Diabetic Complications Consortium

Application Title: Targeting Neuromedin S as a novel therapy for diabetic kidney disease

Principal Investigator: Jinhua Li

1. Project Accomplishments:

The estimated project period was "from October 1, 2015 to September 30, 2016". My animal ethics application was approved on February 12, 2016. I started this project from March 2013. The no-cost extension request has been approved by the Division of Sponsored Program Administration, Augusta University to reflect the dates 08/29/16-03/31/17 to incorporate the additional time.

We have completed the preventive study in vivo to determine whether administration of neutralizing α-Neuromedin S (NMS) Ab can reduce the development and progression of diabetic kidney disease in eNOS-/- db/db mice in a preventive strategy. We also completed part of the in vitro study to investigate whether diabetic podocyte and mesangial cell injury and apoptosis is dependent upon NMS.

Specific Aims:

Aim of the study: To determine whether administration of α-NMS Ab can reduce development and progression of DN in eNOS$^{-/-}$ db/db mice.

Specific Aim 1. Pharmacologic blockade of NMS in DN.
Experimental groups: (i) Control IgG (week 8 to 26); (ii) α-NMS Ab (week 8 to 26); (iii) Control IgG (week 16 to 26); (iv) α-NMS Ab (week 16 to 26). eNOS-/- mice are used as controls.

Results: We completed the preventive strategy experiments: treated ***eNOS$^{-/-}$db/db*** mice using control IgG (week 8 to 26) or α-NMS Ab (week 8 to 26).

Early intervention with α-NMS Ab inhibits the development and progression of diabetic renal injury

Groups of diabetic eNOS-/-db/db mice were fed standard chow and treated with control IgG or α-NMS Ab from week 8 until being killed on week 26. Compared with control

IgG, α-NMS Ab had no effect upon HbA1c levels (Fig 1). A substantial increase in urinary albumin excretion was evident in eNOS-/-db/db group treated with control IgG. The treatment with α-NMS Ab significantly reduced proteinuria compared with control IgG (Fig 2). There was also a reduction in the number of glomerular WT1+ podocytes at week 26. The loss of podocytes was partially rescued by the administration with α-NMS Ab (Fig. 3). The decline in renal function at week 26 after the development and progression of diabetic kidney disease, as assessed by increased serum creatinine levels, was significantly improved by α-NMS Ab treatment (Fig. 4).

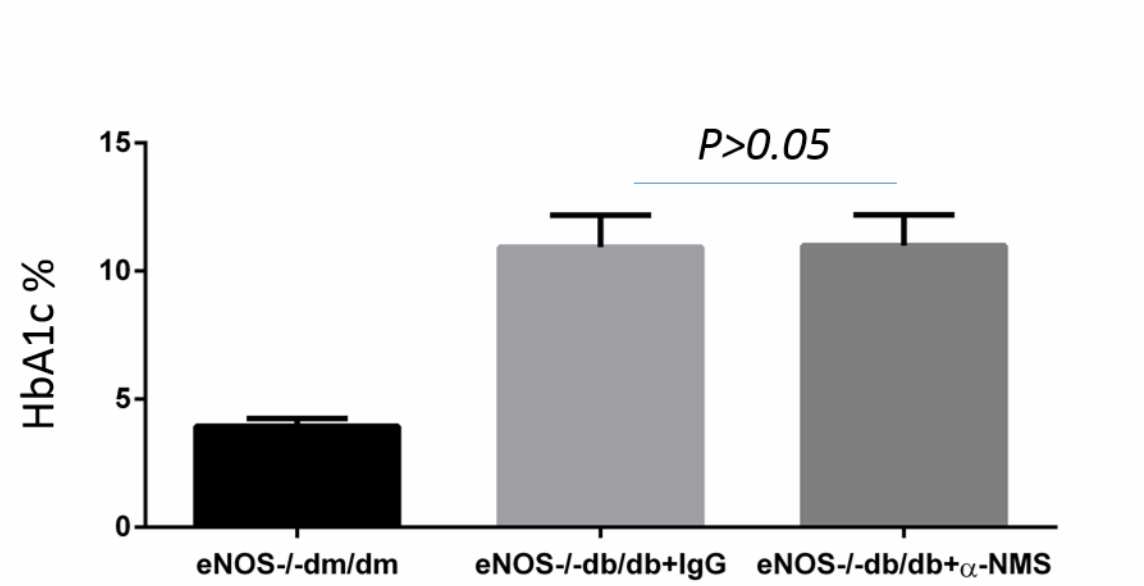

Figure 1. *Effect of α-NMS Ab on HbA1c during the development and progression of diabetic kidney disease in eNOS-/-db/db mice. Groups of diabetic eNOS-/-db/db mice were fed standard chow with treatment with control IgG or α-NMS Ab from week 8 until being killed on week 26. Age-matched nondiabetic eNOS-/-dm/dm mice were used as controls.*

