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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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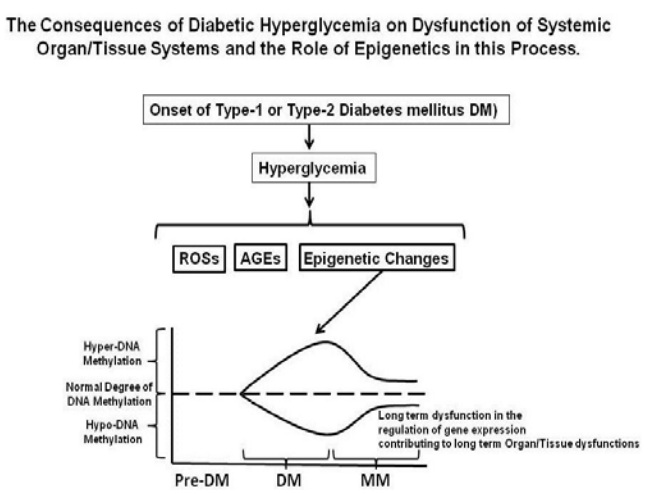

Figure 1: Scheme of the relationship of Hyperglycemia to the long term organ/tissue dysfunctions observed in Diabetes mellitus. ROSs: Reactive Oxygen Species; AGEs: Advanced Glycation End-products; Epigenetic changes as related to gDNA methylation as shown in the graph; Pre-DM: Pre-Diabetes Mellitus; 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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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: 4-14.
2. Inzucchi S, Majumdar S. Glycemic Targets: What is the Evidence? Med Clin North Am. 2015; 99: 47-67.
3. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes. 2005; 54: 1615-1625.
4. Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes. 1991; 40: 405-412.
5. Costa P, Soares R. Neovascularization in diabetes and its complications. Unraveling the angiogenic paradox. Life Sci. 2013; 92: 1037-1045.
6. 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: 844-846.
7. Skyler JS, Bergenstal R, Bonow RO, Buse J, Deedwania P, Gale EAM, et al. 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. Diabetes Care. 2009; 32: 187-192.
8. Duckworth WC, McCarren M, Abraira C, VA DT. Glucose control and cardiovascular complications: the VA Diabetes Trial. Diabetes Care. 2001; 24: 942-945.
9. Ismail-Beigi F, Craven T, Banerji MA, Basile J, Calles J, Cohen RM, 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: 419-430.
10. Gaede P, Valentine WJ, Palmer AJ, Tucker DMD, Lammert M, Parving HH, 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: 1510-1515.
11. Gaede PH, Jepsen PV, Larsen JN, Jensen GV, Parving HH, Pedersen OB. [The Steno-2 study. Intensive multifactorial intervention reduces the occurrence of cardiovascular disease in patients with type 2 diabetes]. Ugeskr Laeger. 2003; 165: 2658-2661.
12. Turner RC, Cull CA, Frighi V, Holman RR. 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 : the journal of the American Medical Association. 1999; 281: 2005-2012.
13. Retinopathy and nephropathy in patients with type 1 diabetes four years after a trial of intensive therapy The Diabetes Control and Complications TrialEpidemiology of Diabetes Interventions and Complications Research Group. 2000; 342 SRC - GoogleScholar: 381-389.
14. Intine RV, Sarras MP. Metabolic memory and chronic diabetes complications: potential role for epigenetic mechanisms. Current diabetes reports. 2012; 12: 551-559.
15. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus The Diabetes Control and Complications Trial Research Group. 1993; 329 SRC - GoogleScholar: 977-986.
16. Sustained effect of intensive treatment of type 1 diabetes mellitus on development and progression of diabetic nephropathy: the Epidemiology of Diabetes Interventions and Complications (EDIC) study. JAMA. 2003; 290 SRC - GoogleScholar: 2159-2167.
17. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. N Engl J Med. 2008; 359: 1577-1589.
18. Ceriello A, Ihnat MA, Thorpe JE, J. Clinical review2 : The "metabolic memory": is more than just tight glucose control necessary to prevent diabetic complications? Endocrinol Metab. 2009; 94: 2 SRC - Google Scholar: 410-415.
19. Ihnat MA, Thorpe JE, Kamat CD, Szabo C, Green DE, Warnke LA, et al.

Reactive oxygen species mediate a cellular 'memory' of high glucose stress signalling. Diabetologia. 2007; 50: 1523-1531.

20. Roy S, Sala R, Cagliero E, Lorenzi M. Overexpression of fibronectin induced by diabetes or high glucose: phenomenon with a memory. Proc Natl Acad Sci U S A. 1990; 87: 404-408.

21. 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: 194-199.

22. Hammes HP, Klinzing I, Wiegand S, Bretzel RG, Cohen AM, Federlin K. Islet transplantation inhibits diabetic retinopathy in the sucrose-fed diabetic Cohen rat. Investigative ophthalmology & visual science. 1993; 34: 2092-2096.

23. Engerman RL, Kern TS. Progression of incipient diabetic retinopathy during good glycemic control. Diabetes.1987; 3: 808-812.

24. Olsen AS, Sarras MP, Leontovich A, Intine RV, D. Heritable Transmission of Diabetic Metabolic Memory in Zebrafish Correlates With and Aberrant Gene Expression. Diabetes. 2012; 61: 485-91.

25. Kowluru RA. Effect of reinstitution of good glycemic control on retinal oxidative stress and nitrative stress in diabetic rats. Diabetes.2003; 52: 818-823.

26. Li SL, Reddy MA, Cai Q, Meng L, Yuan H, Lanting L, et al. Enhanced proatherogenic responses in macrophages and vascular smooth muscle cells derived from diabetic db/db mice. Diabetes. 2006; 55: 2611-2619.

