cystatin C, and induced glomerular hypertrophy, glomerulosclerosis, extracellular matrix expansion, and tubular atrophy. Treatment with drug X yielded improved renal function, reduced induced glomerular and podocyte injury. Increased NETs formation in diabetes was paralleled by an increase in NOX-dependent ROS production and mTOR signaling pathway activation. Our findings were further confirmed in transcriptomic analysis of podocyte-specific NETs, and positive correlation between NETs and DKD was observed. Querying protein-protein interaction databases also revealed an association between NETs markers, mTOR signaling proteins, and NOXs.

Conclusions: To our knowledge, this study is the first to describe the role of NETosis in DKD, identifying NETosis as one of the final mechanistic drivers of DKD.

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PO0679

Alteration of Autophagy-Related Protein 5 (ATG5) Levels and Atg5 Gene Expression in Diabetes Mellitus with and Without Complications

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Background: Autophagy is a catabolic mechanism that involves lysosomal-dependent degradation of unnecessary or ineffective intracellular components. Autophagy is the process responsible for normal cellular homeostasis, by recycling organelles and proteins. Autophagy pathway and its key participant ATG5 are associated with several pathologies such as diabetes mellitus and its complications.

Methods: Levels and expression of autophagy key components ATG5 and LC3B were analyzed in both human model and murine tissues. One hundred and twenty human subjects were divided into four groups: Healthy (control), diabetic without complications, diabetic nephropathy and diabetic retinopathy. Additionally, we used kidneys from diabetic mice model (WT healthy mice and DN mice). Lesion data were derived from human peripheral blood mononuclear cells, and murine renal cortex lysates were subjected to western blot analyses of ATG5 and LC3B and immunohistochemical analysis was performed on mouse renal tissues.

Results: Western blot and immunohistochemical analysis demonstrate that ATG5 protein levels were significantly decreased in DM, DN and DR patients (0.59±0.07; 0.67±0.06; 0.72±0.06 A.U. units respectively), vs. healthy controls (0.96±0.16 A.U. units), and in DN mice compared to healthy mice (0.65±0.04; 1.15±0.13 A.U. units respectively). Quantitative staining of area (%) of ATG5 mice tissue expression also decreased in DN vs. healthy mice (4.42±1.08%; 10.87±1.01% respectively). LC3B levels and expression correlates with ATG5 results: significant reduction in peripheral blood mononuclear cells diabetic patients (with or without complications) vs. healthy controls (0.44±0.05; 0.42±0.03; 0.8±0.60 compared with 0.81±0.05 A.U. units). Renal LC3B levels were lower in DN vs. healthy mice (0.6±0.03; 0.68±0.07 A.U. units). Renal LC3B staining quantification revealed significant reduction in DN vs. healthy mice (1.7±0.23%; 8.5±1.17%)

Conclusions: We conclude that ATG5, as well as LC3B, are down regulated in diabetic patients with or without complications. This diminution contributes to deficiencies in the autophagy process. Our observations show a novel association between autophagy-related protein 5 (ATG5) and diabetic kidney and retinal diseases, with ATG5 as a candidate protein for diabetic nephropathy and retinopathy.

PO0680

The Emerging Role of the mTORC2/Rictor Signaling Complex in Autophagy Dysfunction-Associated Diabetic Kidney Disease

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Background: Podocyte injury has been implicated in the pathogenesis of many renal diseases, including diabetic kidney disease (DKD). Dysregulation of podocyte autophagy has been positively correlated with podocyte loss and progression of proteinuria in patients with diabetes. Yet, the exact mechanisms behind diabetes-induced autophagy dysfunction remain to be elucidated. Various signaling pathways including the mTORC1 complex have been implicated in podocyte autophagy dysfunction. However, the role of mTORC2 in autophagy and its interaction with key mechanistic pathways involved in DKD, including the ROS-producing enzymes, are still unknown. Herein, we investigated the role of mTORC2, its crosstalk with the NADPH oxidases 4 (Nox4)-induced ROS, and its effect on autophagy, and the possible link to podocyte integrity in animal models of type 1 and type 2 diabetes.

Methods: Type1 diabetes was induced in mice by streptozotocin (STZ) injections, and type 2 diabetes was initiated by a ‘western’ diet followed by low-dose STZ injections. Mice were divided into control, diabetic, and diabetic treated with a selective mTORC2 inhibitor (XR-AB2-011). Functional, pathological, and biochemical studies were performed.

Results: Diabetes-induced podocyte injury is reflected by alterations of the slit diaphragm proteins, paralleled by podocyte depletion as assessed by decreased WT1 staining and accompanied by autophagy dysregulation. The effect of autophagy was further highlighted in control mice treated with the autophagy inhibitor hydroxycyclochrome, that mirrored the effect of diabetes on functional, phenotypic, histological, and molecular changes in the kidney. These observations were concomitant with an observed activation of the mTORC2/Rictor protein expression and increased levels of superoxide generation through Nox4. Of interest, these results were paralleled by activation of the mTORC1/p70S6K pathway. Moreover, specific inhibition of mTORC2 curbed the homeostatic function of the kidneys and restored the histological and phenotypic changes, consistent with regulating the Nox4/mTORC1 signaling axis. More importantly, JR treatment regulated diabetes-induced autophagy protein dysregulation (Beclin, Atg3, and LC3).

