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Use of zebrafish as a model to investigate the role of epigenetics in propagating the secondary complications observed in diabetes mellitus☆

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ABSTRACT

Diabetes mellitus (DM) is classified as a disease of metabolic dysregulation predicted to affect over 400 million individuals world-wide by 2030. The debilitating aspects of this disease are the long term complications involving microvascular and macrovascular pathologies. These long term complications are related to the clinical phenomenon of metabolic memory (MM) that is defined as the persistence of diabetic complications even after glycemic control has been pharmacologically achieved. The persistent nature of MM has invoked involvement of epigenetic processes. Current research with the DM/MM zebrafish model as described in this review as well as human and mammalian studies has established that changes in DNA methylation patterns appear to contribute to tissue dysfunctions associated with DM. This review will describe studies on an adult zebrafish model of type I diabetes mellitus that allows analysis of both the hyperglycemic (HG or DM) phase and MM phase of the disease. The review will discuss the model in regards to: 1) its hyperglycemic phase, 2) its MM phase, 3) biochemical õpathways underlying changes in DNA methylation patterns observed in the model, 4) loci specific changes in DNA methylation patterns, and 5) strengths of the adult zebrafish model as compared to other MM animal models.

1. Introduction

This review will focus on how DNA methylation changes contribute to the long term secondary complications of diabetes mellitus (DM) using an adult zebrafish model of type I DM that is uniquely qualified for this type of epigenetic analysis. In this regard, diabetes mellitus is classified as a disease of metabolic dysregulation that results in reduced life expectancy due to disease specific microvascular (retinopathy, nephropathy, neuropathy, impaired wound healing) and macrovascular (heart disease and stroke) complications (Brownlee, 2005). A unifying mechanism for the induction of complications due to hyperglycemia has been proposed by Brownlee and central to this mechanism is the increased production of reactive oxygen species (ROS) which in turn promotes flux through the polyol, hexosamine, protein kinase C and AGE formation pathways leading to altered gene expression profiles of affected cells (Baynes, 1991; Brownlee, 2005). The results of several large scale clinical trials indicate that once initiated, these complications persist and continue to progress unimpeded even when glycemic control is achieved through pharmaceutical intervention (Gaede et al., 2003; Holman et al., 2008; Turner et al., 1999). This persistence was first documented in a canine model of DM and has been supported by multiple lines of experimental evidence using a variety of animal models and in vitro culture systems (El-Osta et al., 2008; Ihnat et al., 2007b). Collectively, these studies clearly show that the initial hyperglycemic period results in permanent abnormalities (including aberrant gene expression) in the target organs/cells and this harmful phenomenon has been termed, Metabolic Memory (MM) (Ceriello et al., 2009; Ihnat et al., 2007a). The ability to sustain these complications in the absence of hyperglycemia invokes a role for the epigenome in perpetuating diabetic complications and MM.

1.1. Description of the adult zebrafish type I model of diabetes mellitus and metabolic memory.

As many cellular processes are highly conserved throughout vertebrate evolution, zebrafish models spanning a wide range of human pathologies including genetic disorders and acquired disease have been

Abbreviations: HG, Hyperglycemia; DM, Diabetes mellitus; MM, Metabolic memory; DM/MM, Diabetes mellitus/Metabolic Memory; GLUT, Glucose transporter; ROS, Reactive oxygen species; VEGF, Vascular endothelial growth factor; STZ, Streptozotocin; AGE, Advanced glycation end product; Tet, Ten–Eleven Translocase; CpG, Cytosine–Phosphate–Guanine; MR, Methylated DNA region; Parp, Poly-ADP ribose polymerase; TF, Transcription factor; dnmt1, DNA methyl transferase (gene 1); mcm2, Mini-chromosome maintenance protein (gene 2); orc3, Origin of replication (gene 3).

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developed (Lieschke and Currie, 2007). In the context of diabetes mellitus, recent data indicate that zebrafish regulate glucose metabolism via the same enzymes and pathways as mice and humans. For example, high glucose in zebrafish stimulates insulin expression (Elo et al., 2007), negatively regulates gluconeogenesis (Elo et al., 2007), increases cortisol levels (Powers et al., 2010) and induces expression of VEGF (Alvarez et al., 2010). In addition, characterization of the GLUT transporter family has revealed remarkable conservation of structure, function and glucose affinity from zebrafish to human (Castillo et al., 2009). From a pharmacological perspective, zebrafish have been documented to respond to anti-diabetic drugs reducing blood glucose levels which further illustrates the physiological conservation of glucose regulation (Elo et al., 2007). As in mammals, the zebrafish pancreas is comprised of two types of both exocrine and endocrine tissue with the later responsible for regulation of glucose metabolism through secretion of insulin, somatostatin, and glucagon directly into the bloodstream (Gnugge et al., 2004). Due to the above factors it was hypothesized that the zebrafish would make a suitable model for examination of type I DM and its complications.

Experimental models of type I diabetes can be induced through pancreatic beta-cell destruction utilizing the diabetogenic drug streptozotocin (STZ) and this approach was used to induce hyperglycemia in the zebrafish X. Characterization of this model demonstrated that zebrafish exhibit the similar characteristics as patients with diabetes mellitus including: 1) hyperglycemia (HG), FBGLs increasing from 59 mg/dl to 307 mg/dl, 2) loss of pancreatic beta-cells, 3) significantly reduced serum insulin, 4) increased serum nonenzymatic glycated proteins and 5) the known secondary complications of diabetes mellitus including: retinal thinning, renal glomerular basement membrane thickening, and impaired epithelial wound healing (Olsen et al., 2010). Other endocrine cells of zebrafish islets are not affected by STZ treatment as previously reported (Olsen et al., 2010, 2012). It should be noted that STZ-treated fish also exhibit the additional complication of impaired tissue regeneration which uniquely provides a quantifiable bioassay of hyperglycemia induced tissue dysfunction (Olsen et al., 2010). Recently published studies have extended this work to the process of angiogenesis in the zebrafish (Sarras et al., 2014).

