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Genetic and chemically-induced Zebrafish models for the study of diabetes mellitus

Abstract

Diabetes mellitus (DM) will likely affect over 400 million worldwide by 2030 and is considered a disease resulting from metabolic dysfunction related to the homeostasis of systemic glucose levels. While management of systemic glucose levels is essential to the health of the patient, the long term secondary complications of diabetes poses an equally critical problem. In this regard, evidence from both the laboratory and large scale clinical trials has revealed that complications from the onset of hyperglycemia (such as impaired blood vessel growth) progress unimpeded via the phenomenon of "metabolic memory" (MM) even when glycemic control is pharmaceutically achieved. This applies to both type 1 and type 2 diabetes. Over the past decade considerate effort has been placed toward developing animal models that allow molecular analysis of both 1) glucose regulation processes, and 2) the mechanisms underlying the secondary complications of the disease. A powerful model that has been developed is the teleost, Zebrafish (*Danio rerio*) because its physiology matches that of the human in regard to glucose regulation and the occurrence of secondary complications resulting from the diabetic state in this model. The development of genetic methods and chemical methods to induce either type 1 and type 2 diabetes in Zebrafish has allowed investigators to better understand the underlying mechanisms of DM and MM. This review will summarize and discuss these genetic and chemically-induced zebrafish DM models with the aim to point out their particular strengths in the study of DM and MM.

Keywords: diabetes, metabolic memory, Type 1 and 2 diabetes, epigenetics, genetic models, chemically-induced DM models, zebrafish

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Abbreviations: DM, diabetes mellitus; MM, metabolic memory; HG, hyperglycemic; CFP, cyan fluorescent protein; NTR, nitroreductase; Met, metronidazole; AGE, advanced glycation end products; STZ, streptozotocin

Introduction

Diabetes mellitus (DM) will likely affect over 400 million worldwide by 2030[1] and is classified as a disease of metabolic dysfunction related to the homeostasis of systemic glucose levels [e.g. elevated glucose levels or what is termed hyperglycemia (HG)].[2,3] DM can be subdivided into type-1 DM and type-2 DM. In type-1 DM the ability of insulin to be produced is impaired while in type-2 DM, the ability of the body to respond to insulin is impaired. While management of systemic glucose levels is essential to the health of the patient, the long term secondary complications of diabetes poses an equally critical problem. In this regard, data from the laboratory[4–10] and from clinical trials[11–23] indicate that complications from the onset of hyperglycemia progress unimpeded via the phenomenon of "metabolic memory" (MM) even when glycemic control is pharmaceutically achieved.[11–23] This applies to both type 1 and type 2 diabetes. The underlying molecular mechanisms of hyperglycemic complications and metabolic memory involve: 1) the involvement of excess reactive oxygen species (ROS), 2) the involvement of advanced glycation end products (AGE), and 3) alterations in tissue-wide gene expression patterns.[2,3] The heritable nature of metabolic memory[24,25] points to involvement of the epigenome. In general terms, the epigenome is comprised of all chromatin modifying processes including DNA methylation and histone modifications allowing cells/organisms to quickly respond to changing environmental stimuli.[26–28] Furthermore, these processes allow for quick adaptation to environmental stimuli but also impart the ability of the cell to "memorize" these encounters.[24,25] The underlying molecular mechanism/s of MM has been examined via both animal model approaches and *in vitro* based studies.[4–10] These studies have established that the initial hyperglycemia results in permanent aberrant gene expression in DM target tissues such as the: kidney, retina, cardiovascular system, skin as related to wound healing, and impaired blood vessel growth such as seen in wound healing.

Over the past decade considerate effort has been placed toward developing animal models that allow molecular analysis of both 1) the glucose regulation processes, and 2) the mechanisms underlying the secondary complications of the disease. Toward this aim, the teleost, Zebrafish (*Danio rerio*) has immerged as a powerful model to study DM because its physiology matches that of the human in regard to glucose regulation and the occurrence of secondary complications in the fish following an induced diabetic state. In this review a number of 1) genetic and 2) chemically-induced Zebrafish DM models will be discussed.

