female IRS-2+/+, IRS-2-/+ and IRS-2-/- C57Bl/6 mice. Male IRS-2-/- mice become spontaneously diabetic at 10-12wks, with a fasting blood glucose >20mmol/L. Females follow a similar disease progression but at a later stage. We detected evidence of renal kidney damage with microalbuminuria and decreases in urinary creatinine in IRS-2-/- vs wild-type mice. Significant alterations in the expression of genes which are implicated in tubulointerstitial fibrosis were also detected e.g. CTGF and gremlin. Evidence suggests that downregulation of FOXO regulated genes occurs in the IRS-2-/- mice compared to wild-types. Markers for EMT will also be assessed in the post diabetic time points in IRS-2-/- vs wild-types.

Together these approaches will aim to elucidate a signalling pathway centering on IRS-2 in diabetic nephropathy.

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F-PO1343

Type 2 Diabetes and Nephropathy in db/db Mice: Role of MKK3-p38 MAPK Signaling Andy K. H. Lim,[1] David J. Nikolic-Paterson,[1] Frank Y. Ma,[1] Elyce Ozols,[1] Richard A. Flavell,[2] Roger J. Davis,[3] Gregory H. Tesch.[1] *[1]Dept Nephrology and Monash Uni Dept of Medicine, Monash Medical Centre, Clayton, Victoria, Australia; [2]School of Medicine, Yale University, New Haven, CT; [3]Uni of Massachesetts Medical School, Howard Hughes Medical Institute, Worchester, MA.*

Increased intracellular p38 mitogen-activated protein kinase (MAPK) signaling occurs with obesity and diabetes, and may promote tissue inflammation and injury. Although p38 MAPK can be activated by either of the two upstream kinases (MKK3 or MKK6), recent evidence suggests that MKK3 has non-redundant roles in pathological p38 MAPK activation. The aim of this study was to determine whether MKK3 deficiency affects the development of obesity, type 2 diabetes and diabetic nephropathy. C57BL/6 db/db mice deficient in the Mkk3 gene were assessed between 8 and 32 weeks of age. Mkk3+/+ db/db and Mkk3-/- db/db mice developed equivalent obesity and an equivalent incidence and severity of diabetes. Kidney levels of phospho-p38 and MKK3 protein were increased in diabetic Mkk3+/+ db/db mice but remained normal in diabetic Mkk3-/- db/db mice despite a 4-fold compensatory increase in MKK6 protein. Reduced p38 MAPK signaling in Mkk3-/- db/db mice was associated with a reduction in albuminuria, podocyte loss, mesangial cell activation and glomerular fibrosis. MKK3 deficiency also protected diabetic db/db mice from tubular injury and interstitial fibrosis, which was associated with reduced kidney levels of MCP-1 and interstitial macrophage accumulation. Thus, MKK3-p38 MAPK signaling is not required for the development of obesity or type 2 diabetes but plays a distinct pathogenic role in the progression of diabetic nephropathy in db/db mice.

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F-PO1344

Diabetes-Induced Loss of Podocyte Density Is a Novel Phenotypic Trait in C57BL/6J (Resistant) and DBA2/J (Susceptible) Mouse Strains Haiying Qi,[1] Kremena Star,[2] Shaolin Shi,[1] Erwin Bottinger.[1] *[1]Medicine, Mount Sinai School of Medicine, New York, NY; [2]Medicine, Albert Einstein College of Medicine, New York, NY.*

The onset of diabetes triggers a significant loss of podocytes in type 1 and type 2 diabetic patients and murine models. Similar to differential genetic susceptibility in human diabetic nephropathy, diabetic inbred DBA2/J (D2) mice are more prone to albuminuria and mesangial expansion compared with diabetic C57BL/6J (B6). We hypothesized that D2 mice are susceptible and B6 mice are resistant to podocyte loss after STZ-induced diabetes. 8-week-old D2 and B6 mice were subjected to multiple low-dose STZ injections for 5 constitutive days. The time of onset and the levels of hyperglycemia (more than 400mg/dl) were comparable between both strains. Control B6 (B6C) and D2 (D2C), and diabetic B6 (B6DM) and D2 (D2DM) mice were sacrificed after 3, 6, and 12 wks of diabetes,