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Translational Implications of the β Cell Epigenome in Diabetes Mellitus

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Abstract

Diabetes mellitus is a disorder of glucose homeostasis that affects over 24 million Americans and 382 million individuals worldwide. Dysregulated insulin secretion from the pancreatic β cells plays a central role in the pathophysiology of all forms of diabetes mellitus. Therefore an enhanced understanding of the pathways that contribute to β cell failure is imperative. Epigenetics refers to heritable changes in DNA transcription that occur in the absence of changes to the linear DNA nucleotide sequence. Recent evidence suggests an expanding role of the β cell epigenome in the regulation of metabolic health. The goal of this review is to discuss maladaptive changes in β cell DNA methylation patterns and chromatin architecture and their contribution to diabetes pathophysiology. Efforts to modulate the β cell epigenome as a means to prevent, diagnose, and treat diabetes will also be discussed.

Keywords

Epigenetics; β cell; diabetes; islet; insulin; Pdx-1

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INTRODUCTION

Diabetes mellitus (DM) is a disorder of glucose homeostasis that affects over 24 million Americans and 382 million individuals worldwide (1). There are two main forms of DM, which can be categorized as either Type 1 or Type 2 diabetes mellitus. Nearly 90–95% of worldwide DM cases result from Type 2 diabetes (T2D) and occur secondary to the combined effects of insulin resistance, dysregulated hepatic gluconeogenesis, and progressive β cell dysfunction. Type 1 diabetes (T1D) comprises 5–10% of all DM cases and is secondary to autoimmune destruction of the pancreatic β cell. Despite somewhat disparate etiologies, altered β cell secretory function and survival play a central role in the pathophysiology of both major forms of diabetes mellitus.

Genetic susceptibility to diabetes has been postulated since the early 20th century, and completion of the Human Genome Project in 2003 only increased the intensity of investigation in this area (2–5). Genetic studies now suggest that T2D is largely a polygenic disorder. Underscoring the importance of the β cell, the majority of susceptibility genes identified in genome-wide association studies (GWAS) point to the presence of gene polymorphisms that predispose certain individuals towards β cell failure and diabetes in settings where insulin sensitivity is compromised. In contrast, T1D GWAS studies have primarily identified polymorphisms in genes that regulate autoimmunity (6). However, emerging data indicate that many genes previously thought to only regulate immune function are also expressed in the islet and may impact β cell function and/or susceptibility to immune-mediated destruction (7).

Genetics alone cannot fully account for the observed heritability of diabetes, and recent evidence indicates an ever-more expansive role for epigenetics in the regulation of metabolic health. Epigenetics is defined as heritable changes in DNA transcription that occur in the absence of changes in the primary DNA sequence itself (8, 9). Given the central focus on the β cell during the development and progression of both major forms of diabetes, the goal of this review is to summarize the current literature surrounding the role of the β cell epigenome in the pathophysiology of diabetes mellitus. There are three major categories of epigenetic regulation, and these include DNA methylation, post-translational modification of histone proteins, and effects of non-coding RNA molecules (9–11). Here, we will focus on how DNA methylation patterns and histone modifications modulate gene expression and the β cell phenotype in DM. The emerging role for non-coding RNAs within the β cell under conditions of health and disease has been covered in detail recently elsewhere (12–14).

DNA METHYLATION

DNA methylation is the covalent attachment of a methyl group to the 5-position of a cytosine ring at sites where a cytosine is adjacent to guanine in the linear sequence of DNA. This dinucleotide pair is referred to as a cytosine-phosphodiester-bond-guanine or a CpG site. DNA methylation at CpG sites serves as a key determinant of gene expression and plays an essential role in a number of developmental processes including X-chromosome inactivation and gene imprinting. Methylated CpGs are found throughout the genome and may be located within genes or in intergenic regions, and approximately 70% of CpG

