Diabetic Complications Consortium

Application Title: A Novel Vertebrate Model of Diabetic Nephropathy

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1. Project Accomplishments:

We have evaluated the renal phenotypes in two different models of pancreatic beta cell ablation in zebrafish, which serves as the zebrafish model of Type 1 Diabetes. We found that in these two models, the pronephric glomeruli appear to be affected by the insulin insufficiency: significant ultrastructural change was observed but no proteinuria was detected. On the other hand, we have also performed the in vivo analyses of ROS in zebrafish pronephric renal cells. Interestingly, the ROS levels were found to be lower in the pronephric tubular cells in the diabetic zebrafish larvae. This is consistent with the DHE staining results we previously got. Thus this establishes a useful tools for determine the cytoplasmic ROS level in renal cells in live zebrafish.

2. Specific Aims:

SA1: To establish and characterize a zebrafish model of diabetic nephropathy

To establish a zebrafish model of diabetic nephropathy, we examined three previously established models of hyperglycemia and type 1 diabetes. The first model is glucose incubation of larval fish for 30 minutes. This is an acute hyperglycemia model as longer incubation in glucose solution adversely affected the gross growth of larval fish. However, such a short period of incubation did not result in significant changes in renal glomerular ultrastructure. We did not observe any obvious abnormality in foot processes of glomerular podocytes in this model. We further analyzed renal glomerular ultrastructure of two other type 1 diabetes models. One is DTA-mediated pancreatic beta cell ablation (DTA model) and the other is MTZ-mediated pancreatic beta cell ablation (MTZ model). In the DTA model, diphtheria toxin A is expressed specifically under the control of insulin promoter so beta cells are completely eliminated beyond about 30 hours post fertilization. This resulted in growth retardation and majority of the transgenic larvae died between 10-14 days post fertilization. We thus examined the glomerular structure using TEM at around 10 days post fertilization when the larval fish have not display any gross morphological abnormality. TEM revealed mild foot process effacement, reminiscent of minimal change disease (**Figure 1**). We observed widened foot processes, but no podocyte detachment or loss. The GBM is largely normal. In addition to the TEM analyses, we have performed the functional characterization of glomerular filtration barrier. However, neither the GFP fluorescence intensity assay nor the ELISA-based proteinuria assay revealed significant leakage through the glomerular filtration barrier (**Figure 2**). Given the foot process effacement was very mild in the diabetic fish, it is likely that the GFB assay is not sensitive enough to detect micro-proteinuria commmonly associated with early stages of diabetic nephropathy.

SA2: To study mitochondria metabolism and mitochondrial ROS levels in vivo in a zebrafish model of diabetes.

Diabetes is known to disturb the mitochondrial metabolism, but in contrast to the previous findings that indicate an increased level of ROS is observed in cells under diabetic conditions, our preliminary studies suggested that there is a reduction in mitochondrial metabolism and ROS in diabetes. In order to investigate this in kidney cells in live fish larvae in vivo, we constructed a transgene that express the mitochondria-targeted RFP in kidney cells in zebrafish such that we can employ fluorescence imaging to assess mitochondrial mass in the kidney cells in diabetic fish. We introduced the Tg(pod:mito-RFP) transgene into the Type 1 Diabetes models through

genetic crossing and imaged and quantified the red fluorescence in the pronephric glomeruli in 7 day old larvae. We did not find any significant difference in red fluorescence intensity between the diabetic fish and the normal control (**Figure 3**). This suggested that the mitochondrial biogenesis was affected by diabetic condition. To further investigate the ROS level in live fish, we constructed another transgene for the measurement of mitochondrial ROS in live fish. We expressed the ROS-sensitive GFP (roGFP12) in the zebrafish kidney cells (podocytes and tubular epithelial cells). The GFP fluorescence intensity indicates the level of mitochondrial ROS in vivo. We thus found that the GFP fluorescence intensity in podocytes was not affected by the diabetic condition, but the GFP fluorescence intensity in the renal tubular cells appeared significantly lower in diabetic fish than in normal controls (**Figure 4**). This result was consistent with our previous finding using DHE staining. Thus our study provided a new in vivo tool for the analyses of ROS in zebrafish models of diabetes.

