Diabetic Complications Consortium

Application Title: Molecular Characterization of Detrusor Interstitial Cells in Diabetic Detrusor Overactivity

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1. Project Accomplishments:

Our lab has discovered a novel type of interstitial cells in detrusor muscles. We have identified with antibodies against platelet-derived growth factor receptor-α (PDGFRα). These PDGFRα^+ cells have been identified in human, guinea pig and murine detrusor muscle. PDGFRα^+ cells are associated with varicose nerve processes in detrusor muscles. PDGFRα^+ cells may be innervated and receive and transduce neurotransmitters. Activation of small-conductance Ca^{2+}-activated K^+ (SK) channels is involved in membrane stabilization in the detrusor smooth muscle. Functional expression of SK channels was reported in detrusor smooth muscle cells (SMCs) previously. However, the current density attributable to SK channels in detrusor SMC is minimal, even at positive (i.e. non-physiological) potentials. In contrast, PDGFRα^+ cells display a very high current density attributable to SK channels. Defects of SK channels in PDGFRα^+ cells amplify contractile responses leading to a detrusor overactivity phenotype. In this study, we characterized the molecular and protein components of these novel PDGFRα^+ cells and determine how these cells participate in the regulation of diabetic bladder dysfunction (DBD).

We used Akita mice in type 1 DM animal model studies, the transcriptional and protein expressions of PDGFRα receptors and SK channels are down-regulated in the detrusor muscles in Akita mice and thus, disruption of membrane stabilization (i.e. depolarization) might display detrusor overactivity. These findings provide novel information about changes in relative expression of PDGFRα receptor and SK channels in detrusor muscles. In high fat diet (HFD) animal model studies which as a type 2 DM, the data support that PDGFRα receptors and SK channels play an important pathological role in DBD.

We collected purified cells from FACS using PDGFRα^+/eGFP and smMHC/Cre/eGFP mice. Collected cells from control mice were used for RNA sequencing (RNA-seq) analysis to determine which genes are highly expressed in PDGFRα^+ cells. Unfortunately, delayed animal delivery due to Covid-19 made us delayed experiments. We collected detrusor PDGFRα^+ cells in normal diet (ND) and HFD mice, sent these samples to the BGI Genomics and are waiting for the results of RNA-seq. These data from RNA-seq will provide important information to explore more targets that involved in DBD developments and generate new hypotheses about sites of pathophysiological responses in diabetic bladder dysfunction.

In conclusion, current data obtained from DiaComp support showed that PDGFRα^+ cells express important genes in regulating detrusor contractility. Loss of PDGFRα^+ cells or reduced expression of key proteins in PDGFRα^+ cells may underlie detrusor overactivity in both type 1 and type 2 DM. Based on these findings, I submitted the COBRE grant as a project leader and am waiting for funding decision (July, 2021).

2. Specific Aims:

Aim 1. Determine how T1 and T2 DM affect the native phenotypes of detrusor PDGFRα$^+$ cells:

In order to study the mechanisms of early stage DBD in particular detrusor overactivity, we have developed novel techniques to identify and isolate PDGFRα$^+$ cells and SMCs to characterize the molecular phenotype of these cells. The PDGFRα$^+$/eGFP mice (Jackson Lab) express eGFP in PDGFRα$^+$ cells.

Akita mice, a recognized model of type 1 DM: 12-week old Akita mice were puchased from Jackson Lab. Average non-fasted blood glucose level was 322± 41 mg/dL (control: 134±15mg/dL). Detrusor muscles were used to compare with wild type (C57BL/6).

High fat diet (HFD)-induced Obesity (Type 2 DM) using PDGFRα$^+$/eGFP mice. HFD can induce type 2-like DM in male mice.

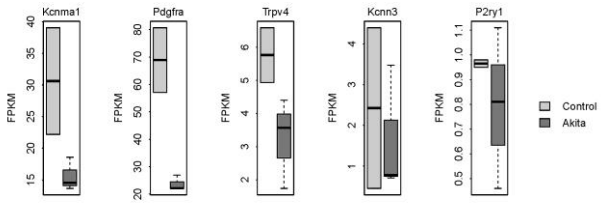

Figure 1. Comparison of key transcripts in detrusor muscles from control and Akita mice from RNA-seq data.