Discussion

Zebrafish genetic models of type 1 diabetes

Beginning in 2007, Zebrafish genetic models began to be published as indicated in the references cited. The studies of Pisharath et al.[29] used molecular approaches in Zebrafish to ablate pancreatic beta cells of the fish thereby inducing a diabetic state. This DM state was then followed by a natural regenerative phase in which the beta cells re-differentiated because of the epimorphic capabilities of the organism. To achieve the initial ablation of the beta cells they expressed an Escherichia coli gene called nfsB in the beta cells of embryonic Zebrafish. The gene nfsB encodes a nitroreductase (NTR) enzyme, that is able to convert prodrugs [e.g. metronidazole (Met)] to

cytotoxins. Use of NTR rendered the model a hybrid between a pure genetic DM model and a chemically inducted DM model. By fusing nfsB to mCherry, they were able to make beta cells susceptible to the prodrug and visualize Met-dependent cell ablation. They found that the other cells of pancreatic Islets such as the alpha and delta cells were un-affected by prodrug treatment and that ablation was beta cell specific. After Met was removed from the treatment solution, recovery of beta cells through regeneration was observed by 36 hours post Met treatment. It was also shown that beta cell regeneration occurred independently of the presence of the exocrine pancreas. These studies traced cell lineage using cytomarkers and cell proliferation was monitored using BrdU incorporation. This beta cell regenerative model provided the opportunity to induce a short-term DM state and therefore a short-term hyperglycaemic state. The main objective in the model was the ability to analyze beta cell regeneration so that large-scale screens for pharmacological and genetic modifiers of beta cell regeneration could later be conducted. The Pisharath et al.[29] studies were followed in 2012 by the studies of Andersson et al.[30] who again utilized the nitroreductase (NTR) enzyme/metronidazole (Met) approach to induce beta cell ablation followed by beta cell regeneration after Met treatment was terminated. The study evaluated thousands of small molecules to identify enhancers of beta cell regeneration for possible treatment of type 1-DM. To more clearly monitor beta cell regeneration, transgenic larvae were used that expressed the fusion protein of cyan fluorescent protein (CFP) and NTR under the control of the insulin promoter, Tg(ins:CFP-NTR). CFP was only weakly fluorescent so they crossed the Tg(ins:CFP-NTR) line with another transgenic line that expressed the more robust fluorescent protein Kaede, under the control of the insulin promoter, (Tg(ins:Kaede). Using these double-transgenic Tg(ins:CFP-NTR);Tg(ins:Kaede) line, larvae were treated with Met for ablation of beta cells. At 4 days post fertilization, Met treatment was terminated and the larvae were treated with the compounds of interest as beta cell regeneration occurred. They observed that at 6 days post fertilization, control larvae carrying only the Tg(ins:Kaede) transgene were not affected by Met and had a normal number of beta cells, whereas Met-treated Tg(ins:CFP-NTR);Tg(ins:Kaede) larvae had only a few beta cells. Following this protocol, 100,000 larvae were utilized to identify enhancers of beta cell regeneration by screening 7,186 compounds. They found that adenosine signalling molecules promoted regeneration of Pancreatic beta cells. It should be noted however, that in these models hyperglycemia served only as a temporary bi-product of the model (3-4 days of a DM state).

Therefore, if one delays removal of Met in the model, the DM state continues; thus allowing more long-term hyperglycemic effects. Conversely, if one allows the NTR-transgenic zebrafish line to reach maturity before Met treatment is begun, one has the ability to study more prolonged periods of DM/hyperglycemia followed by a normal adult state when glucose homeostasis returns to normal following removal of Met treatment and subsequent beta cell regeneration. These conditions would require extensive study to establish blood glucose and insulin levels during the DM phase and post DM phase. The pre and post-DM phases would also have to monitor for tissue dysfunction as related to the long term complications associated with DM and metabolic memory. Such DM characterization studies were not performed by Pisharath et al.[29] or Andersson et al.[30] but were performed in other Zebrafish DM/MM models.[8,31,32]

Lastly, in 2017 this same NTR-Zebrafish model was used to demonstrate that ROS can be generated in a beta cell-specific manner[33] in Zebrafish larvae as monitored using a Cell ROX green reagent. In these studies larvae were analyzed during the Met-treatment phase in which hyperglycemia would exist. As discussed in the introduction, ROS has been proposed to be an important compound involved in inducing and maintaining hyperglycemia[2,3] and these studies clearly demonstrated its production in beta cells.

These studies therefore establish a well characterized Zebrafish genetic/chemically-induced type-1 DM model that can be expanded upon to include further analysis of the DM/hyperglycemic state and the mechanisms underlying the long term complications of DM with some modifications to its use so that one is able to study DM and MM in the adult Zebrafish as opposed to just the larvae.

