Final Progress Report

"Novel Model System for Monitoring Multiple Diabetic Complications in Tandem"

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A. Specific Aims – not changed

The goal of this project was to establish a battery of novel preclinical models that will facilitate the identification of compounds that delay, ameliorate, or reverse the progression of diabetic complications. To develop in vivo corneal confocal microscopy (IVCCM) methodology to study the cornel innervation in normal and diabetes induced zebrafish model and establish this technique to diagnose diabetes induced complications by detecting corneal sensory nerve damage.

Aim 1) Validate zebrafish models for monitoring multiple diabetic complications. The onset of different cellular pathologies, e.g., loss of corneal innervation, accumulation of dying cells, neovascularization, will be monitored in living fish and compared between controls, type 1, and type 2 diabetes models. Disease onset and progression will be correlated to relative glucose levels using a novel non-invasive transgenic method and/or direct glucose measurements.

Aim 2) Test current therapies for the ability to slow or reverse progression of diabetic complications. We will determine the degree to which currently available drugs serve to ameliorate diabetic complications in transgenic fish.

B. Studies and Results

We have established an *in-vivo* time series confocal methodology that allows us to track cellular phenotypes /pathologies over time in individual fish ranging from larval to juvenile stages (e.g., 4 days to one month old, see Fig 1). We used this technique to quantify changes in corneal innervation patterns over time, comparing control and diabetic models. The results show clear deficits in corneal innervation patterns following exposure to high glucose – the proposed type 2 diabetic model (Fig. 2). However, our osmotic control for high glucose treatments (mannitol) also showed similar corneal innervation deficits (Fig. 2). These results suggest that high glucose treatments may be inadequate to properly model type 2 diabetes in an aquatic species due to effects resulting from changes in osmolarity as opposed to hyperglycemia. Thus, we were unable to assess current therapies using high glucose models. Instead, we focused on crossing type 1 diabetic models—inducible targeted beta cell ablation—into various transgenic backgrounds facilitating monitoring of cellular phenotypes. Future studies will be devoted to testing the effects of acute and chronic beta cell ablation on corneal innervation and other diabetic complication pathologies.

Effects of high glucose treatments

Transgenic lines facilitating time-lapse monitoring of corneal innervation [*Tg(isl1-Crest3:YFP-2A-nfsB*)] were exposed to glucose or mannitol at 2% final concentration for 24 hrs every other day starting at 7 days postfertilization (7 dpf, i.e., late larval stage). In our hands, such treatments lead to lethality issues; all treated fish expired by 17 dpf precluding our ability to track pathologies beyond 14 dpf. Nevertheless, we were able to collect confocal time series images of corneal innervation patterns at 7 and 14 dpf (Fig. 2). The images show clear differences in innervation density, nerve, number, and branch number between fish treated with glucose and non-treated controls at 14 dpf (Fig 2, compare series A to series B). However, similar changes were observed in mannitol treated fish (Fig. 2C series), a control for osmolarity changes attending high sugar treatments. Our conclusion from these studies was that high glucose treatment regimens are not an adequate means of modeling type 2 diabetes in fish. We next turned to a type 1 diabetic model that we developed.

Type I diabetic models for monitoring multiple diabetic complications.

Previously, we created an inducible cell ablation system that when applied in zebrafish provides a means to explore cell-specific regeneration paradigms (Mumm and Schroeter, 2002; Curado et al., 2007; Walker et al., 2012). In order to address issues which arose with high glucose treatments, we have crossed new versions of a type 1 diabetes model—inducible ablation of pancreatic beta cells—with compound transgenic lines facilitating monitoring of cellular phenotypes associated with diabetic complications. This process ended up taking considerably more time than we anticipated due to low yields. To date, we have successfully created a number of new compound transgenic lines (summarized in Section F) useful for inducing beta cell ablation either acutely or chronically [using either the *Tg(ins:PhiYFP-Eco.NfsB,sst2:TagRFP)lum01*, or *Tg(ins:TagCFP-Eco.NfsB,sst2:TagRFP)lum11* lines, #1 - #8 below] and

monitoring peripheral innervation patterns (#1 and #5, below); heart morphology (#2 and #6, below); cell death (#3 and #7, below); and kidney morphology (#4 and #8, below)

As stated above, future studies will be devoted to testing the effects of acute and chronic beta cell ablation on corneal innervation and other diabetic complication pathologies which these compound transgenic lines facilitate.

C. Significance – not changed

D. Plans – not applicable, funding was only for 1 year (but see above)

E. Publications – None

F. Project-Generated Resources
The following compound transgenics lines were made for this project and can be accessed by contacting our laboratory personnel.

