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Dissertations-Kurzfassung

The increased concentration of 4-Hydroxynonenal in *aldh3a1* zebrafish mutants disrupts pancreas development, leading to hyperglycaemia and retina hyaloid vasculature alteration

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Background and Aim: The increased formation of methylglyoxal (MG) under hyperglycaemia is associated with the development of microvascular complications in patients with diabetes. However, in zebrafish, a permanent knock out of glyoxalase 1(Glo1), the central MG detoxifying system, only led to a two-fold elevation of endogenous MG levels. Importantly, a two-fold increase in aldehyde dehydrogenases (ALDH) activity, a group of enzymes which catalyse the oxidation of aldehydes, was observed. Besides, RT-qPCR based results identified elevated *aldh3a1* mRNA level in *glo1*$^{-/-}$ mutants. Thus, this dissertation aims to generate Aldh3a1 knockout zebrafish and analyse its function in glucose homeostasis and diabetes.

Materials and Methods: *aldh3a1*$^{-/-}$ zebrafish were generated by using CRISPR/Cas9. Vasculature, pancreatic and β-Cell mass area size were analysed in *Tg(fli1:EGFP)*, *Tg(hb9:GFP)* and *Tg(ins:nfsB-mCherry)*zebrafish larvae. mRNA expression was examined by RT-qPCR and RNA-seq analysis. 4-Hydroxynonenal (4-HNE) amount was measured by ELISA (BioVision) and whole-body glucose was measured by Glucose Assay Kit (Merck).

Results: Aldh3a1 knockout zebrafish were successfully generated and validated by significantly decreased ALDH activity. In *Tg(fli1:EGFP)* zebrafish larvae, loss of Aldh3a1 increased abnormal intersegmental vessels formation in the trunk and widened branch diameters in retina hyaloid vasculature, which can be further enhanced via *pancreatic and duodenal homeobox 1* (*pdx1)* expression silencing. A combination of Aldh3a1 knockout and knockdown strategies identified a decreased pancreatic size in *Tg(hb9:GFP)* and reduced β-Cell mass dimension in *Tg(ins:nfsB-mCherry)* zebrafish larvae. Also, mRNA expression of *pdx1* and *insulin* were decreased and RNA-seq data have further confirmed disruption of the endocrine pancreas development in the *alda3a1* mutants. Consequently, *aldh3a1* $^{-/-}$ larvae exhibited hyperglycaemia by 40% whole-body glucose elevation. Moreover, disruption of the pancreas in *alda3a1*mutants is driven by an increased 4-HNE amount and external 4-HNE in wild type zebrafish larvae mimics the phenotype in *aldh3a1* mutants.

Conclusion: Overall, this dissertation provided patent evidence for the contribution of deficient 4-HNE detoxification and subsequent increased 4-HNE concentration to the development of hyperglycaemia via pancreas dysfunction in *aldh3a1* mutants, as a novel direction for future research regarding diabetic pathophysiology and therapy.