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dinucleotides within the mammalian genome are methylated (15, 16). The majority of unmethylated CpGs are located in regions referred to as CpG islands. These are sections of DNA consisting of large stretches of unmethylated CpG repeats and are typically found within promoter regions of highly expressed genes (17, 18). In contrast,silent or suppressed genes typically have hypermethylated CpG sites (19). DNA methylation-induced suppression of gene activity occurs either through direct inhibition of transcription factor binding or may also be mediated by activity of methyl-CpG binding proteins (20). The proteins that are responsible for adding methyl groups to DNA are members of the evolutionarily conserved DNA methyltransferase (Dnmt) family. All known Dnmt isoforms utilize S-adenosyl methionine (SAM) as a methyl donor (21).

Genes that have already been suppressed by other epigenetic means are more likely to be further silenced by DNA methylation (22–25), with the net result being that transiently silenced genes may become suppressed via longer lasting mechanisms. The additive effect of DNA methylation paired with suppressive histone modifications may silence genes completely, whereas under some circumstances transcription factors are able to overcome repression imposed by histone modification alone (26, 27). DNA methylation is also a more thermodynamically favorable means of gene suppression since continual transcription of noncoding RNA, repeated modification of histones, and transcription and translation of repressive proteins is not required for suppression if the gene promoter remains methylated (28).

Methylation of DNA is also an important regulator of cellular differentiation (29). During development inappropriate genes for a given cell type are methylated at CpG islands, thereby stably and heritably repressing their expression (30–32). For example, methylation of CpG islands at the insulin gene in non-insulin-producing cell types prevents its inappropriate expression, while the insulin promoter is appropriately demethylated in the pancreatic β cell (33–35). Likewise, in order to maintain β cell identity and function, certain essential genes must remain methylated, while detrimental genes should remain methylated and suppressed.

Alterations in DNA methylation patterns may have important metabolic effects and therefore contribute to diabetes pathophysiology. For example, lack of maternal methylation on the chromosome region 6q24 results in a rare form of transient neonatal diabetes (TND). In this disorder, diabetes related to β cell dysfunction occurs within a few days of birth but remits during infancy, while those affected typically develop T2D later in life (36). Three genetic categories of TND have been identified, and these include paternal uniparental disomy of chromosome 6, duplication of the 6q24 region, or loss of maternal methylation of an imprinted 6q24 CpG island (36). While the gene product responsible for TND has not been definitively identified, the 6q24 region contains both an untranslated transcript with no known function as well as the gene for pleomorphic adenoma gene 1 (PLAG1 or ZAC) (37). PLAG1 or ZAC is a zinc finger transcription factor that serves as the leading gene candidate for TND and has been linked to regulation of cell cycle and apoptosis. In the β cell, this gene has also been linked to impaired insulin secretion (38–40). Interestingly, chromosome 6q24 is associated with obesity and T2D in adults, particularly in the African American community. Whether this could be due to a similar epigenetic effect remains an open

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question (41, 42). It is clear, however, that heritable changes in DNA methylation are relevant to the development of metabolic disease.

Research into the role of DNA methylation as a component of diabetes susceptibility and pathophysiology has been carried out using unbiased screens as well as candidate-based approaches. A recent comprehensive DNA methylation profile of cadaveric human islets revealed significant changes in overall methylation patterns in islets from donors with T2D compared to islets from non-diabetic controls. Specifically, this analysis revealed 276 CpG sites corresponding to 254 unique genes with altered CpG island methylation in diabetic compared to non-diabetic islets. Interestingly, only ten sites were hypermethylated in T2D samples, compared to 266 sites that showed decreased methylation. Groups of proteins demonstrating altered methylation included genes related to three broad categories: signaling pathways essential for β cell adaptation, pathways relating to survival or apoptosis of the β cell, and pathways of unknown significance in the β cell (43). Importantly, these same patterns were not inducible in non-diabetic islets treated with high glucose, suggesting they may be causative of β cell dysfunction as opposed to a response to hyperglycemia. However, the diabetic milieu is also characterized by high levels of pro-inflammatory cytokines and free fatty acids (44). Whether changes in methylation patterns might result from these varied insults is unclear. Furthermore, none of the differentially methylated promoter sites belonged to genes previously identified by GWAS as diabetes susceptibility genes (45, 46).