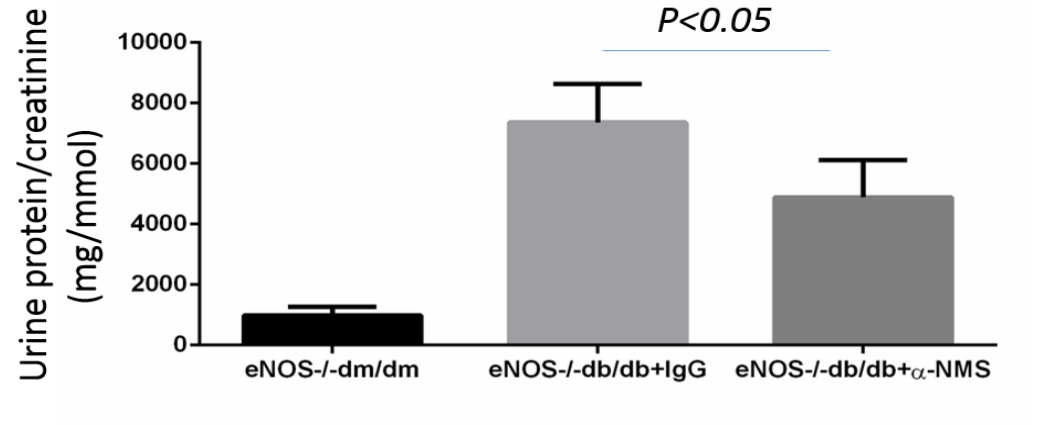

Figure 2. *Effect of α-NMS Ab on proteinuria during the development and progression of diabetic kidney disease in eNOS-/-db/db mice. Groups of diabetic eNOS-/-db/db mice were fed standard chow with treatment with control IgG or α-NMS Ab from week 8 until being killed on week 26. Age-matched nondiabetic eNOS-/-dm/dm mice were used as controls. Urine protein-to-creatinine ratio was calculated as mg/mmol.*

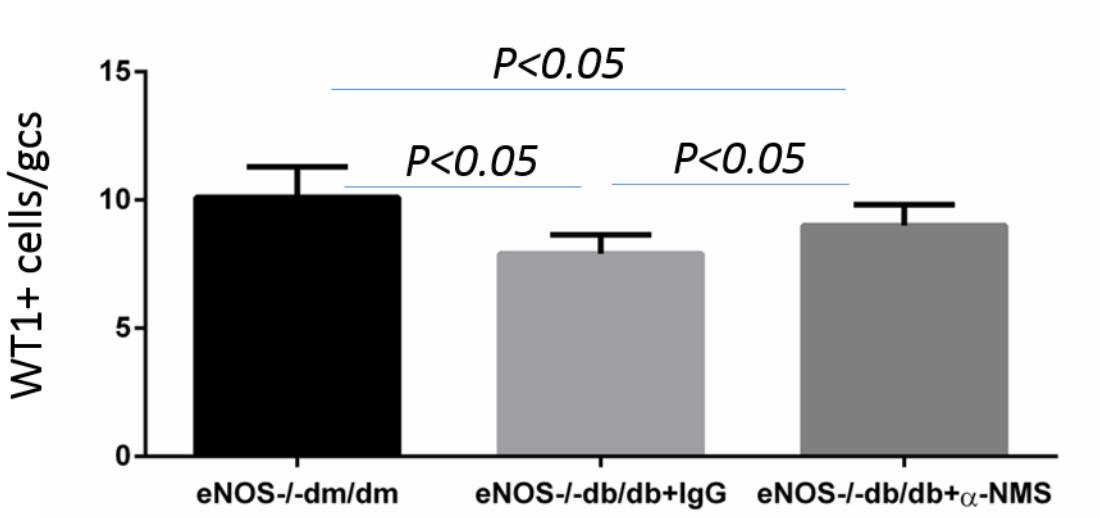

Figure 3. *Effect of α-NMS Ab on podocytes during the development and progression of diabetic kidney disease in eNOS-/-db/db mice. Groups of diabetic eNOS-/-db/db mice were fed standard chow with treatment with control IgG or α-NMS Ab from week 8 until being killed on week 26. Age-matched nondiabetic eNOS-/-dm/dm mice were used as controls. Number of WT1+ podocytes per glomerular cross-section.*

