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

Introduction
Diabetes mellitus (DM) will likely affect over 400 million worldwide by 2030 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)]. 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 and from clinical trials indicate that complications from the onset of hyperglycemia 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. 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. The heritable nature of metabolic memory 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. Furthermore, these processes allow for quick adaptation to environmental stimuli but also impart the ability of the cell to “memorize” these encounters. The underlying molecular mechanism/s of MM has been examined via both animal model approaches and in vitro based studies. 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. 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
Lastly, in 2017 this same NTR-Zebrafish model was used to demonstrate that ROS can be generated in a beta cell-specific manner 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 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 genetically-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/hyperglycemic state. 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 AIC, etc.). 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. It was found that STZ alone did not induce tissue dysfunctions unless hyperglycemia was induced via IP induction. A follow-up study in 2012 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. 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 glibenpiride 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