A prominent example of a gene affecting cellular survival noted to be hypomethylated and presumably activated in islets from donors with T2D is the *CDK5R1* gene. This gene encodes cyclin dependent kinase 5 regulatory subunit protein 1 or p35, which is a direct activator of cyclin dependent kinase 5 (CDK5). CDK5 is activated in the β cell by high glucose and the pro-inflammatory cytokine interleukin 1β (47). CDK5 activation leads to a number of detrimental effects in the β cell including decreased transcription of the insulin gene and decreased expression of the homeobox transcription factor pancreatic and duodenal homeobox 1 (Pdx-1), a protein that plays a central role in β cell development as well as the maintenance of the mature β cell phenotype. Lack of Pdx-1 leads to pancreatic agenesis in humans and mice, while haploinsufficiency causes a form of maturity onset diabetes of the young (MODY4) (48). In addition to its effect on Pdx-1, CDK5 activation also leads to increased ER stress through alterations in gene expression of the sarco endoplasmic reticulum calcium ATPase 2b (SERCA2b) (49, 50). Other genes with a functional role in survival and apoptosis that demonstrated altered DNA methylation in T2D islets compared to healthy islets included *CASP10*, *BCL2*, *PPP2R4* and *GSTP1* (43).

Emerging evidence from pre-clinical rodent models suggests an additional role for β cell de-differentiation in the pathophysiology of T2D (51), raising the intriguing possibility that alterations in epigenetic pathways in the diabetic state might lead to a loss of expression of key genes required for the specification of β cell identity. In agreement with this model, the β cell specific transcription factor paired box 4 (Pax4) was noted to be hypermethylated and therefore silenced in islets from human donors with T2D (43). Similar to Pdx-1, Pax4 is essential for β cell development in the early pancreas and also plays a role in the mature β cell (52, 53). Lack of Pax4 during development results in maturity onset diabetes of the

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young type 9 (MODY9), and mutations in Pax4 have also been associated with susceptibility to both type 1 and type 2 diabetes (54–58). Additionally, Pax4 is required for proliferation of β cells in the adult pancreas, and without Pax4 adaptation of the pancreas to chronic hyperglycemia is impaired (52, 59). Exogenous expression of Pax4 has also been shown to reprogram adult α cells into a β cell phenotype (60, 61). Taken together, these data suggest that aberrant methylation may play a role in the loss of the normal β cell transcriptional program and contribute to a loss of β cell identity under pathophysiological conditions.

Widespread screening studies have been performed to test interactions between diabetes susceptibility genes identified through GWAS studies and DNA methylation patterns. Using human islets from 84 cadaveric donors, Dayeh and colleagues tested the idea that common single nucleotide polymorphisms (SNPs) identified as Type 2 diabetes risk variants might introduce or remove a novel CpG dinucleotide, thereby influencing patterns of DNA methylation. Of 40 SNPs tested, 19 SNPs either introduced or removed a CpG site and 16 of these sites were associated with differential DNA methylation patterns in human islets. Further analysis revealed that several of these CpG-SNPs impacted β cell function and hormone secretion (62).