Zebrafish chemically-induced models of type 1 and 2 diabetes

In 2010 and 2012 studies were published that focused on adult Zebrafish and the use of streptozotocin (STZ) to ablate beta cells thereby inducing a DM/hyperglycaemic state.[8,31] Due to the ablation of beta cells, this model represented a type-1 DM model. These studies first conducted a detailed characterization of all diabetic parameters following continued injection of STZ (e.g. blood glucose levels, blood insulin levels, loss of beta cells, production of ROS, AGEs, and A1C, etc.).[31] They found that a true diabetic hyperglycemic state was reached by one week of STZ treatment and this state was maintained as long as STZ treatment was continued. During the hyperglycemic state, tissue dysfunction characteristic of DM was observed to include: thickening of the kidney basement membrane, thinning of retinal cell layers, impaired tissue wound healing as monitored by fin regeneration assays, and impaired angiogenesis.[34] It was found that STZ alone did not induce tissue dysfunctions unless hyperglycemia was induced via IP induction. A follow-up study in 2012[8] focused on the metabolic memory state that occurred once STZ treatment was terminated. They reported that normal blood glucose and insulin levels were reached by 14 days following termination of STZ treatment. This was accompanied by reappearance of insulin producing beta cells in the pancreas. Despite obtaining euglycemia, all parameters of tissue function remained impaired. This suggested that something occurred in the DM/hyperglycemic state that was "remembered" and this pointed to the involvement of an epigenetic process. When DNA was sequenced in the Control, DM, and MM states of the STZ-induced DM model it was found that extensive hypo and hyper-methylation had occurred. The degree of DNA methylation changes that occurred in the DM state did not return to normal in the MM state and this correlated with altered mRNA profiles for the various tissues analyzed as compared to the Control and DM states. This clearly implicated DNA methylation as one epigenetic process contributing to the secondary long term effects resulting from the initial hyperglycemic event.

Finally, Capiotti et al.[32] developed a type-2 like Zebrafish DM model using immersion of Zebrafish in 111 mM glucose for 14 days to induce hyperglycemia. A MM state was induced by 1) treating DM fish with anti-diabetic drugs such as glimepiride or metformin or 2) returning DM fish to normal water conditions. These purely chemically-induced DM/MM Zebrafish models offer the advantage that adult fish can be studied which allows for more detailed tissue analysis to be performed. Because one is studying adult fish in these models, physiological and molecular aspects of true metabolic memory can therefore be analyzed.

Conclusion

A better understanding of the underlying mechanisms of DM and MM are essential if effective treatments are to be developed. The Zebrafish provides a powerful model to analyze such mechanisms because of the combination of both genetic and chemically-induced DM/MM states. Future studies using these models are anticipated to provide a deeper understanding of both DM and MM.

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Conflict of interest

The author has no conflict of interests or commercial interests as related to the information provided in this review.

