Diabetic Complications Consortium

Application Title: **Role of Interstitial Cells in Diabetic Bladder Dysfunction**

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1. Project Accomplishments:

We have recently discovered a novel type of interstitial cells in detrusor muscles. These cells were identified with antibodies against platelet-derived growth factor receptor-α (PDGFRα). PDGFRα^+ cells have been identified in human, guinea pig and murine detrusor muscle. PDGFRα^+ cells are associated with varicose nerve processes in detrusor muscles. Thus, 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. Thus, 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).

In type 1 DM animal model studies, the transcriptional and protein expressions of PDGFRα receptors and SK channels are down-regulated in the streptozotocin (STZ)-injected detrusor 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 animal model studies as a type 2 DM, the data support that PDGFRα receptors and SK channels plays an important pathological role in DBD.

Since we can collect purified cells from FACS using PDGFRα^+/eGFP and smMHC/Cre/eGFP mice, collected cells from control mice were used for deep sequencing analysis to determine which genes are highly expressed in PDGFRα^+cells. The data from deep sequencing analysis 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 (DBD).

In conclusion, these preliminary data 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 type 1 and type 2 DM. Based on these findings, we are aiming to submitting the grant [Type 1 Diabetes Complications IMPACT Award (DP3): RFA-DK-14-017]. Based on these findings, we are preparing the grant submission (**Type 1 Diabetes Complications IMPACT Award (DP3): RFA-DK-14-017)**

2. Specific Aims:

Aim 1. To examine the molecular and protein expression of PDGFRα$^+$ cells, purinergic receptors and SK channels in streptozotocin-induced DM mice.

In order to study the mechanisms of early stage DBD (detrusor overactivity), we have developed novel techniques to identify and isolate PDGFRα$^+$ cells and smooth muscle cells (SMCs) to characterize the molecular phenotype of these cells. The PDGFRα$^+$/eGFP mice (Jackson Lab) express eGFP in PDGFRα$^+$ cells. *Streptozotocin (STZ)-induced diabetes:* Eight-week old STZ-injected PDGFRα$^+$/eGFP mice were puschased from Jackson Lab. Average non-fasted blood glucose level was 317± 39 mg/dL (control: 123±5mg/dL). *Purification of freshly dispersed cells:* To study suburothelium and detrusor muscle, we separated the detrusor from suburothelium by sharp dissection and dispersed PDGFRα$^+$ cells specifically from suburothelium and detrusor layers. Enzymatically dispersed PDGFRα$^+$ cells were purified using fluorescence activated cell sorting (FACS) using the cell-specific expression of reporters.

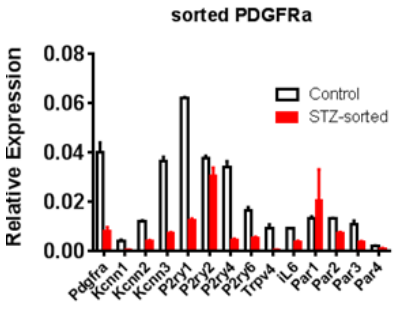

Figure1. Quantitative analysis of key transcripts in sorted PDGFRα$^+$ cells from control (saline injected) and STZ-injected detrusor muscle layers.

RESULTS:

Molecular Analysis: Hypoxanthine guanine phosphoribosyl transferase (*Hprt*), *Gapdh* (housekeeping), *Kit* (ICC), *Myh11* (SMC), *Uchl1* (neurons) were monitored to establish relative purity of PDGFRα$^+$ cells and SMCs by FACS. Using quantitative RT-PCR (*qPCR*), transcriptional expression of PDGFRα$^+$ cells in non-diabetic (control) and STZ-induced diabetic detrusor were examined. PDGFRα (Pdgfra), SK (Kcnn), purinergic receptors (P2ry1, P2ry4, P2ry6) and Trpv4 genes in PDGFRα$^+$ cells were down-regulated in STZ-induced diabetic detrusor compared with control sorted PDGFRα$^+$ cells (Fig 1). To compare with transgenic diabetic mouse, we used Akita mice. Akita detrusor muscle also showed the down-regulation of *Pdgfra, Kcnn1, Kcnn3 and Trpv4* but rather up-regulation of purinergic receptors (Fig. 2). Consistent findings from two animal models can be interpreted with bladder overactivity since SK channels and mechanosensitive TRPV4 channels are important factor for the membrane stabilization in detrusor PDGFRα$^+$ cells. Unexpected result from STZ-injected PDGFRα$^+$ cells were down-regulation of interleukin 6 which is a marker for inflammation (Fig.1). Thus, we tested the inflammation related genes using RT^2 qPCR array Kit (Qiagen). Interestingly all immune related genes are down-regulated in STZ-induced diabetic PDGFRα$^+$ cells. The results suggest that STZ could have non-specific effects on immune system and work as an immunosuppressant. We also performed the deep sequencing (RNA-seq) analysis from sorted PDGFRα$^+$ cells in suburothelium and detrusor, and smooth muscle cells in detrusor. These data

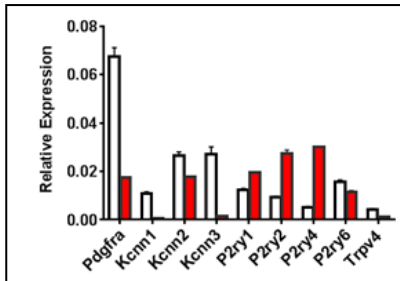

Figure 2. Quantitative analysis of key transcripts in detrusor muscles from control and Akita mice.

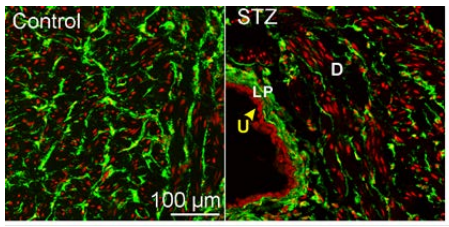

Figure 3. Immunoreactivity of PDGFRα (green) in control (wild type) and STZ-injected bladder. Red staining denotes propidium iodide for nuclei. U:urothelium, LP:lamina propria, D: Detrusor.

are currently under analysis to raise further hypothesis for the role of PDGFRα$^+$ cells during diabetic progress.

Protein Analysis (Immunohistochemistry and Mass Spectrometry): Immunohistochemistry were utilized for the protein expression of PDGFRα in suburothelium and detrusor smooth muscle from control (non-diabetic) and STZ-induced DM. Immunohistochemistry showed the decrease in PDGFRα$^+$ cells in the detrusor layer compared with age-matched control detrusor (see Fig. 3). Double immuno-labeling with SK1, SK2 and SK3 (Alomone labs Abs) are in progress. We are also making progress in changes in suburothelium to investigate fibrosis mechanisms through phenotype changes of PDGFRα$^+$ cells. Antibodies (α-SMA and cadherin 11) as fibrosis markers have purchased. These data will support the changes in molecular expression of suburothelium and detrusor PDGFRα$^+$ cells from diabetic mice. We are attempting to use Mass Spectrometry (with UC Davis Proteomics Core) with plasma membrane fractions from sorted PDGFRα$^+$ cells. This is an innovative approach and feasible. The data will be obtained soon.

Isometric force measurement: Apamin did not increase the EFS-induced contraction suggesting that SK channels are down-regulated in STZ-injected detrusor muscle strips (Fig. 4). Taken together, STZ-induced detrusor overactivity is due to the down-regulation of PDGFRα$^+$ cells and SK channels since SK channels in detrusor PDGFRα$^+$ cells regulate membrane stabilization.