Focused candidate-based assessments seeking to link changes in DNA methylation patterns and β cell dysfunction have also been undertaken. The incretin hormone glucagon like peptide-1 (GLP-1) signals through the GLP-1 receptor to increase insulin secretion from the β cell. Treatment with GLP-1 receptor agonists or dipeptidyl peptidase 4 (DDP-IV) inhibitors that block GLP-1 breakdown are currently used clinically in the treatment of T2D. These drugs are associated with improved insulin secretion and glycemic control, while GLP-1 receptor agonists promote weight loss (63, 64). Conversely, inhibition of the GLP-1 receptor leads to β cell death and worsening of glycemia in rodent models (65). Given the importance of incretin hormone signaling in β cell function, methylation and suppression of the gene for the GLP-1 receptor has been assessed in islets from cadaveric donors with T2D. Interestingly, DNA methylation at a CpG site in the proximal promoter correlated with decreased GLP-1 receptor expression in islets, and was positively associated with increased body mass index and hemoglobin A1C (66).

Whether changes in DNA methylation patterns are passed to subsequent offspring is also a question of grave significance, especially considering the increasing prevalence of obesity and diabetes in persons of reproductive age. A recent study addressed this question by analyzing offspring of mothers who were previously obese and then underwent weight reduction following gastric bypass surgery. DNA methylation patterns were compared in siblings born both before and after surgery, and 5698 genes were found to be differentially methylated betweens sibling pairs. Furthermore, a number of genes found to be differentially methylated were identified to be involved in glucose regulation and inflammation. While this study did not specifically address changes in the β cell, the data do suggest an important impact of intrauterine environment and/or maternal metabolic health on patterns of DNA methylation and metabolic phenotype in offspring (67, 68).

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HISTONE MODIFICATIONS

Histones are proteins that package DNA into structures referred to as nucleosomes, and this combination of histone proteins and DNA is referred to as chromatin. Histone proteins are found binding both nuclear and mitochondrial DNA, and are evolutionarily conserved in all eukaryotes (69, 70). The histones H2A, H2B, H3, and H4 form the core of the nucleosome with 146 base pairs of DNA wrapped around each histone octamer, with each octamer including two of each histone protein isoform. It is clear that histones are not merely inert structural proteins; rather they play an important and indispensable role in the dynamic regulation of gene transcription. Covalent modifications of the basic N-terminal tails of histone proteins impact rates of gene transcription either by altering spatial aspects of chromatin packaging or through recruitment of proteins that act as part of the basal transcriptional machinery, extending and building upon the information stored in the genetic code itself. First proposed over a decade ago, this idea is widely referred to as the “histone code hypothesis” (71). Several types of covalent modifications to the N-terminal tails have been described including acetylation, methylation, ubiquitination and phosphorylation. Similar to DNA methylation, modification of histone proteins can be passed from parent cell to daughter cells (72–75).

Acetylation is perhaps the most extensively investigated of histone modifications, and generally, this modification has been associated with gene activation (74). Specific N-terminal lysine (Lys) residues are acetylated and deacetylated by histone acetyltransferase proteins (HATs) and histone deacetylase proteins (HDACs), respectively (76, 77). All eleven known HDAC proteins are expressed in the pancreatic β cell (78), while HDAC inhibitors (HDACis) have been tested as potential modulators of maladaptive epigenetic changes under disease conditions (79).

Multiple studies have demonstrated that transcriptionally active genes in regions of euchromatin are correlated with hyperacetylation of histones H3 and H4, while inactive genes are associated with regions of “closed” chromatin (heterochromatin) and histone hypoacetylation (80, 81). Two mechanisms have been proposed to explain the relationship between acetylation and gene transcription. First, acetylation of Lys residues diminishes the positive charge of the N-terminal tail, which promotes unfolding of chromatin structure through mitigation of the electrostatic interactions between DNA and the histone proteins (82). Secondly, acetylation may also serve to “mark” Lys residues that are recognized by proteins containing a conserved bromodomain (83). Acetylation itself is a transient effect, but by either opening DNA to allow promoter access or by attracting bromodomain proteins to a given gene, the follow-on effects of acetylation can be long-lasting (84).