References

1. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract*. 2010;87(1):4–14.
2. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. *Diabetes*. 2005;54(6):1615–1625.
3. Baynes JW. Role of oxidative stress in development of complications in diabetes. *Diabetes*. 1991;40(4):405–412.
4. Roy S, Sala R, Cagliero E, et al. Overexpression of fibronectin induced by diabetes or high glucose: phenomenon with a memory. *Proc Natl Acad Sci USA*. 1990;87(1):404–408.
5. Kowluru RA, Chakrabarti S, Chen S. Re-institution of good metabolic control in diabetic rats and activation of caspase-3 and nuclear transcriptional factor (NF-kappaB) in the retina. *Acta Diabetol*. 2004;41(4):194–199.
6. Hammes HP, Klinzing I, Wiegand S, et al. Islet transplantation inhibits diabetic retinopathy in the sucrose-fed diabetic Cohen rat. *Invest Ophthalmol Vis Sci*. 1993;34(6):2092–2096.
7. Engerman RL, Kern TS. Progression of incipient diabetic retinopathy during good glycemic control. *Diabetes*. 1987;36(7):808–812.
8. Olsen AS, Sarras MP, Leontovich A, et al. Heritable transmission of diabetic metabolic memory in zebrafish correlates with and aberrant gene expression. *Diabetes*. 2012;61(2):485–491.
9. Kowluru RA. Effect of reinstitution of good glycemic control on retinal oxidative stress and nitrative stress in diabetic rats. *Diabetes*. 2003;52(3):818–823.
10. Li SL, Reddy MA, Cai Q, et al. Enhanced proatherogenic responses in macrophages and vascular smooth muscle cells derived from diabetic db/db mice. *Diabetes*. 2006;55(9):2611–2619.
11. Costa P, Soares R. Neovascularization in diabetes and its complications. Unraveling the angiogenic paradox. *Life Sci*. 2013;92(22):1037–1045.
12. Riddle MC. Effects of intensive glucose lowering in the management of patients with type 2 diabetes mellitus in the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial. *Circulation*. 2010;122(8):844–846.
13. Skyler JS, Bergenstal R, Bonow RO, et al. American Diabetes Association, American College of Cardiology Foundation, A.H. Association, Intensive glycemic control and the prevention of cardiovascular events: implications of the ACCORD, ADVANCE, and VA diabetes trials: a position statement of the American Diabetes Association and a scientific statement of the American College of Cardiology Foundation and the American Heart Association. *Circulation*. 2009;119(2):351–357.
14. Duckworth WC, McCarren M, Abraira C, et al. Glucose control and cardiovascular complications: the VA Diabetes Trial. *Diabetes Care*. 2001;24(5):942–945.
15. Ismail-Beigi F, Craven T, Banerji MA, et al. Effect of intensive treatment of hyperglycaemia on microvascular outcomes in type 2 diabetes: an analysis of the ACCORD randomised trial. *Lancet*. 2010;376(9739):419–430.
16. Gaede PH, Jepsen PV, Larsen JN, et al. The Steno-2 study. Intensive multifactorial intervention reduces the occurrence of cardiovascular disease in patients with type 2 diabetes. *Ugeskr Laeger*. 2003;165(26):2658–2661.
17. Gaede P, Valentine WJ, Palmer AJ, et al. Cost-effectiveness of intensified versus conventional multifactorial intervention in type 2 diabetes: results and projections from the Steno-2 study. *Diabetes Care*. 2008;31(8):1510–1515.
18. Turner RC, Cull CA, Frighi V, et al. Glycemic control with diet, sulfonylurea, metformin, or insulin in patients with type 2 diabetes mellitus: progressive requirement for multiple therapies (UKPDS 49). UK Prospective Diabetes Study (UKPDS) Group. *JAMA*. 1999;281(21):2005–2012.
19. Intine RV, Sarras MP. Metabolic memory and chronic diabetes complications: potential role for epigenetic mechanisms. *Curr Diab Rep*. 2012;12(5):551–559.
20. Nathan DM, Genuth S, Lachin J, et al. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med*. 1993;329(14):977–986.
21. Holman RR, Paul SK, Bethel MA, et al. 10-year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med*. 2008;359(15):1577–1589.
22. Ceriello A, Ihnat MA, Thorpe JE. Clinical review 2: The "metabolic memory": is more than just tight glucose control necessary to prevent diabetic complications?. *J Clin Endocrinol Metab*. 2009;94(2):410–415.
23. Ihnat MA, Thorpe JE, Kamat CD, et al. Reactive oxygen species mediate a cellular 'memory' of high glucose stress signalling. *Diabetologia*. 2007;50(7):1523–1531.
24. Morgan DK, Whitelaw E. The case for transgenerational epigenetic inheritance in humans. *Mamm Genome*. 2008;19(6):394–397.
25. Dolinoy DC, Jirtle RL. Environmental epigenomics in human health and disease. *Environ Mol Mutagen*. 2008;49(1):4–8.
26. Gluckman PD, Hanson MA, Beedle AS. Non-genomic transgenerational inheritance of disease risk. *Bioessays*. 2007;29(2):145–154.
27. Whitelaw NC, Whitelaw E. Transgenerational epigenetic inheritance in health and disease. *Curr Opin Genet Dev*. 2008;18(3):273–279.
28. Bjornsson HT, Fallin MD, Feinberg AP. An integrated epigenetic and genetic approach to common human disease. *Trends Genet*. 2004;20(8):350–358.
29. Pisharath H, Rhee JM, Swanson MA, et al. Targeted ablation of beta cells in the embryonic zebrafish pancreas using *E. coli* nitroreductase. *Mech Dev*. 2007;124(3):218–229.
30. Andersson O, Adams BA, Yoo D, et al. Adenosine signaling promotes regeneration of pancreatic beta cells *in vivo*. *Cell Metab*. 2012;15(6):885–894.
31. Olsen AS, Sarras MP, Intine RV. Limb regeneration is impaired in an adult zebrafish model of diabetes mellitus. *Wound Repair Regen*. 2010;18(5):532–542.
32. Capiotti KM, Antonioli R Jr, Kist LW, et al. Persistent impaired glucose metabolism in a zebrafish hyperglycemia model. *Comp Biochem Physiol B Biochem Mol Biol*. 2014;171:58–65.
33. Kulkarni AA, Conteh AM, Sorrell CA, et al. An *in vivo* zebrafish model for interrogating ROS-mediated pancreatic beta-cell injury, response, and prevention. *Oxid Med Cell Longev*. 2018;2018:1324739.
34. Sarras MP Jr, Mason S, McAllister G, et al. Inhibition of poly-ADP ribose polymerase enzyme activity prevents hyperglycemia-induced impairment of angiogenesis during wound healing. *Wound Repair Regen*. 2014;22(5):666–670.