27. Rando OJ. Combinatorial complexity in chromatin structure and function: revisiting the histone code. Current opinion in genetics & development. 2012; 22: 148-155.

28. Zhong Q, Kowluru RA. Epigenetic changes in mitochondrial superoxide dismutase in the retina and the development of diabetic retinopathy. Diabetes. 2011; 60: 1304-1313.

29. Zhong Q, Kowluru RA. Role of histone acetylation in the development of diabetic retinopathy and the metabolic memory phenomenon. J Cell Biochem. 2010; 110: 1306-1313.

30. Brasacchio D, Okabe J, Tikellis C, Balcerczyk A, George P, Baker EK, et al. Hyperglycemia induces a dynamic cooperativity of histone methylase and demethylase enzymes associated with gene-activating epigenetic marks that coexist on the lysine tail. Diabetes. 2009; 58: 1229-1236.

31. El-Osta A, Brasacchio D, Yao D, Pocai A, Jones PL, Roeder RG, et al. Transient high glucose causes persistent epigenetic changes and altered gene expression during subsequent normoglycemia. J Exp Med. 2008; 205: 2409-2417.

32. Miao F, Smith DD, Zhang L, Min A, Feng W, Natarajan R. Lymphocytes from patients with type 1 diabetes display a distinct profile of chromatin histone H3 lysine 9 dimethylation: an epigenetic study in diabetes. Diabetes.2008; 57: 3189-3198.

33. Miao F, Wu X, Zhang L, Yuan YC, Riggs AD, Natarajan R. Genome-wide analysis of histone lysine methylation variations caused by diabetic conditions in human monocytes. The Journal of biological chemistry. 2007; 282: 13854-13863.

34. Miao F, Gonzalo IG, Lanting L, Natarajan R. *In vivo* chromatin remodeling events leading to inflammatory gene transcription under diabetic conditions. The Journal of biological chemistry. 2004; 279: 18091-18097.

35. Li Y, Reddy MA, Miao F, Shanmugam N, Yee JK, Hawkins D, et al. Role of the histone H3 lysine 4 methyltransferase, SET7/9, in the regulation of NF-kappaB-dependent inflammatory genes. Relevance to diabetes and inflammation. The Journal of biological chemistry. 2008; 283: 26771-26781.

36. Yu J, Auwerx J. Protein deacetylation by SIRT1: an emerging key post-translational modification in metabolic regulation. Pharmacol Res. 2010; 62: 35-41.

37. Prattichizzo F, Giuliani A, Ceka A, Rippo MR, Bonfigli AR, Testa R, et al. Epigenetic mechanisms of endothelial dysfunction in type 2 diabetes. Clin Epigenetics.2015; 7: 56.

38. Putta S, Lanting L, Sun G, Lawson G, Kato M, Natarajan R. Inhibiting microRNA-192 ameliorates renal fibrosis in diabetic nephropathy. Journal of the American Society of Nephrology : JASN. 2012; 23: 458-469.

39. Wang B, Koh P, Winbanks C, Coughlan MT, McClelland A, Watson A, et al. miR-200a Prevents renal fibrogenesis through repression of TGF-+¦2 expression. Diabetes. 2011; 60: 280-287.

40. Kato M, Zhang J, Wang M, Lanting L, Yuan H, Rossi JJ, et al. MicroRNA-192 in diabetic kidney glomeruli and its function in TGF-beta-induced collagen expression via inhibition of E-box repressors. Proc Natl Acad Sci U S A. 2007; 104: 3432-3437.

41. McArthur K, Feng B, Wu Y, Chen S, Chakrabarti S. MicroRNA-200b regulates vascular endothelial growth factor-mediated alterations in diabetic retinopathy. Diabetes. 2011; 60: 1314-1323.

42. Feng B, Chen S, McArthur K, Wu Y, Sen S, Ding Q, et al. miR-146a-Mediated extracellular matrix protein production in chronic diabetes complications. Diabetes. 2011; 60: 2975-2984.

43. Ruan Q, Wang T, Kameswaran V, Wei Q, Johnson DS, Matschinsky F, et al. The microRNA-21-PDCD4 axis prevents type 1 diabetes by blocking pancreatic beta cell death. Proc Natl Acad Sci USA. 2011; 108: 12030-12035.

44. Carmeliet P. Angiogenesis in life, disease and medicine. Nature. 2005; 438: 932-936.

45. Menegazzo L, Albiero M, Avogaro A, Fadini GP. Endothelial progenitor cells in diabetes mellitus. Biofactors. 2012; 38: 194-202.

46. Olsen AS, Sarras MP, Intine RV. Limb regeneration is impaired in an adult zebrafish model of diabetes mellitus. Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society. 2010; 18: 532-542.

47. Sarras MP Jr, Leontovich AA, Intine RV. Use of zebrafish as a model to investigate the role of epigenetics in propagating the secondary complications observed in diabetes mellitus. Comp Biochem Physiol C Toxicol Pharmacol. 2015.

48. Leontovich AAIRV, Sarras MP Jr. Epigenetic Studies Point to DNA Replicaton/Repair Genes as a Basis for the Heritable Nature of Long Term Complications in Diabetes. J Diabetes Res. 2016; 1-10.

49. Leontovich A, Sarras MP Jr. Distribution of Methylated regions within gDNA in Acute and Chronic Phases of Diabetes mellitus. The Handbook of Nutrition, Diet and Epigenetics. 2019.

50. Ma H, Marti-Gtierrez N, Park SW, Wu J, Lee Y, et al. Correction of a pathogenic gene mutation in human embryos. Nature. 2017; 548: 413-419.

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