Conclusions: Our data suggest that targeting mTORC2 signaling could be a potential therapeutic target for DKD.

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PO0681

Mitophagy-Related Renal and Proximal Tubular Protection During the Normoalbuminuric Stage of Diabetes Mellitus

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Background: Oxidative stress during the normoalbuminuric stage of type 1 diabetes mellitus (DM) damages renal cortical mitochondria. Because accumulation of damaged mitochondria can contribute to renal dysfunction, we aimed to determine a) if oxidative stress triggers mitophagy as a mitochondrial quality control mechanism, and b) if the renal cortical structures in which these events occur.

Methods: Rats receiving i.p. injection of streptozotocin (STZ, 65 mg/kg) or vehicle (Sham) were either left untreated or treated with telmisartan (TLM, an angiotensin II receptor blocker; 10 mg/kg/d). Two weeks later, blood pressure (BP), renal filtration rate, and urinary excretion of albumin and N-acetyl-β-D-glucosaminidase (NAG) were measured. The oxidative stress marker, 3-nitrotyrosine (3-NT), was detected by HPLC. Mitophagy-related proteins (LC3-II, p62, PINK1, PINK2) were measured by western blot and immunohistochemical analysis was performed on tissue samples.

Results: STZ rats displayed hyperglycemia and hyperfiltration that were unaltered by TLM. BP, albumin excretion, and NAG excretion were similar in all groups. Renal cortical LC3-II levels were increased in STZ rats, a change that was prevented by TLM (STZ+TLM). Renal cortex from STZ rats displayed TLM-sensitive increases in LC3-II and PINK1 (all P<0.05), although BNIP3 and p62 levels did not differ among groups. HistoScore data failed to reveal mitophagy-related proteins in glomeruli. In contrast, in diabetic mice model (BTBR) without EMPA treatment, renal cortical 3-NT levels were increased in STZ rats; this effect was blunted in STZ+TLM rats. Mitophagy-related protein immunostaining was also apparent in distal tubules, but the HistoScores tended to be less than that of proximal tubules and were unaffected by STZ or TLM.

Conclusions: During the normoalbuminuric stage of DM, renal cortical mitophagy is most prominent in the proximal tubule. This effect is blunted by TLM in association with its antioxidant effect, suggesting a mitophagy-related proximal tubular protection mechanism triggered by oxidative damage.

PO0682

The Molecular Effect of the Sodium-Glucose Transporter 2 (SGLT-2) Inhibitor Empagliflozin on the Autophagy Pathway in Diabetes Mellitus and Its Vascular Complications

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Background: Diabetes mellitus (DM) is a severe metabolic disorder characterized by chronic hyperglycemia. DM is associated with increased oxidative stress that can lead to metabolic and vascular complications. The kidney is a major target for both diabetes and its adrenergic diabetic drug, known as SGLT2, in T2DM patients. Autophagy is a catabolic mechanism that involves lysosomal-dependent degradation of unnecessary or dysfunctional intracellular components, and is known to have an important role in DM complication (Diabetic Nephropathy-DN). Two weeks after the leptin-deficiency mutation that develops severe type II DM which is presented with hyperglycemia and DN. EMPA was administered to the diabetic mice via drinking water for a period of 12 weeks. Routine monitoring of blood and urine standard DM parameters will be carried through experiment duration. At the end of the experiment, mice kidneys will be removed and subjected to further biochemical and histological analysis. Western blot analysis demonstrated the protective role of EMPA treatment on DN via the autophagic proteins ATG5 & LC3B.

Methods: We used T2DM animal model-mouse strain BTBR with the ob/ob leptin-deficiency mutation that develops severe type II DM which is presented with hyperglycemia and DN. EMPA will be administrated to the diabetic mice via drinking water for a period of 12 weeks. Routine monitoring of blood and urine standard DM parameters will be carried through experiment duration. At the end of the experiment, mice kidneys will be removed and subjected to further biochemical and histological analysis. Western blot analysis demonstrated the protective role of EMPA treatment on DN via the autophagic proteins ATG5 & LC3B.

Results: Blood glucose concentration was normal in control mice (C57) throughout the experiment. In DM mice (BTBR) without EMPA, blood glucose concentration was higher than control, and lower in diabetic mice treated with EMPA compared to DM. Urine volume of BTBR mice treated with EMPA increased throughout the experiment and was higher in comparison to DM mice without EMPA treatment. Renal cortical

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Underline represents presenting author.