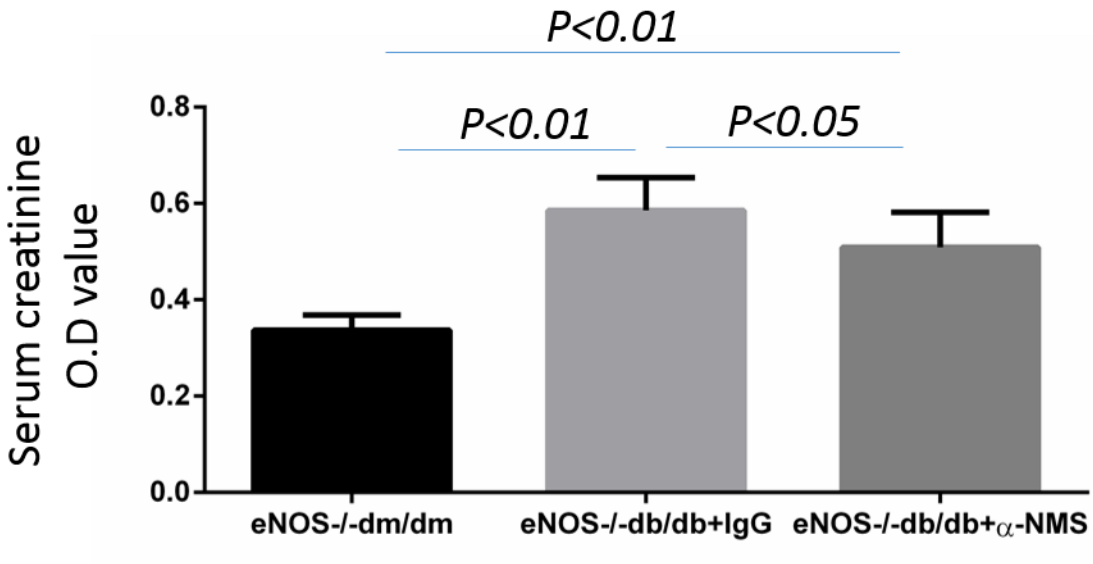

***Figure4**. Effect of α-NMS Ab on serum creatinine during the development and progression of diabetic kidney disease in eNOS-/-db/db mice. Groups of diabetic eNOS-/-db/db mice were fed standard chow with treatment with control IgG or α-NMS Ab from week 8 until being killed on week 26. Age-matched nondiabetic eNOS-/-dm/dm mice were used as controls. Serum creatinine was calculated as O.D.*

<u>Early intervention with α-NMS Ab inhibits the development and progression of renal fibrosis</u>

The administration of α-NMS Ab also reduced glomerulosclerosis as demonstrated by PAS (Fig 5A-D), collagen IV staining (Fig 5E-G) and CD68 macrophage infiltration (Fig 5H).