In contrast, the traditionally understood role of histone methylation was to maintain DNA as tightly packed heterochromatin inaccessible to transcription factors. It is now known, however, that methylation can have differing effects on gene transcription depending on the specific histone residue that is modified (85). For example, some types of methylation, especially dimethylation of Lys-4 of histone H3 (H3-K4), are associated with an open euchromatin conformation, whereas other types of methylation, for example Lys-9 methylation of histone H3 (H3-K9), are associated with compacted and inaccessible

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heterochromatin (86). Methylated Lys residues also serve as binding sites for chromodomain-containing ATP-dependent chromatin remodeling complexes (76). These complexes alter the positioning of nucleosomes, and hence the overall accessibility ("openness") of genes. Methylation is catalyzed by histone methyl-transferases (HMTs), and until recently methylation was thought to serve as a long-term epigenetic marker for heterochromatin since no histone demethylases had yet been identified (85). With the identification of the amine oxidase lysine specific demethylase 1 (LSD-1) and jumonji C family enzymes capable of demethylating Lys residues, histone methylation is now also viewed as a dynamic mechanism capable of altering short-term rates of gene transcription (87–89).

Acetylation and methylation are not the only possible covalent modification of histones, but other modifications exert their effect largely by influencing either methylation or acetylation patterns. Histones are known to be ubiquitinated, but this modification does not target the histone for degradation. Rather, ubiquitination of histones H2A, H2B and H3 is correlated with increased gene transcription (90). For example, radiation dependent protein 6 (Rad6/Ubc2) ubiquitinates histones allowing for H3-K4 dimethylation thereby opening the DNA conformation and enhancing transcription of specific genes (91). Histones can also be phosphorylated, and phosphorylation generally prevents acetylation and contributes to maintenance of DNA in a closed heterochromatin conformation (92). In this way, the histone covalent modifications ubiquitination and phosphorylation add further dynamic control to the already versatile mechanisms of histone acetylation and methylation.

During development histone modifications also contribute to the specification of β cell identity by directly turning off certain genes, increasing expression of others, or by priming non β cell genes for inhibitory methylation at promoter CpG islands. For example, within the β cell, histones regulating expression of arista-less related homeobox protein (Arx) are methylated prior to DNA methylation in order to prevent a program of α cell differentiation (31). In fact, a mutually antagonistic relationship exists between Arx and Pax4, such that Pax4 must be expressed and Arx suppressed to specify β cell identity, illustrating a critical role for histone modifications and gene methylation in cell fate determination (93). HDAC expression and activity are also developmentally regulated during pancreatic organogenesis. Administration of small molecule HDAC inhibitors to embryonic pancreatic explants has been shown to suppress acinar differentiation, promote ductal differentiation, increase Pax4 expression, and enhance development of the β cell pool (94). These findings were confirmed using genetic mouse models and lentiviral gain of function strategies in pancreatic explants, where HDAC 5 and 9 knockout mice had increased numbers of β cells, while HDAC 4 and 5 knockout mice had increased numbers of somatostatin producing δ cells. HDAC4 and HDAC5 gain of function experiments demonstrated a decreased pool of β cells and δ cells (95).

Arguably, one of the most important genes in the β cell is the insulin gene. Studies performed in β cell lines and rodent islets have revealed the presence of activating marks such as H3 hyperacetylation and H3-K4 hypermethylation at the proximal insulin promoter. In β cell lines, H3 acetylation was highly correlated with recruitment of the HAT p300 to the insulin gene, but this pattern was absent in fibroblasts and α cells, suggesting that cell

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specific expression of insulin may be epigenetic in origin (96). Recent studies have sought to map histone modifications in human islets, and activating histone marks were found on the promoters of multiple important and highly expressed β cell genes including Pdx-1. In contrast to patterns previously observed in cell lines and rodent islets, several groups found that activating histone marks were not dramatically enriched at the insulin promoter. Instead a relative enrichment of histone acetylation and H3K4 dimethylation was found along the entire insulin coding region, and this pattern was absent in other cell types (97–99). Taken together, these data indicate the presence of a unique and unusual chromatin architecture around the β cell insulin gene.

