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Mini Review

Global but Loci Specific Gdna Methylation Changes are Induced by Hyperglycemia in the Both the Acute and Metabolic Memory States of Type-1 Diabetes

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Abstract

Although glycemic control in diabetes can be managed through appropriate medications, diet, and exercise; the long term complications of the disease pose a severe health threat to both the type 1 and type 2 diabetic patient. These long term complications mostly stem from dysfunctions in the cardiovascular system that lead to organ failures in the renal, retinal, and integument systems; to name only a few affected in the disease. The long term complications arise in patients groups that are 1) both well controlled and 2) poorly controlled for their hyperglycemic episodes. This fact has generated the term "diabetic metabolic memory" that hypothesizes that initial hyperglycemia causes systemic changes that are "remembered" in the long term diabetic and result in the organ dysfunctions that are observed. As discussed in this review, mounting evidence indicates that one contributing factor in establishing metabolic memory is the occurrence of gDNA methylation changes that likely underlie organ dysfunction due to induced problems in normal gene regulation patterns.

Keywords: Diabetes mellitus; Zebrafish; Metabolic memory; Epigenetics; Gdna methylation; Chromatin; promoters; Hypomethylation; Hypermethylation; Bioinformatics

Introduction

Diabetes mellitus (DM, both type-1 and type-2) is a disease of metabolic dysfunction and currently affects 23.6M Americans with a projection of 400M worldwide by 2030 [1]. Although glycemic control in diabetes can be managed through appropriate medications, diet, and exercise; the long term complications of the disease pose a severe health threat to both the type 1 and type 2 diabetic patients [1-5]. These long term complications involve a broad array of tissue/organ systems such as the cardiovascular system, renal system, retinal system, and integument as related to problems with wound heading [1-5]. Clinical trials have established that once hyperglycemia is initiated, complications can be observed to persist and continue to progress even when glycemic control is achieved through medical intervention; a process termed, "Metabolic Memory" (MM) [5-19]. The mechanism(s) of metabolic memory have been examined through both animal model approaches and *in vitro* type studies [20-26] and these. These studies indicate that hyperglycemia results in permanent aberrant gene expression in tissues affected by the disease. The ability to sustain these complications in the absence of hyperglycemia indicates a role for the epigenome to perpetuate tissue dysfunction. While epigenetic research has been conducted regarding histone modifications [27-36] and microRNA mechanisms [37-43], less is known about the role of hyperglycemia-induced persistent gDNA methylation changes; although data from animal models and humans indicate that aberrant gDNA methylation does occur in diabetes. Moreover, the hyperglycemic environment induces changes in the cardiovascular system as seen in endothelial cells that undergo structural, metabolic, and functional alterations such as aberrant blood vessel formation [2,5,44,45]. It should be noted that, blood vessel formation is a critical fundamental process found to be altered in a broad spectrum of organs/tissues affected in diabetes [2,5,44,45] and therefore; any pathology associated with blood vessel formation can lead to systemic problems that diminish the long term health and survival of the diabetic patient. Epigenetic mechanisms underlying this pathology thereby provide a partial explanation for the basis of metabolic memory and the consequences of its continuance in both the type-1 and type-2 diabetic patient.

Discussion

Analysis of the acute and metabolic memory states of type 1 diabetes as studied in a zebrafish animal model of the disease

Previous articles and reviews by our laboratory have described in detail the development and use of a type-1 diabetes zebrafish model for study of the acute and metabolic memory states of this disease [24,46,47] The reader is referred to these articles for an in-depth description of this zebrafish DM/MM model. In brief, zebrafish was chosen to develop a DM/MM model because of its high regenerative capacity. Ablation (either surgically or chemically) of almost any tissue/organ in the zebrafish results in subsequent regeneration of this tissue/organ. Taking advantage of this fact, studies were begun to determine the feasibility of chemically ablating the beta cells of the zebrafish pancreas using the beta cell degenerative agent, streptozotocin (STZ); thus producing a type-1 DM state. Studies found that STZ induced an increased fasting glucose levels from 60 mg/dL to 315 mg/dL with one week of treatment. Hyperglycemia was accompanied by tissue/organ dysfunction of the cardiovascular