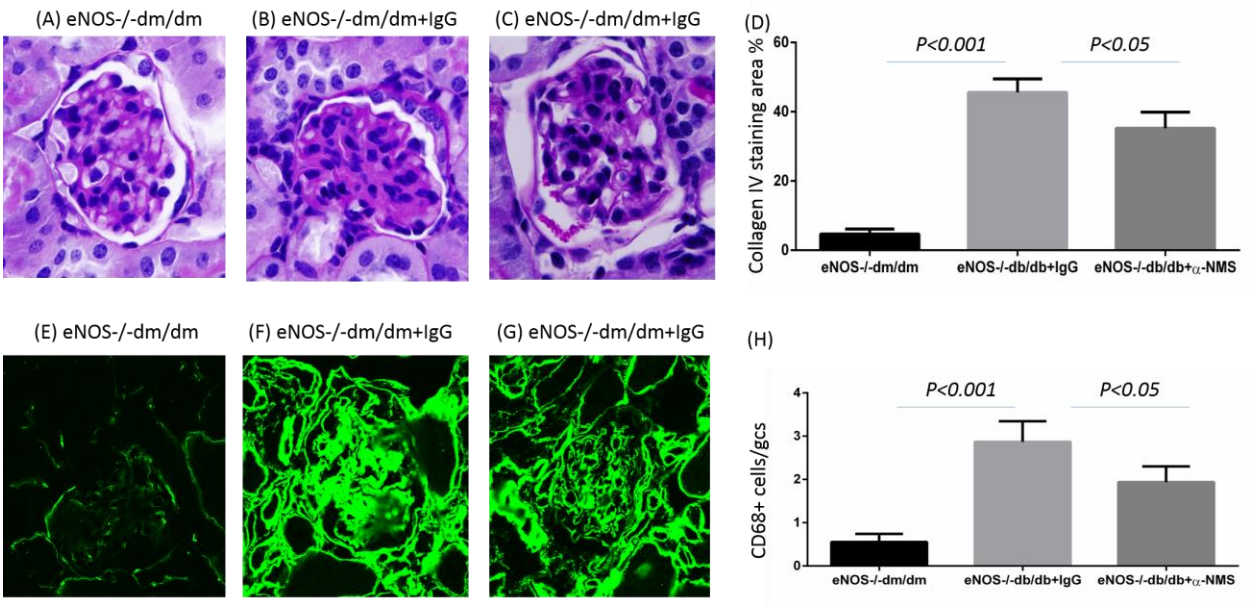

***Fig 5.** Effect of α-NMS Ab on glomerular damage during the development and progression of diabetic kidney disease in eNOS-/-db/db mice. Groups of diabetic eNOS-/-db/db mice were fed standard chow with treatment with control IgG or α-NMS Ab from week 8 until being killed on week 26. Age-matched nondiabetic eNOS-/-dm/dm mice were used as controls A–C: PAS-stained kidney sections. A: Nondiabetic control eNOS-/-dm/dm kidney. B: Significant PAS-stained deposits were seen in glomeruli after 26 weeks of diabetes in mice with no treatment. C: Treatment with α-NMS Ab over weeks 8-26 reduced glomerular PAS-stained deposits. E–G: Confocal microscopy demonstrated immunostaining for collagen IV (green. E: Nondiabetic control eNOS-/-dm/dm kidney showing staining of collagen IV in the glomerular basement membrane. F: Significant mesangial deposition of collagen IV after 26 weeks of diabetes with no treatment. G: Treatment with α-NMS Ab over weeks 8-26 suppressed glomerular collagen IV deposition. D: Quantification data for collagen IV staining area, %. H: Quantification of the number of glomerular CD68+ macrophages.*

Specific Aim 2. Is diabetic podocyte and mesangial cell injury and apoptosis dependent upon NMS?

Results: We have treated mouse endothelial cells, mesangial cells with TGF-β1 and podocytes with TNF-α and at the same time with these cells were also treated with neutralizing anti-NMS antibody or IgG. Western blotting demonstrated TGF-β1 induced fibrotic response in endothelial cells and mesangial cells while TNF-α induced loss of synaptopodin in podocytes. The neutralizing anti-NMS Ab, not control IgG significantly reduced TGF-β-induced fibrotic response in endothelial cells and mesangial cells and TNF-α-induced injury in podocytes, demonstrating the critical role of NMS in glmoerulosclerosis and podocyte injury (Fig 6).

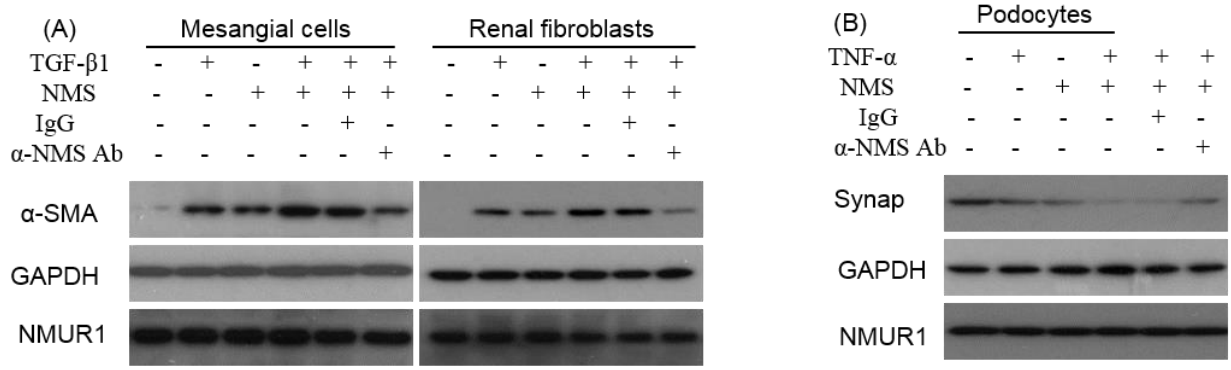

Figure 6. *Western blotting shows that the NMS peptide enhances TGF-β1-induced expression of α-smooth muscle actin (α-SMA) in mesangial cells and renal fibroblasts (A) and that NMS enhances TNF-α-induced down-regulation of podocyte expression of synaptopodin (Synap, B). Effects of NMS were neutralized by our mouse anti-NMS antibody, but not by control mouse IgG (A&B). These renal cells express the NMS receptor, NMUR1 (A&B).*

2. Publications:

Not yet.