Mechanistic studies have also provided important insight into the impact of altered chromatin architecture on β cell function. Acute and chronic palmitate-induced lipotoxicity, in a clonal β cell line, resulted in increased histone acetyltransferase activity and changes in expression of a number of important β cell genes, coupled with coordinated changes in either activating or silencing histone acetylation and methylation patterns (100). Under high glucose conditions, changes in histone acetylation has been shown to activate both beneficial and detrimental pathways related to β cell survival and function. For example, in the context of hyperglycemia, the HAT protein p300 cooperates with carbohydrate response element binding protein (ChREBP) to acetylate histone H4 of the *TXNIP* gene, leading to decreased thioredoxin activity (101). In models of T1D and T2D, decreased thioredoxin activity increased reactive oxygen species and increases β cell apoptosis (102, 103), while p300 has also been shown to play an essential role in activating β cell iNOS gene transcription in response to IL-1β signaling (104). Together, these data suggesting that p300 may play a negative role in the β cell. However, the actions of p300 are not exclusively detrimental as p300 also cooperates with the transcription factor pancreatic and duodenal homeobox protein 1 (Pdx-1) to open the histone architecture of the insulin gene, thereby facilitating transcription under high glucose conditions (105, 106). Conversely, under low glucose conditions, Pdx-1 is not complexed with p300 and instead associates with HDAC1 and HDAC2 to inhibit insulin gene transcription. By alternately recruiting HAT or HDAC proteins, Pdx-1 provides dynamic regulation of insulin transcription in response to glucose (107, 108), and by varying its interactions, p300 plays a dynamic role in β cell epigenetic function as well.

Many histone methyltransferase (HMT) proteins include the Su(var)3–9 and 'Enhancer of zeste' (SET) domain, and one such HMT that is critical to β cell function is SET7/9 (109). Under normal conditions, SET7/9 helps maintain transcription of essential β cell genes including insulin, glucose transporter 2 (GLUT2), and V-Maf Avian Musculoaponeurotic Fibrosarcoma Oncogene Homolog A (MafA) (109). Under conditions of hyperglycemia and ER stress, β cell SET7/9 displayed prominent nuclear to cytoplasmic localization, and exclusion of SET7/9 from the nucleus correlated with a loss of β cell secretory function as well as H3K4 dimethylation of the insulin promoter (110).

Another important β cell histone lysine methyltransferase that lacks a SET domain is enhancer of zeste homolog 2 (EZH2), and its role in β cell development and proliferation has been studied by several groups. EZH2 is a polycomb group protein that represses the cell-cycle inhibitors Ink4a and Arf through H3K27 trimethylation. As a result, EZH2 prolongs

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the lifetime window of replication for the β cell, but the activity of EZH2 decreases over the lifespan of the organism (111). Conditional ablation of EZH2 in the β cell results in impaired β cell proliferation, decreased β cell mass, and diabetes (112). In contrast, EZH2 overexpression results in increased β cell proliferation (113).

Epigenetic changes in response to environmental cues can begin even before birth, and therefore increasing emphasis is now being placed on the role of parental metabolism and intrauterine environment in patterning the adult epigenome. Intrauterine growth restriction (IUGR) occurs in response to poor maternal nutrition or a lack of nutrient exchange at the level of the placenta. IUGR offspring are predisposed to obesity and T2D in adulthood, and epigenetic changes are hypothesized to play a prominent role in this observed susceptibility metabolic disorders (114, 115). The β cell-specific effects of IUGR have been studied using a rat model of bilateral uterine artery ligation. This intervention reduced placental blood flow by approximately 50% resulting in low birth weight offspring (114). Analysis of 7 week-old offspring from dams treated with bilateral uterine artery ligation revealed mild hyperglycemia, hyperinsulinemia, and reduced β cell mass. By 26 weeks of age IUGR pups progressed to frank obesity and severe hyperglycemia, with even more significant alterations in β cell mass (114). Mechanistic studies performed in islets from IUGR offspring also revealed reduced Pdx-1 expression that was associated with H3 and H4 deacetylation, H3K4 demethylation, and increased H3K9 methylation at the Pdx-1 promoter. Loss of activation of the Pdx-1 gene resulted from diminished binding of the transcription factor upstream stimulation factor 1 (USF1) to the Pdx-1 promoter, while suppression was indicated by the binding of paired amphipathic helix protein Sin3a (Sin3a) and HDAC-1 at the Pdx-1 promoter. These changes were subsequently followed by inhibitory DNA methylation at this same locus.