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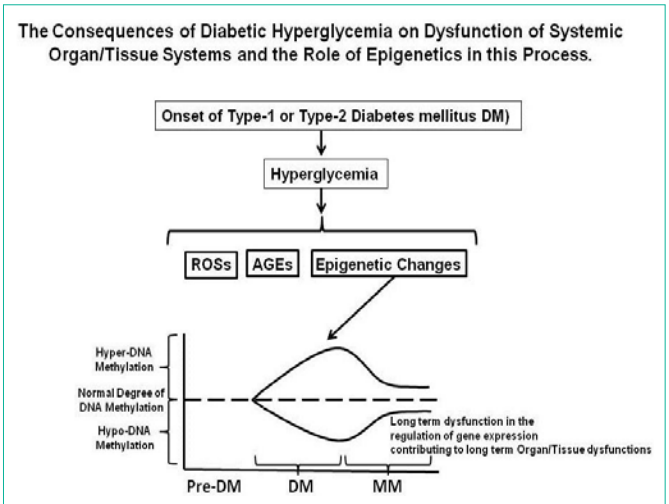

Figure 1: Scheme of the relationship of Hyperglycemia to the long term organ/tissue dysfunctions observed in Diabetes mellitus. ROSs: Reactive Oxygen Species; AGEs: Advanced Glycation End-products; Epigenetic changes as related to gDNA methylation as shown in the graph; Pre-DM: Pre-Diabetes Mellitus; DM: Diabetes Mellitus; MM: Metabolic Memory.

system, renal system, retinal system, limb regenerative function, and integument (as related to wound healing) throughout the time of STZ treatment. Subsequent removal of STZ treatment resulted in a return to normal glucose levels within two weeks; however, tissue/organ dysfunction was retained. Therefore, the model allowed one to induce a DM state and then return the fish to a normal glycemic state after a defined period of hyperglycemia. The fact that tissue/organ dysfunction was retained after normal glycemic levels returned, indicates that the fish had entered a true "metabolic memory" state upon termination of STZ treatment.

Methylated gDNA Patterns in the Acute and Metabolic Memory States in the Zebrafish Type 1-DM model

The DM/MM type-1 zebrafish model allows one to study the mechanisms of metabolic memory without the continuance of hyperglycemic episodes that are seen in the type-1 DM patient or mammalian animal models of the disease. These hyperglycemic episodes result in continue metabolic dysfunction as related to the generation of Reactive Oxygen Species (ROSs) and Advance Glycation End-products (AGEs). Such reagents create "metabolic noise" that complicates discernment of mechanisms that are unique to metabolic memory and unrelated to ROS and AGE affects.

When epigenetic changes related to gDNA methylation patterns were studied by MeDIP sequencing and micro-array analysis in zebrafish in the metabolic memory state, specific molecular patterns were observed. Specifically, as compared to controls, DM fish underwent alterations in the amount of gDNA methylation (both Hypomethylation and Hypermethylation) in specific loci for a given tissue/organ [48]. These patterns were retained in fish that entered the metabolic memory state; although the degree of methylation in any given gene loci could be observed to change (either higher or lower amounts of methylation). Gene expression changes accompanied the gDNA methylation patterns. These gene expression patterns were observed in both DM and MM as compared to controls.

Analysis of the specific loci affected found gDNA methylation changes in regulatory gene groups such as members of the DNA replication/repair process group. This included such genes as *apex1, mcm2, mcm4, orc3, lig1*, and *dnmt1* [48]. Of these genes, dnmt1 is of particular interest due to its critical function in the gDNA methylation process. Bioinformatic analysis of the data found that gDNA methylation changes occurred as far as 6-13 kb upstream of the transcription start site of these genes, indicating potential effects regarding enhancer elements [48].

