

The role of DNA methylation in type 1 and 2 diabetes as related to endothelial cell dysfunction

Abstract

A major clinical problem associated with type 1 and 2 diabetes is the debilitating long term complications seen in this disease. This review will discuss 1) the role of epigenetics in type 1 and 2 diabetes, and 2) the dysfunction of endothelial cells in this disease, especially as related to blood vessel growth. While many tissues are affected in DM, pathologies associated with the endothelial cell are often central to these tissue problems. With the identification of epigenetic changes, (e.g. DNA methylation, histone modification, etc.) as processes associated with both DM and MM, investigators now have another potential target for investigation of the molecular mechanisms that may explain the persistent dysfunction seen in the many tissues affected in this disease.

Keywords: type 1 and 2 diabetes, epigenetics, DNA methylation, endothelial dysfunction in diabetes, alterations in blood vessel growth in diabetes

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Abbreviations: DM, diabetes mellitus; MM, metabolic memory; EC, endothelial cell; BVG, blood vessel growth; HG, hyperglycemic; RNA, ribonucleic acid; DNA, deoxyribonucleic acid; 5mC, 5- methylcytosine; AGE, advanced glycation end products; CpG, cytosine-phosphate-guanine; H3K9/K14, histone acetylation groups; EPCs, endothelial progenitor cells

Introduction

Diabetes mellitus (DM) is projected to affect over 400 million worldwide by 2030.¹ Diabetes mellitus is a disease of metabolic dysregulation^{2,3} resulting in microvascular and macrovascular complications.^{2,4-6} As such, the endothelial cell (EC) is a fundamental cell type targeted by the hyperglycemic (HG) episodes that occur in the disease.^{2,4-6} Endothelial cell dysfunction in DM is seen to take on different pathologies such as

- i. Altered compliance,⁷
- ii. Acquired vascular flow abnormalities,⁸
- iii. Altered blood vessel growth (BVG) {through angiogenesis^{5,9,10} and neovascularisation}.^{6,11-14}

Alterations in BVG affects a wide spectrum of organs/tissues in DM thereby causing systemic problems.⁹ In this regard, evidence from both the laboratory¹⁵⁻²¹ and large scale clinical trials^{4,22-35} has revealed that complications from the onset of hyperglycemia (such as impaired BVG) progress unimpeded via the phenomenon of “metabolic memory” (MM) even when glycemic control is pharmaceutically achieved.^{4,22-35} This applies to both type 1 and type 2 diabetes. The underlying molecular mechanisms of hyperglycemic complications and metabolic memory may include:

- i. The involvement of excess reactive oxygen species,
- ii. The involvement of advanced glycation end products (AGE),
- iii. Alterations in tissue-wide gene expression patterns.^{2,3}

However, the heritable nature of metabolic memory^{36,37} suggests a role for the epigenome. 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.³⁸⁻⁴⁰ These processes not only allow for quick adaptation but also confer the ability of the cell to “memorize” these encounters.³⁸⁻⁴⁰ The underlying molecular mechanism/s of MM has been examined via both animal model approaches and *in vitro* based studies.¹⁵⁻²¹ These studies establish that the initial hyperglycemia results in permanent aberrant gene expression in DM target tissues (e.g. cardiovascular system, kidney, retina, skin as related to wound healing, and impaired BVG such as seen in the wound healing process).