In the human, the histopathology of type 1 diabetes is defined by a decreased β-cell mass in association with insulitis, a characteristic lymphocytic infiltration limited to the islets of Langerhans and prominent in early stage disease in children (In't, 2011). Animal models designed to mimic type 1 diabetes use various approaches, both genetic and pharmacological. In the later case, as indicated above, streptozotocin is often used as a diabetogenic drug (see Table 2). Depending on the dose of STZ used, beta-cell death may or may not be accompanied by insulitis in mammalian animal models (Rossini et al., 1977). Recent studies indicate however, that onset of hyperglycemia is not related to insulitis, but rather is solely the result of STZ-induced beta-cell death (Deeds et al., 2011; Lu et al., 1998; O'Brien et al., 1996). Our studies do not report the occurrence of insulitis associated with STZ treatment of zebrafish (Olsen et al., 2010).

One of the main reasons zebrafish was chosen is because as a regeneration competent organism it was hypothesized that if allowed, the fish would restore glucose control via beta-cell replenishment. When STZ was removed, the fish did indeed regain glucose control and as expected this was accompanied by new beta cell production (Olsen et al., 2012). In contrast, tissue regeneration, wound healing and angiogenesis remained impaired to the same extent in the newly euglycemic fish as their acutely DM counter parts (Olsen et al., 2012). Moreover it was shown that the impairment was transmissible from mother to daughter cell indicating a (epi)genetic component to the persistence of these complications (Olsen et al., 2012). As such, this model provides a unique opportunity to examine hyperglycemia-induced changes within a wide variety of tissues as the fish transverse through the normal, DM, and MM states. This allows for the study of important regulatory systems underlying both DM and MM linking the relationship between the two. Furthermore, the contribution of epigenetic mechanisms to the MM phenomenon can be studied independent of the potentially complicating factors of AGE, ROS, etc, of the previous diabetic state (Olsen et al., 2012).

1.2. Correlation of DNA epigenetic changes to DM and MM zebrafish

Studies with the DM/MM zebrafish model (Olsen et al., 2010, 2012) were begun to address the epigenetic aspect of MM by first examining hyperglycemia induced DNA methylation changes as, it is well documented that the methylation status of DNA is heritable through mitosis. Through methylated DNA immuno-precipitation followed by sequencing experiments, it was documented that hyperglycemia-induced specific CpG island demethylation (assayed by genome wide CpG island methylation status identification at a fifty base pair resolution) which was followed by re-methylation in the MM state, but this re-methylation was not restored to "normal levels" for a subset of loci (Olsen et al., 2012). When this data was viewed within the context of global gene expression (via microarray analysis), a correlation of CpG island DNA demethylation changes and altered expression was observed (Olsen et al., 2012; Sarras et al., 2013). Therefore, the persistence of hyperglycemia-induced retardation of fin regeneration correlated directly with aberrant DNA methylation and continued gene expression alterations in the MM state. This led us to conclude that the epigenetic DNA methylation mechanism may be responsible, in part, for the metabolic memory phenomenon. Additional DNA methylation studies (data not published) indicate that this epigenetic event is not limited to fin tissue but is also observed in others zebrafish tissues with clinical relevance to the human diabetic condition, to include: 1) renal, 2) retinal, and 3) skin tissue. The clinical relevance of these findings is strengthened by the recent report that DNA de-methylation is also observed in the case of patients with diabetics mellitus as related to epigenetic changes in the Connective Tissue Growth Factor Gene (Zhang et al., 2014). No other diabetic zebrafish models have focused on epigenetic changes associated with the DM and MM states. It should be emphasized that a number of controls were conducted to determine that the changes associated with DM and MM were not due to spurious affects of the STZ. For example, direct injection of STZ into fins does not affect fin regeneration or induce DNA methylation changes. Other controls are described in detail in the original article (Olsen et al., 2010). In summary, these controls indicate that tissue deficits, epigenetic changes, and gene expression pattern changes are solely due to hyperglycemia and not due to a non-specific effect of STZ.

Preliminary studies not yet published related to the DNA methylation state of the caudal fin in control, DM, and MM conditions indicate that DNA de-methylation has very specific characteristics. These studies focus on 1) what functional gene groups are prominent during DM, 2) which genes of these groups persist into MM, and 3) what is the positional genomic relationship of DNA methylation to these genes. Our data indicates that the DM/MM states are associated with alterations in gene expression within the *DNA replication and DNA metabolism* groups. Methylated DNA regions (MRs) were found 6–13 kb upstream of the transcription start site for a subset of functionally important genes (*dnmt1*, *mcm2*, and *orc3*) within these groups and MRs were associated with in silica identified transcription factor (TF) binding sites whose methylation is known to perturb TF binding. Translational application to the human genome is currently being investigated as it applies to the human *DNMT1*, *MCM2* and *ORC3* genes and these studies suggest that zebrafish MRs correspond to high identity regions in the counterpart human genes.

One of the main advantages of the zebrafish model is that it is experimentally very approachable and amenable to genetic manipulation thereby allowing for the dissection of molecular pathways and mechanisms. The above mentioned de-methylation events occurring during hyperglycemia raise the question as to the pathways involved in this process. Studies from human cells, rats, and zebrafish have documented that hyperglycemia induces the demethylation of specific cytosines throughout the genome. The authors have published studies that at