As a follow-up to these studies, global gDNA methylation patterns were then studied [49]. These studies focused on gDNA regions 10Kb upstream, 1Kb upstream, and 300bp downstream of the transcription start site for all genes of the zebrafish genome [49] in the control, DM, and MM zebrafish groups. Analysis of the general pattern of gDNA methylation in the three regions found no distinct pattern of spatial distribution. Methylation was found to occur anywhere along the Minus or Plus DNA strand; suggesting a random distribution. However if one analyzes the counts of methylated CpG dinucleotides, a different trend was observed. For the three regions analyzed, the number of methylated CpG dinucleotides was distinctly different between the Control, DM and MM groups. The number of methylated CpG dinucleotides in the DM group appears to be overall increased as compared to the controls, while the number of methylated CpG dinucleotides in the MM group appears to be overall decreased. Therefore, while hyperglycemia triggers gDNA methylation changes, these changes can involve both Methylation (DM) and De-Methylation (MM). If one focuses on specific gene groups, it is found that specific patterns can be observed. For example, methylation changes for the genes involved in blood vessel formation, predominantly occurs 10Kb upstream of the transcription start site in these genes. Approximately a total of sixty genes at this time can be identified involved in the regulation of blood vessel formation based on human and vertebrate data bases [49]. Of these sixty genes, the greatest number of methylated CpG dinucleotides were observed in the Control group and the majority of these were found on the "+" strand of DNA (20 genes) with only 6 genes having methylated CpG dinucleotides on the "-" Strand. Additionally, five genes of the "+" strand were found to have no methylated CpG dinucleotides in DM, indicating that complete de-methylation had occurred with genes of this group. All six genes on the "-" strand retained methylated CpG dinucleotides, although this analysis does not tell one that the number of methylated CpG dinucleotides remains the same in these genes in DM. By comparison, all 20 genes in the Control group with methylated CpG dinucleotides were lost in the MM group indicating a complete de-methylation had occurred with these genes. One gene of the DM group (*mmp2*) showed a loss of all methylated CpG dinucleotides in the MM group indicating that in the transition from the DM to the MM stage, *mmp2* is completely de-methylated. Moreover, *pola2*, a gene that was not seen in the Control or DM groups, becomes methylated in the MM group. In the 1Kb and 300bp regions, only two genes were methylated. For these regions, tet3 becomes methylated in the DM state but is not methylated in Control or MM groups. To add to this complexity, one finds that the other gene with methylated CpG dinucleotides in the 1Kb and 300bp regions is *mbd2. mbd2* is a gene located on both the Plus and Minus DNA strands with its own transcription start site on both of these strands. In total, these studies indicated the high degree of complexity involved during gDNA methylation that is induced by hyperglycemia.

From a functional standpoint, one may ask how this relates to the tissue/organ dysfunctions observed in the MM state following hyperglycemia? The answer rests in the fact that gDNA methylation changes can occur in promoter and enhancer regions as well as a gene's UTRs and open reading frame. Because it is known that methylation in promoter and enhancer regions affects the ability of transcription factors to bind to their respective sites, one can hypothesize that the methylation changes induced by hyperglycemia can alter the regulation of gene expression patterns for a given tissue/organ; thereby leading to the induction of dysfunction and the long term pathology that is observed. Additional studies are required to further elucidate these DM/MM mechanisms.

The application of such data to the treatment of DM and MM has limitations, but also has distinct avenues of application for the human disease. The limitation is the broad number of genes, such as genes that regulate the formation of blood vessels that are affected by gDNA methylation. On the other hand, new technical approaches are available that offer hope in this regard. For example, recent studies using non-viable embryos have shown that mutated genes could be targeted using CRISPR technology for correection of the heritable blood disorder, beta thalassemia. There were limitations however, in that the few embryos that took up the change made by CRISPR were found to be a patchwork of edited and unchanged cells. In addition, the embryos affected bore unintended edits outside the targeted gene. Later, another group reported repairing disease-causing mutations *in viable* embryos, but some still contained a patchy mix of edited cells; a phenomenon called mosaicism. It should be noted that none of these groups went on to implant the manipulated embryos in women. Most recently, the laboratory of Mitalipov produced tens of successfully edited embryos, and avoided the issue of mosaicism by injecting eggs with CRISPR right as they were fertilized with donor sperm [50]. These advances suggest that embryonic gene editing may be possible and applied to those with at least Type-1diabetes.

Conclusion

While the mechanisms underlying metabolic memory are multifaceted, the present review indicates that epigenetics likely has an important role in its pathology. There are, of course, many aspects of epigenetics not discussed in this review such as the role of histone modifications; but the maintenance of gDNA methylation changes after initial episodes of hyperglycemia occur, argues for changes in gDNA methylation patterns being an important contributing factor in the prolonged pathology associated with the long term complications observed in MM. A schematic flow-chart of the major points discussed in this review regarding the inter-relationship of diabetic hyperglycemia, organ/tissue dysfunctions, and the role of epigenetics in these processes is shown in Figure 1.

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