Discussion

Potential Role of DNA methylation in metabolic memory of patients with type 1 and 2 diabetes

While extensive epigenetic research has been conducted regarding histone modifications⁴¹⁻⁵⁰ and microRNA mechanisms^{5,51-56} much less is known about the role of HG-induced persistent DNA methylation changes that occur in both type 1 and 2 DM. We have previously reported that hyperglycemia induces aberrant DNA methylation with concomitant altered gene expression patterns that correlate with persistent diabetic complications.¹⁹ The role of HG-induced DNA methylation changes within endothelial cells as related to persistent MM dysfunction remains unclear, particularly as related to alterations in BVG that is common to so many tissues targeted in DM. The role of epigenetics in diabetes is an important and expanding area of study. In this context it is important to note that the role of DNA methylation (as opposed to histone modifications) in DM is much less understood or studied. Epigenomes consist of all the chromatin modifications for a given cell type and are responsible for a cell's unique gene expression pattern. These chromosome modifications support cell differentiation and are dynamic throughout development.⁵⁷⁻⁵⁹ In addition, they are responsive to external stimuli, can be altered in disease,⁶⁰ and are mitotically stably inherited.^{36,37} Epigenetic mechanisms include post-translational histone modifications, non-canonical histone variant inclusion in octomers, chromatin access changes through DNA methylation, and gene expression control through non-coding

microRNAs.⁶¹⁻⁶⁴ Altogether, these processes allow cells/organisms to quickly respond to changing environmental stimuli,³⁸⁻⁴⁰ and confer the ability for the cell to “memorize” these encounters once the stimulus is removed.³⁸⁻⁴⁰ Therefore, because gene expression changes resulting from epigenetic processes are stable in the absence of the signal that initiated them and are heritable through cell division, they have gained great interest as underlying molecular mechanisms of metabolic memory (MM). Significant advances have been made towards understanding the roles that histone modifications⁴¹⁻⁵⁰ and microRNAs^{5,51-56} play in the metabolic memory phenomenon; however, as indicated much less has been documented regarding the role of dynamic DNA Methylation.^{19,65-67} In this regard, DNA methylation occurs predominantly as 5-methylcytosine (5mC); mostly in the context of CpG dinucleotides. In vertebrate genomes, these dinucleotides are clustered into regions (in order of decreasing CpG density) termed islands, shores, shelves, and open seas.^{68,69} Multiple roles for DNA methylation including gene silencing, silencing of transposable elements, developmental regulation of transcription, cell cycle control, and differentiation have been documented.⁷⁰⁻⁷⁴ Historically, hypermethylation of CpG islands in promoter regions was thought to inhibit promoter activity by maintaining chromatin in a stably repressed state with alterations to this resulting in gene expression changes. However, more recent studies indicate that while this is correct for some loci; the majority of tissue specific expression and cancer-induced aberrant expression is governed by variations in the shore regions.⁶⁹ Additionally, genome wide DNA methylation analyses have indicated that methylation in the “bodies” of active genes is significantly higher than those of inactive genes.^{75,76} This feature appears to be evolutionarily conserved and may function to suppress inappropriate transcription, regulate mRNA splicing, modulate elongation, and regulate tissue specific alternative promoter usage.⁷⁷⁻⁸¹ Not unexpectedly, due to its critical role in gene expression, altered DNA methylation is associated with several human diseases including many cancers.⁸²⁻⁸⁶ Variations in “normal” DNA methylation are correlated with many aspects of DM including: susceptibility to DM,⁸⁷⁻⁸⁹ insulin resistance,⁹⁰ diabetes complication development,⁹⁰ and early detection.⁹¹⁻⁹³ Very recently, a comprehensive genomic DNA methylation profiling of type 2 diabetic islets revealed that CpG loci displayed a significant hypomethylation phenotype and may provide insight on diabetic islets and disease pathogenesis.⁹⁴ The first report demonstrating a cause and effect relationship between hyperglycemia and altered DNA methylation documented that genomic hypomethylation was induced within the liver of type 1 diabetic rats as early as 2 weeks post hyperglycemia onset.⁶⁷ Pirola et al.⁶⁶ examined primary aortic endothelial cells exposed to high glucose (24hr) under *in vitro* conditions and performed a more comprehensive analysis of both histone acetylation and DNA Methylation.⁶⁶ In this study they observed significant alterations in DNA methylation patterns and showed that induced methylation changes localized to regions within five kilobases of transcriptional start sites. They also observed broad changes to H3K9/K14 acetylation and reported that regionalized hyper-acetylation correlated well with DNA hypomethylation and hyperglycemia-induced gene induction. However, these studies were limited to *in vitro* conditions did not examine results from a prolonged hyperglycemic state or the metabolic memory state.