Purification of freshly dispersed cells: To study detrusor muscle, we separated the detrusor from suburothelium by sharp dissection and dispersed PDGFRα$^+$ cells (both ND and HFD) specifically from detrusor layers. Enzymatically dispersed PDGFRα$^+$ cells were purified using fluorescence activated cell sorting (FACS) using the cell-specific expression of reporters. Average non-fasted blood glucose level of HFD mice was 357± 34 mg/dL (ND control: 196±25mg/dL).

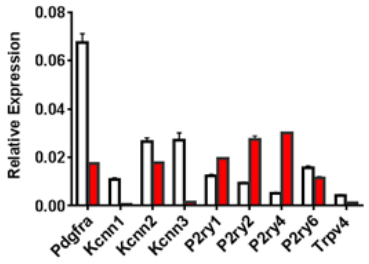

Figure 2. Quantitative analysis of key transcripts in detrusor muscles from control and Akita mice. White and Red bar denote wild type and Akita mice, respectively. Gapdh was used for housekeeping gene to normalize (relative expression).

RESULTS:

To examine the molecular expression of PDGFRα$^+$ cells, purine receptors and SK channels in Akita and high fat diet DM mice.

Quantitative analysis of trnascriptional expression of key genes in Akita and wild type detrusor muscles: Firstly, we compared the key genes from RNA-seq data. Figure 1 showed the downregulation of *Kcnma1*, *Pdgfra*, *Trpv4* and *Kcnn3* in Akita detrusor muscles. We are now analyzing the pathways including excitability-related pathways using KEGG and GOBP anlaysis. These data will be available soon.

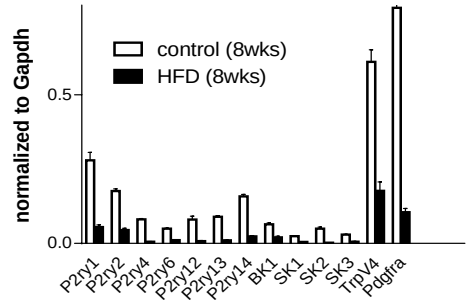

Figure 3. Quantitative analysis of key transcripts in detrusor muscles from control (normal diet) and High-Fat Diet (HFD) mice.

qPCR were employed to confirm the changes in transcriptional expression of *Pdgfra, Kcnn1-3, P2ry1,P2ry2, P2ry4, p2ry6 and Trpv4* which are mainly expressed in the detrusor PDGFRα$^+$ cells. In Akita detrusor muscles, *Pdgfra, Kcnn1, Kcnn3 and Trpv4* were dramatically decreased (Fig. 2). These data

suggest that downregulation of membran-stabilization, *Kcnn3 and Trpv4* can induce detrusor overactivity in Akita mice.

We also performed quantitative analysis of transcripts in HFD exposure for 8wks. Figure 3 showed the down-regulation of purinergic receptors, SK channels, Trpv4 and Pdgfra expressions. These data suggest that HDF detrusor muscle can show overactivity due to decrease in key membrane stabilization genes. These sorted cells for RNA-seq have been sent to the BGI for RNA-seq. These data will be very useful to raise further hypothesis for the role of PDGFRα^{+} cells during diabetic progress.

To examine the protein expression of PDGFRα^{+} cells in HFD mice.

Immunohistochemistry were utilized for the protein expression of PDGFRα in detrusor smooth muscle from control (ND) and HFD type 2-like DM. Immunohistochemical findings with PDGFRα antibody in HFD detrusor also revealed the decrease in PDGFRα immunoreactivity in HFD detrusor muscle layer compared with the control (ND) detrusor muscle layer (Fig. 4). Double immuno-labeling with SK1, SK2 and SK3 (Alomone labs Abs) are in progress. The data will be obtained soon. These data suggest that loss of PDGFRα^{+} cells in HFD mice affect the development of DBD. Taken together, we begin understanding the molecular and protein phenotype of this unique population of cells and discover the cellular apparatus that contributes to development of DBD.

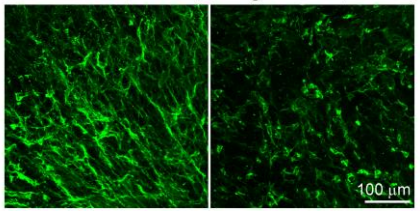

Fig. 4. Immunoreactivity of PDGFRα (green) in normal diet (***A***) and high fat diet (***B***) detrusor.

3. Publications:

None. But I applied the COBRE grant proposal as a project leader.