1) *Tg(isl1-Crest3:YFP-2A-NTR)lum09;Tg(pax6-DF4:GAP-CFP)q01;Tg(ins:PhiYFP-Eco.NfsB,sst2:TagRFP)lum01*

2) *Tg(vegfR2:AcGFP;Tg(isl1-Crest3:YFP-2A-NTR)lum09;Tg(ins:PhiYFP-Eco.NfsB,sst2:TagRFP)lum01*

3) *Tg(tbp:Gal4)f13;Tg(UAS:SEC-Hsa.ANXA5-YFP,myl7:RFP)f12;Tg(pax6D4:M-CFP)q01;Tg(ins:PhiYFP-Eco.NfsB,sst2:TagRFP)lum01*

4) *Et(fos:KalTA4)gmc692;Tg(UAS-E1b:Eco.NfsB-mCherry)c264;Tg(ins:PhiYFP-Eco.NfsB,sst2:TagRFP)lum01*

5) *Tg(isl1-Crest3:YFP-2A-NTR)lum09;Tg(pax6-DF4:GAP-CFP)q01;Tg(ins:TagCFP-Eco.NfsB,sst2:TagRFP)lum11*

6) *Tg(vegfR2:AcGFP;Tg(isl1-Crest3:YFP-2A-NTR)lum09;Tg(ins: TagCFP-Eco.NfsB,sst2:TagRFP)lum11*

7) *Tg(tbp:Gal4)f13;Tg(UAS:SEC-Hsa.ANXA5-YFP,myl7:RFP)f12;Tg(pax6D4:M-CFP)q01;Tg(ins:TagCFP-Eco.NfsB,sst2:TagRFP)lum11*

8) *Et(fos:KalTA4)gmc692;Tg(UAS-E1b:Eco.NfsB-mCherry)c264;Tg(ins:TagCFP-Eco.NfsB,sst2:TagRFP)lum11*

Figure-1

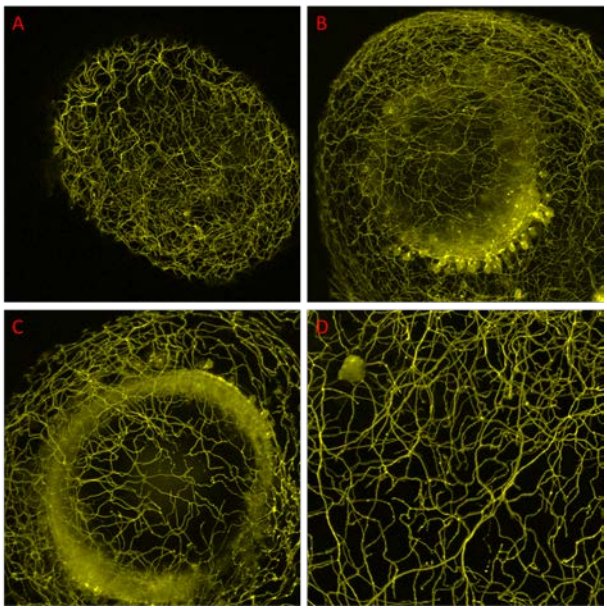

Figure-1 - *In vivo* Corneal Confocal Microscopy
Images of larval and juvenile transgenic zebrafish (see #1 in list above): A) 4 dpf larvae; B) 1 week old larvae; C) 2 week old larvae and D) One month old juvenile.

Figure-2

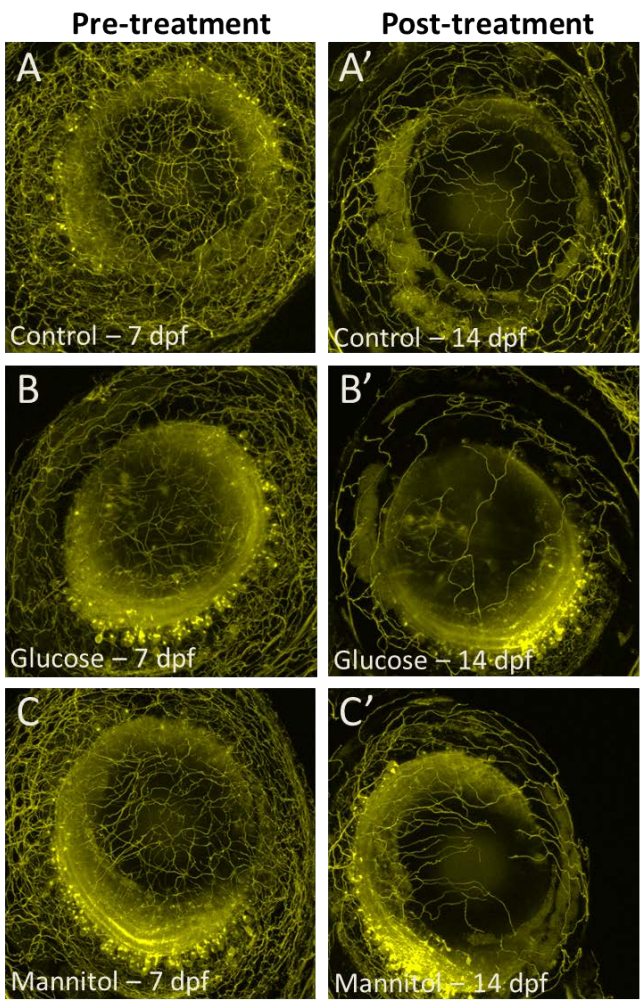

Figure 2 – Time Series Corneal Innervation Imaging
Time series images of representative individual fish from each of three conditions, control (A-A'), 2% glucose treated (B-B'), and 2% mannitol treated (C-C'). Groups treated with high sugar regimens showed clear changes in corneal innervation, however the mannitol controls suggest this may be due to osmolarity changes rather than hyperglycemia.