The central role of endothelial cells to the long term complications seen in patients with type 1 and 2 diabetes

The role of endothelial cell (EC) dysfunction in the long term complications of diabetes is of profound importance and affects a broad array of tissues.⁹ This has been shown in the case of patients

with long term DM as well as in a broad array of DM/MM animal models from rodent^{15,17} to Zebrafish.^{19,95} Endothelial dysfunction in DM is seen to take on different pathologies such as

- i. Altered compliance,⁷
- ii. Acquired vascular flow abnormalities,⁸
- iii. Altered blood vessel growth (BVG) as related to the processes of angiogenesis^{5,9,10} and neovascularization^{6,11-14} in the case of the adult human.

Angiogenesis in adult humans involves multiple stages⁹⁶ to include:

- i. Tip activation,
- ii. Sprouting,
- iii. Anastomosis; each stage has its own set of regulatory genes.^{5,96}

All of these angiogenic stages are affected in the diabetic state.^{5,96} Neovascularization, which was once thought to only occur during embryonic development, has now been shown in mammals to also contribute to BVG in the adult.⁹⁷ This occurs via endothelial progenitor cells (EPCs) which mainly arise from the bone marrow but are also seen in the peripheral blood.⁹⁸ These EPCs differentiate to endothelial cells and seed to blood vessels; thereby contributing to the process of blood vessel growth in the adult mammal (e.g. human). This has potential therapeutic value because of the EPC's ability to promote blood vessel growth/repair. Angiogenesis and neovascularization utilize a “common gene kit” of regulatory genes.^{5,96,97} In the context of diabetes, EPCs from DM patients have been found to be affected by the disease. For example, EPC numbers are reduced in the diabetic patient,⁹⁹ have some alterations in proliferation, adhesion, and incorporation into vascular structures.¹⁰⁰ Additionally, EPCs obtained from diabetic subjects have been shown to have DNA methylation changes in the promoter regions of key blood vessel growth regulator genes.¹⁰¹ Taken in total, these studies indicate that analysis of DNA methylation changes in the endothelial cells of the diabetic state via analysis of EPCs may lead to a better understanding of the underlying mechanisms of the long term complications of type 1 and 2 diabetes. To be complete, it should be noted that in regard to the dysfunction of endothelial cells in diabetes, the role of miRNAs and histone epigenetic modifications are also of critical importance. As recently reviewed by Prattichizzo et al.¹⁰²

- i. Circulating miRNAs such as miR-126 in type 2 DM patients have been linked to inflammatory pathways in the disease and
- ii. Glucose-mediated changes in the transcription and activation of NF-κB in endothelial cells of DM patients is tied to a) recruitment of histone methyltransferase Set7 with increased monomethylation of H3K9 (lysine 4 of histone 3), b) increased recruitment of histone demethylase LSD1 with reduced H3K9 methylation, c) histone acetyltransferase (HAT)-mediated histone H3K9 hyperacetylation. These are just a few examples of the many roles miRNAs and histone epigenetic changes have in the dysfunction of endothelial cells in diabetes. Additionally, a large body of literature also ties these pathological epigenetic processes to cancer,^{103,104} which also involves abnormalities in cell proliferation as seen in diabetes.