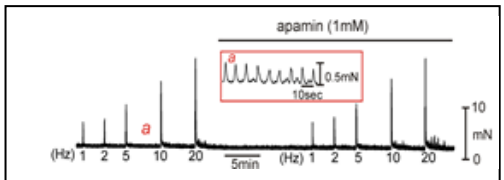

Figure 4. The effect of apamin (300nM) on EFS evoked- contractile responses in STZ-injected detrusor muscles. Inset (expanded time scale in *a*) shows spontaneous contractility.

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.

Aim 2. To examine the molecular and protein expression of PDGFRα$^+$ cells, purine receptors and SK channels in high fat-diet DM mice. As millions of Americans become overweight and obese, type 2 DM and metabolic syndrome are on the rise. High-fat diet can contribute to obesity, which increases the risk for developing type 2 diabetes. In this aim, we purchased high-fat diet mice from Jackson Lab to examine the expression of PDGFRα receptors and SK channels with simultaneous evaluation of body weight and blood glucose level. After 8 wks exposure (12 wks of age) to HFD from 4 wks of age, bladders were excised for molecular, immunohistochemistry and force measurements. Blood glucose level in HFD was higher (175mg/dl) than in control (123mg/dl).

RESULTS:

Molecular Analysis: Quantitative analysis of transcripts in HFD exposure for 8wks showed the down-regulation of purinergic receptors, SK channels, Trpv4 and Pdgfra expressions (Fig. 5). These data suggest that diabetic detrusor muscle can show overactivity due to decrease in key membrane stabilization genes.

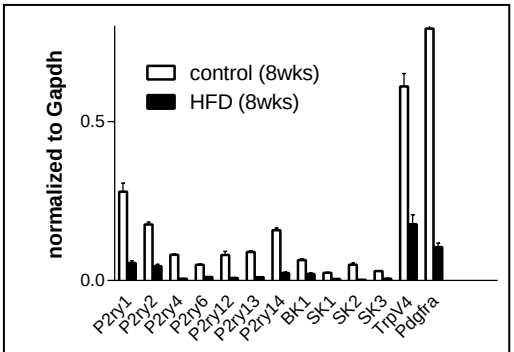

Figure 5. Quantitative analysis of key transcripts in detrusor muscles from control (normal diet) and High-Fat Diet (HFD) mice.

Protein Analysis (Immunohistochemistry):

Immunohistochemical findings with PDGFRα antibody in HFD detrusor also revealed the decrease in PDGFRα immunoreactivity in HFD detrusor muscle layer compared with the control (normal diet) detrusor muscle layer (Fig. 6A&B).

Isometric force measurement: In previous reports, apamin augmented the EFS-evoked responses in a frequency-dependent manner. Interestingly, HFD-treated detrusor muscle strips did not show apamin sensitivity of the EFS-evoked responses (3sec of train duration, from 1-20Hz, Fig. 6C&D). In addition, HFD detrusor strips displayed spontaneous contractility similar to STZ-treated detrusor strips. These data suggest that down-regulation of PDGFRα^{+} cells and SK channels in HFD mice affect the development of DBD.

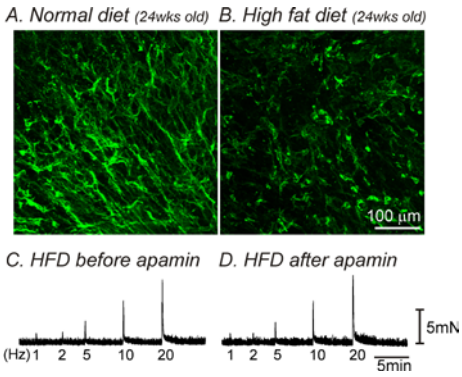

Fig. 6. Immunoreactivity of PDGFRα (green) in normal diet (***A***) and high fat diet (***B***) detrusor. EFS- evoked contractile responses before (***C***) and after (***D***) apamin (300nM) in high fat diet (HFD) detrusor muscles. Apamin did not show significant effect.

3. Publications:

We are planning to submit the grant proposal based on this funding with obtained preliminary data.