Diabetic Complications Consortium

Application Title: Novel Regulators of Diet-Mediated Nephrocyte Dysfunction in Drosophila

Principal Investigator: Ross L. Cagan

1. Project Accomplishments:

The overall goal of this project was to expand on a molecular pathway that we identified that mediates the ability of high dietary sugar to suppress expression of the Nephrin ortholog Sns in the Drosophila nephrocyte. We have successfully established and calibrated a model, but are still screening for specific genes to add to this 'pathway'.

Specific Aims:

This grant had one specific Aim:

Specific Aim: Deficiency screen to identify novel mediators of diet-induced nephrocyte dysfunction

Results: We spent the majority of the funding period establishing the screening platform, which consisted of RNA-interference mediated Sns knockdown targeted to the nephrocyte (*sns>sns(RNAi)*). In this grant we are screening to identify new members of the glucose–hexosamine flux–Polycomb Complex–Knot–Sns pathway. We are performing a 'dominant genetic modifier screen' by crossing in a set of deficiencies; interacting deficiencies are progressively deconvolved with the goal of identifying individual genes.

Two results are worth noting; some of these results have been recently published (Na et al, 2015). First, the assay has proven more noisy than we anticipated for reasons that are unclear. Our initial data indicated that *sns>sns(RNAi)* flies had a 50% reduction in lifespan. Figure 1 shows more recent data, which continues to display a significant difference but one that is smaller than our previous results (see also Na et al, 2015). While performing initial screening, we are continuing to alter temperature and other parameters to improve our ability to identify subtle differences with deficiencies.

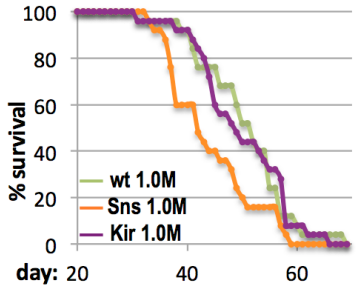

Figure 1. Reduction of Sns, but not Kirre, reduced overall lifespan in adults fed high dietary sugar.

Secondly, we have identified an interesting hit in Cindr, the Drosophila ortholog of mammalian CD2AP/CIN85. This is especially interesting given previous work that has linked CD2AP to Nephrin in mammalian systems. Complete loss of Cindr led to severe nephrocyte disruption (Na et al, 2015). Knockdown of Cindr also led to reduced longevity

in animals fed high dietary sucrose but not a control diet (Figure 2). Cindr in a sense acts as a positive control, but this is the first evidence I am aware of that a CD2AP/CIN85 ortholog is specifically required for proper response to high dietary sucrose.

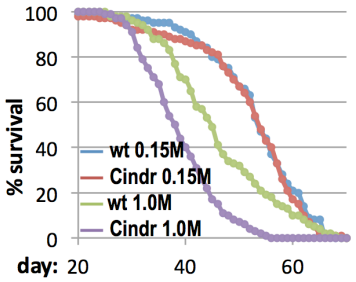

Figure 2. Reduction of Cindr reduced overall lifespan specifically in adults fed high dietary sugar.

Despite this data, to properly complete the deficiency screen we will need to identify a more robust screening platform. We have identified a visual screen that appears promising: expressing a GFP-based reporter of the Sns promoter (*sns-GAL4, UAS-GFP*). Though requiring more effort than viability assays, this assay will provide us the opportunity to more directly identify regulators of Knot and of Sns transcriptional control. Figure 3 demonstrates how high dietary sucrose (HDS) reduced *sns>GFP* expression and also reduces nephrocyte size.

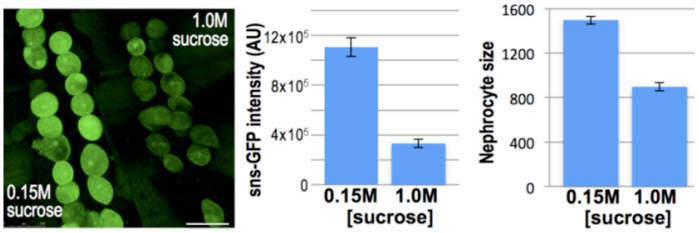

Figure 3. Left panel: high dietary sucross reduced expression of an *sns* reporter. Right panels: quantification of *sns>GFP* expression and nephrocyte size.

2. Publications:

Na J, Sweetwyne MT, Park AS, Susztak K, Cagan RL. Diet-Induced Podocyte Dysfunction in Drosophila and Mammals (2015). Cell Rep. 12(4):636-47.