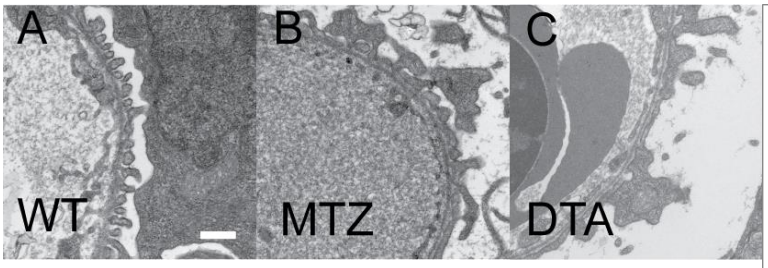

Figure 1:Foot process effacement is observed in podocytes in diabetic larval fish. (A) Wild type control fish display normal foot processes and slit diaphragm. (B) in MTZ-induced beta cell ablation model, foot processes are widened (C) in DTA-mediated beta cell ablation model, foot process effacement is more severe. Scale bar: 400 nm.

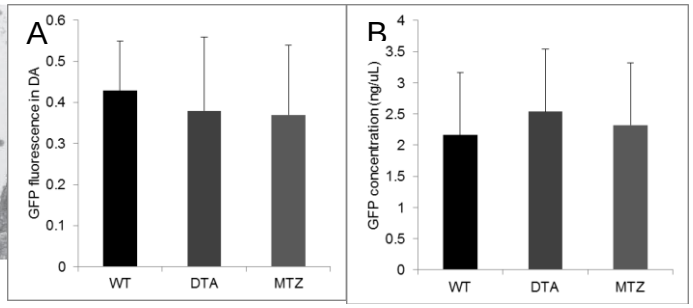

Figure 2: Functional assays do not shown leakage through the glomerular filtration barrier in the two diabetic zebrafish models. (A) VDBP-GFP expressing fish do not show reduced GFP fluorescence in dorsal aorta in the diabetes models. (B) ELISA assay of GFP in fish water do not show increased excretion of GFP in diabetic fish.

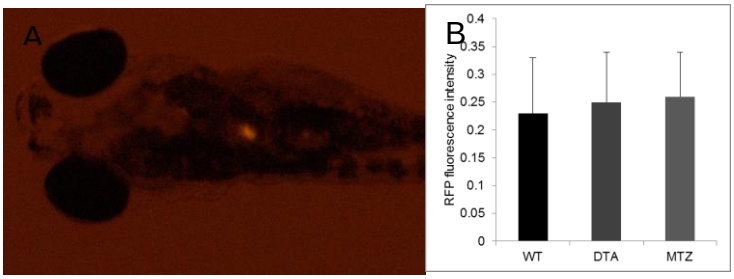

Figure 3:Mitochondrial mass is not increased in the podocytes in diabetic fish. (A) Representative image of Tg (pod:mito-RFP) larva. (B) Quantitation of RFP fluorescence shows no significant difference in wild type control and diabetic fish models.

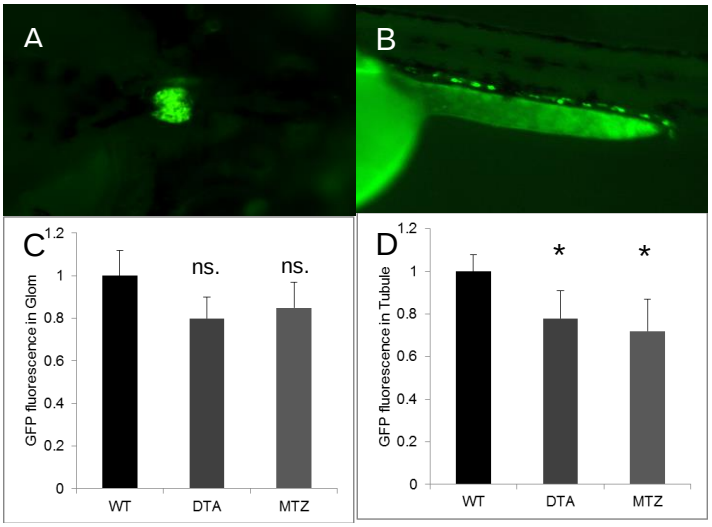

Figure 4: Measuring mitochondrial ROS using genetically encoded sensor roGFP. (A) Representative image of pod:roGFP expression in pronephric podocytes. (B) Representative image of cdh17:roGFP expression in pronephric tubules. (C) Quantitation of GFP fluorescence intensity in glomeruli in diabetic fish. (D) Quantitation of GFP fluorescence intensity in tubules in diabetic fish. ns, not significant; *, p <0.05, n=6

3. Publications:

None