Diabetic Complications Consortium

Application Title: Molecular Mechanism of Diabetic Bladder Dysfunction
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1. Project Accomplishments:

Diabetes mellitus (DM) afflicts 9.4% of the US population, and diabetic bladder dysfunction (DBD) is the most common complication. However, the molecular mechanism of DBD is unclear. We hypothesize that insulin resistance, or disruption of insulin signaling itself in bladder underlies the pathogenesis of DBD. To test this hypothesis, we have generated smooth muscle specific insulin receptor (SMIR) deleted mice. Our data have shown that these SMIR deleted mice exhibit mild overall metabolic disorders. However, they exhibit significant abnormal bladder morphology and voiding dysfunction with voiding frequency and decreased voiding volume per void. Further myography studies show that deletion of SMIR result in significant decrease of bladder smooth muscle contraction forces in response to electrical field stimulation, KCl, carbachol, and α,β -meATP stimulation. These data fully support our hypothesis, and provide a novel mechanism for the pathology of DBD. Based on this accomplishment, we will further use genetic tools and microarray technique to study the molecular pathways involved in DBD.

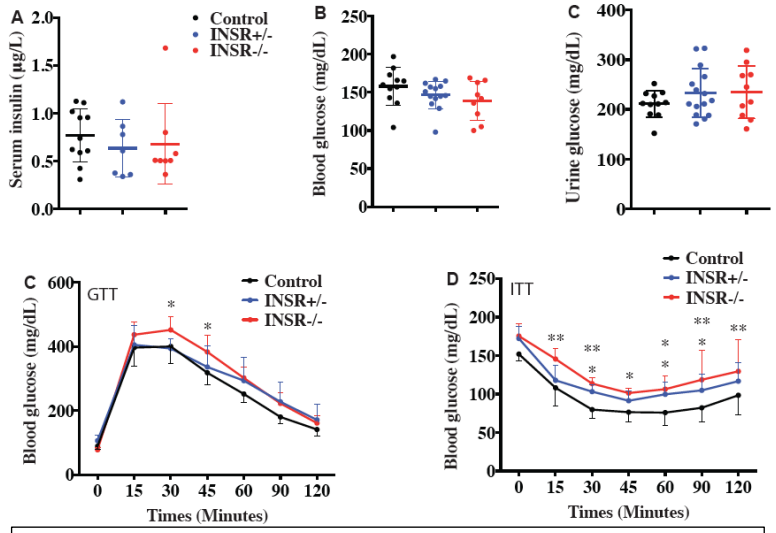

Figure 1. Deletion of insulin receptor in smooth muscle results in abnormal GTT and ITT responses. Insulin and glucose levels were measured in SMIR deleted mice and compared to matching wild type mice. * indicate p≤0.05.

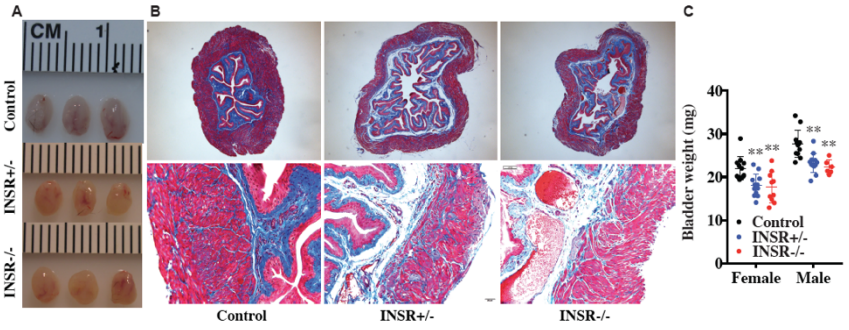

Figure 2. Deletion of insulin receptor in smooth muscle results in abnormal bladder morphology. A. is the gloss morphology of bladders from SMIR deleted mice; B is images from Masson's trichrome stained bladder sections. C is the bladder weight from SMIR deleted bladders. * indicate p≤0.05.

2. Specific Aims:

Specific Aim 1. To test the hypothesis that disruption of insulin receptor mediated signaling in bladder smooth muscle cells underlies the pathogenesis of DBD.

Results: We have generated heterozygous and homozygous smooth muscle specific IR deleted mice (SMIR+/- and SMIR-/-) by breeding *Sm22α-cre* mice (*B6.Cg-Tg(Tagln-cre)1Her/J*, The Jackson Laboratory, Maine) with *INSR$^{f/f}$* mice (Dr. C. Ronald Kahn's Lab at Harvard Medical School).

