



NLRP3-Dependent Mechanisms Downregulate Genes Controlling Urothelial Barrier Function in Diabetic Mice

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Introduction

Diabetic bladder dysfunction (DBD) is a progressive deterioration of urinary function commonly occurring in patients with diabetes. In addition to high glucose levels, diabetes is associated with metabolic derangement and the production of numerous potentially harmful metabolites which accumulate in the circulation as well as the urine. Bladder urothelia normally maintain an impenetrable barrier to protect underlying smooth muscle but this barrier is known to breakdown during diabetes. We have previously shown in the Akita diabetic mouse model that the NLRP3 inflammasome, a multimeric structure which activates inflammatory cascades, is responsible for diabetic bladder inflammation and dysfunction. This increases inflammation and bladder dysfunction. Additionally, the loss of barrier function may be a major factor in the increased susceptibility to urinary tract infections. As an initial investigation, we examined changes in expression of barrier genes in diabetic mice with either intact NLRP3 or in which it has been genetically deleted

Hypothesis

We hypothesize diabetes will reduce barrier function gene expression as a result of NLRP3 activation. Barrier function genes will be preserved in diabetic mice lacking NLRP3.

Methods

Genetically Modified Diabetic Mouse Model

- Type I diabetic Akita mice crossbred with NLRP3 null mice
- Animal groups:
 - Non-diabetic (n=9)
 - Diabetic (n=6)
 - Non-diabetic NLRP3 KO (n=12)
 - Diabetic NLRP3 KO (n=12)
- Animals aged to 15 weeks – time point which diabetic mice demonstrate overactive bladder phenotype

Gene Expression

- Urothelia from each group of 15 wk mice were excised
- Used qPCR to measure gene expression of:
 - Tight junctions:
 - Zona occludins 1 (ZO1)
 - Zona occludins 2 (ZO2)
 - Claudin 4 (CL4)
 - Adherins junctions:
 - Beta Catenin (BCT)
 - Uroplakins:
 - Uroplakin 1 (UP1)
 - Uroplakin 2 (UP2)



Results

Barrier function gene expression is downregulated in diabetic mice but preserved in diabetic mice without NLRP3

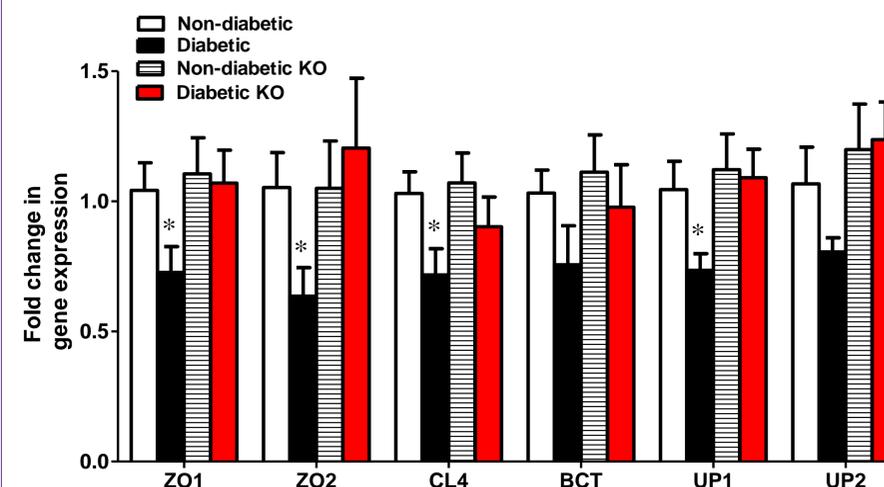


Figure 1. In 15 week old diabetic mice, barrier function genes ZO1, ZO2, CL4 and UP1 are downregulated. Diabetic mice without functional NLRP3 demonstrate no changes in gene expression compared to non-diabetic mice and non-diabetic mice with functional NLRP3. Data are mean±SEM. *p<0.05 vs Non-diabetic,; n=6-12/grp.

Conclusions

Diabetes reduces expression of genes regulating urothelial barrier function in a NLRP3-dependent manner. This finding provides insight into how DBD develops, and may identify a much needed therapeutic target for patients living with DBD.

Future Directions

- Assess how NLRP3-dependent mechanisms activated during diabetes impact *in vivo* barrier function.
- Determine the molecular mechanisms responsible for diabetes-induced break down of the urothelial barrier

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