A Potential gDNA Methylation Epigenetic Mechanism to Explain the Long Term Complications of Type 1 and 2 Diabetes

Abstract

Metabolic memory (MM) is the persistence of diabetic (DM) complications even after glycemic-control is pharmacologically achieved. Using a zebrafish diabetic model that induces a MM state, we previously reported that in this model, tissue dysfunction was of a heritable nature based on cell proliferation studies in limb tissue and this correlated with epigenetic gDNA-methylation changes that paralleled alterations in gene expression. Bioinformatics analysis found that gene expression of the DNA replication/DNA metabolism process group was altered in the DM state and altered expression continued into MM state. In this mini-review, we propose that the underlying mechanism(s) that explain these gene expression changes relate to the long term complications observed in diabetes stems from alterations in the ability of transcription factors to bind to their respective gDNA binding sites. gDNA methylation changes of promoter regions (both proximal and distal to the transcription start site) would disrupt the ability of transcription factors to bind to their gDNA binding sites and this would be tissue specific; thus explaining the variety of problems observed patients suffering from the long term complications of diabetes mellitus (e.g. problems associated with the kidney, retina, skin wound healing processes, and angiogenesis, etc.). Clinical studies are now in planning for the translation of these findings to human DM.

Keywords: Diabetes; Metabolic memory; Zebrafish; Epigenetics; gDNA methylation; Bioinformatics; Transcription factor binding

Introduction

Current research points to hyperglycemia-induced changes to the methylation of gDNA as a contributing factor in the transition from a normal physiological state to one of diabetes mellitus [1-14]. In addition, persistence of this altered gDNA methylated state has been proposed to underlie the induction of changes in tissue-specific gene expression patterns that are associated with the many secondary complications observed in patients with both type-1 and type-2 diabetes [1-14]. The data, to date, are based on correlative findings but functional studies are required to establish a mechanistic link between: hyperglycemia, gDNA methylation changes, altered tissue-specific gene expression patterns, gene regulation systems affected by gDNA methylation, and the subsequent appearance of the secondary complications associated with diabetes mellitus. This mini-review will propose a hypothesis and specific experiments to test this hypothesis that will establish a functional link between the above mentioned pathogenic processes. The review begins with

(i) a review of the literature that points to the role of gDNA methylation in the induction of diabetes and its long term secondary complications, and will then

(ii) propose mechanisms to explain, in part, these processes and present specific functional studies to test these proposed mechanisms.

Current data pointing to gDNA methylation as an underlying epigenetic mechanism(s) of the long term complications observed in diabetes

In addition to issues related to chronic glycemic dysregulation, a major problem with diabetes mellitus (DM) (both type 1 and 2) is its long term complications. In this regard, patients with diabetes encounter a multitude of tissue dysfunctions related to: cardiovascular disease, aberrant angiogenesis, retinopathy, nephropathy, neuropathy, and impaired wound healing [1-14]. Our laboratory has previously developed and reported on an adult zebrafish model of type 1 DM that has unique strengths for elucidating the mechanisms underlying the long term complications of the disease [2,13,14]. In this model, streptozocin (STZ) induced hyperglycemia (serum glucose = 315 +/- 40.96 mg/dL) is accompanied by the full range of diabetic complications seen in patients with DM [13,14]. Additionally, we have shown that withdrawal of STZ results in regeneration of pancreatic ß-cells and the return of previously diabetic fish to a physiologically normal glycemic state within 2 weeks [serum glucose = 62.5 +/- 13.6 mg/dL]. However, in contrast, the tissue deficits associated with hyperglycemia persisted permanently.
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To validate these bioinformatics findings, we will establish that
(a) MRs are functionally related to the genes we have identified and
(b) that transcription factor binding is actually affected by the observed gDNA methylation changes through ChIP analysis studies.

Such an analysis will form a foundation for future studies to determine why particular gene regulatory regions are targeted for gDNA methylation changes following episodes of hyperglycemia.

Clinical studies are now planned to translate these animal model findings to the human DM condition.

**Conclusion**

This review has described data pointing to gDNA methylation as an epigenetic mechanism that at least partially explains the long term complications observed in patients with type 1 or 2 diabetes. Future studies will elucidate these mechanisms and potentially lead to therapeutic approaches to prevent or reverse the long term complications of this disease. Table 1 summarizes the findings and proposals of this mini-review.

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**Table 1. Summary of Data/Proposals Discussed in this Mini-Review**

1. STZ IP injection induces reversible T1 DM in adult zebrafish.
2. The physiological changes induced by STZ mimic those seen in the human DM condition to include: hyperglycemia, aberrant angiogenesis, retinopathy, nephropathy, and impaired wound healing; as well as impaired tissue regeneration as seen in the case of impaired fin regeneration.
3. While withdrawal of STZ leads to a return to normal glycemic levels, the tissue abnormalities described in Point 2 persist indefinitely in the adult zebrafish thereby creating a Metabolic Memory state.
4. Hyperglycemia is accompanied by hypomethylation of gDNA and this epigenetic change persists into the Metabolic Memory state.
5. gDNA methylation changes of Point 4 are accompanied by alterations in fin mRNA expression patterns.
6. Bioinformatics analysis indicates that genes of the DNA replication/DNA metabolism process group (with up-regulation of the apex1, mocm2, mcm4, orc3, lig1, and dnmt1 genes) were altered in the DM state and these molecular changes continued into Metabolic Memory state as impaired cell division in the fin was observed.
7. Persistent gDNA-methylation changes could be found as far as 6-13 kb upstream of the transcription start site for these genes, thus suggesting potential higher levels of epigenetic control.
8. Preliminary bioinformatics analyses indicate that both proximal and distal promoter regions (relative to each gene's transcription start site [TSS]) have alterations in their gDNA methylation of cytosine bases (termed Methylated Regions, MRs).
9. Based on these findings, the hypothesis is proposed that: “Persistent tissue dysfunction in the DM/MM states correlates with hyperglycemia-induced DNA methylation changes within transcription factor binding sites”.
10. Specific studies are proposed to test this hypothesis and clinical studies are planned to expand these animal model findings to the human DM condition.

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