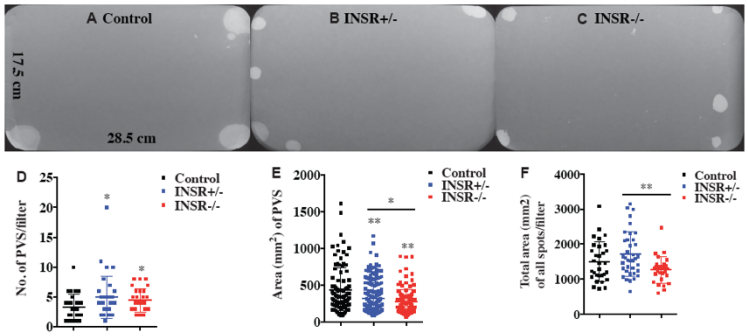

Figure 3. Deletion of insulin receptor in smooth muscle results in abnormal voiding pattern. A-C. are representative voiding patterns from SMIR deleted mice; D-F are quantitated data. * indicate p≤0.05.

Our results show that these heterozygous and homozygous SMIR deleted mice exhibit mild overall metabolic disorders as shown in Figure 1. Briefly, these mice have normal serum insulin, blood glucose, and urine glucose levels (Figure 1 A-C). However, they have increased blood glucose levels in response to GTT and ITT tests (Figure 1 D & E), indicating that these mice have impaired capability in maintaining normal blood glucose levels in response to GTT and ITT conditions.

Interestingly, these mice exhibit significant abnormal bladder morphology as shown in Figure 2. Overall, bladders from these SMIR deleted mice are lighter but floppy. Furthermore, they have thinner muscle layer with disorganized muscle bundles. Fibrosis can be observed. Significantly, dilated blood vessels are obviously. These morphological phenotypes mimic the diabetic bladder and suggest a critical role of insulin signaling in bladder smooth muscle function.

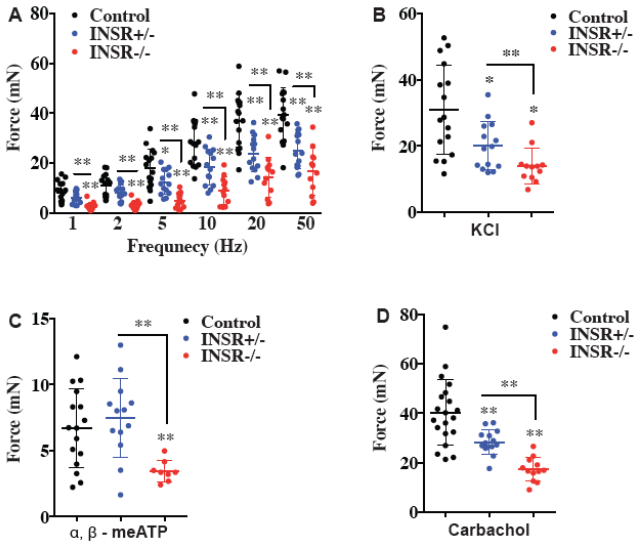

Figure 4. Deletion of insulin receptor in smooth muscle results in abnormal bladder contractility. Bladder muscle strips were subject to myography studies in response to EFS (A), KCl (B), α,β -meATP (C), and carbachol (D). * indicate p≤0.05.

Function test by voiding spot assay indicate these SMIR deleted mice exhibit significant voiding frequency and decreased voiding volume per void as shown in figure 3, indicating abnormal bladder function in these mice.

Myography studies show that deletion of SMIR result in significant decrease of bladder smooth muscle contraction forces in response to electrical field stimulation, KCl, carbachol, and α,β - meATP stimulation, especially in homozygous SMIR deleted mice (Figure 4). These results provide some mechanistic insight that insulin signaling in smooth muscle itself is critical for normal muscle function.

We have the following ongoing studies to further understand the mechanism of diabetic bladder dysfunction and the role of insulin receptors in bladder smooth muscle function. 1. We have created a tamoxifen inducible SMIR deleted mouse model to mimic type II diabetic bladder dysfunction, which was basically developed during adulthood. This will allow us to mimic the adulthood onset of the disease and eliminate the potential involvement of IR signaling during growth and development. 2. We are using microarray studies to understand the molecular pathways involved in these SMIR deleted mice. 3. We will study the potential signal pathways in these SMIR deleted mice based on the microarray data.

3. Publications:

Chen H, Xie X, Zeidel M, Yu W. Role of insulin receptor-mediated signaling in diabetic bladder dysfunction. Neurourology and Urodynamics; 2019 (38): S44. SUFU winter meeting abstract.

As soon as we finish the ongoing studies, we expect that at least one manuscript (full-length article) will be submitted to premier journal(s) for publication during 2020-2021.