Conclusion

A major clinical problem associated with type 1 and 2 diabetes is the

debilitating long term complications seen in this disease. While many tissues are affected in DM, pathologies associated with the endothelial cell are often central to these tissue problems. With the identification of epigenetic changes, (e.g. tissue-specific DNA methylation, direct DNA methylation within pancreatic b-cells,^{105,106} histone modification, etc.) as processes associated with both DM and MM, investigators now have another potential target for investigation of the molecular mechanisms that may explain the persistent dysfunction seen in the

many tissues affected in this disease. The combination of epigenetic analysis and endothelial cell function could lead to the identification of molecular targets for therapeutic intervention for the treatment and possible prevention of secondary complications of this metabolic disease. A general summary of the events associated with changes in DNA methylation patterns in diabetes is depicted in the article (Figure 1).

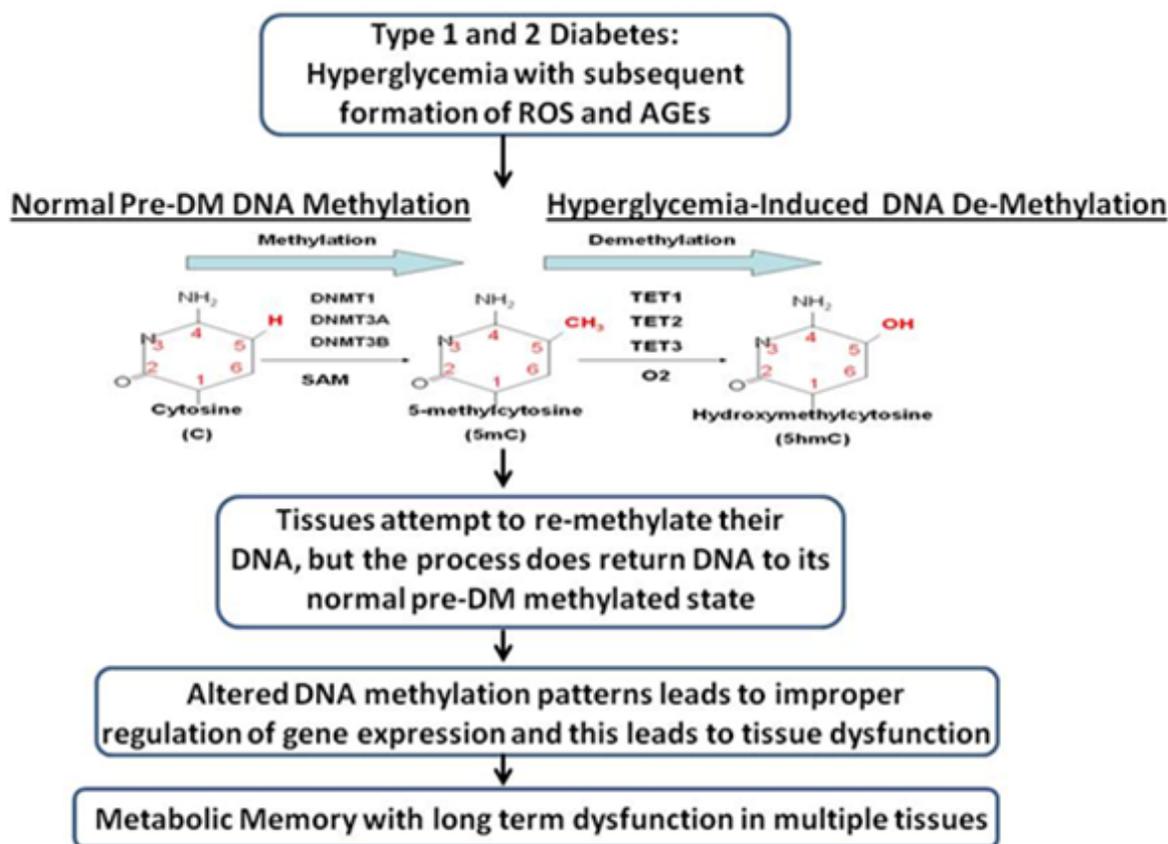


Figure 1 Events in type 1 and type 2 diabetes that are associated with DNA methylation changes and subsequent long-term tissue dysfunction.

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Conflict of interest

Author declares that there is no conflict of interest.

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