Interestingly, treatment of IUGR rat pups with the GLP-1 analogue exendin-4 increased Pdx-1 levels in islets and prevented the onset of T2D (116), and analysis of islets from treated pups revealed that H3K4 methylation and USF1 binding to the Pdx-1 promoter were increased with exendin-4 treatment (117). Furthermore, islets from IUGR rat pups were treated in vitro with trichostatin A (TSA) to inhibit HDAC-1 activity, and this intervention also led to restoration of Pdx-1 expression (32). A subsequent DNA methylation screen revealed nearly 1,400 loci that were differentially methylated in 7 week old IUGR pup islets compared to sham-treated controls, suggesting the involvement of a large number of genes in addition to Pdx-1. Many of the differentially methylated sites were situated within genes known to regulate β cell proliferation, insulin secretion, and survival pathways (118).

The metabolic health of offspring may also be heavily influenced by paternal contributions in addition to intrauterine and/or maternal conditions. A recent study demonstrated that high fat diet exposure in rat fathers led to β cell dysfunction and altered β cell mass in female offspring. Paternal high fat diet also resulted in altered expression of nearly 650 genes in the islets of female offspring. *IL13ra2*, which functions as a component of the Jak-Stat signaling pathway, was found to have the highest fold change in gene expression and was upregulated approximately 6-fold compared to controls. As confirmation this change was epigenetic in origin, methylation status of the IL13ra2 gene promoter was assessed and was found to be hypomethylated (119).

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TRANSLATIONAL IMPLICATIONS OF THE β CELL EPIGENOME

Histone deacetylases have been linked with pro-inflammatory effects, and a number of small molecule inhibitors of histone deacetylases (HDACis or KDACis) have been tested in vitro and subsequently applied in preclinical models of T1D and T2D (79, 120, 121). A recent study by Christensen et al. tested the effect of the lysine deacetylase inhibitors vorinostat and gininostat in the non-obese diabetic (NOD) model of spontaneous autoimmune-mediated β cell destruction (122). Both compounds, when orally administered, prevented T1D in female NOD mice, which was accompanied by decreased islet inflammation and increased numbers of regulatory T cells. Interestingly, changes in H3 acetylation in either the pancreas or immune tissues was not observed with either compound, suggesting the effects were not directly mediated through chromatin remodeling. Rather, the authors propose a model whereby hyperacetylation of p65 protein led to decreased inflammation through decreased p65 promoter occupancy at the inducible nitric oxide (iNOS) gene promoter (122). In a separate report, vorinostat was not able to induce diabetes remission in hyperglycemic NOD mice, indicating that timing of these interventions is important (123). HDAC inhibition has also been tested in models of T2D, where suppression of HDAC activity in the livers of obese diabetic db/db mice and high-fat-diet fed mice led to improved blood glucose. In this report, the effects of HDAC inhibition in the pancreatic β cell were not directly tested (124).

Several drugs currently approved by the FDA for other indications are proposed to have chromatin-modifying effects. For example, valproic acid (VPA) is approved for the treatment of epilepsy and mood disorders, and it is also a well-described HDACi. VPA stimulates in vitro insulin secretion in isolated islets. Unfortunately clinical use of VPA leads to weight gain and insulin resistance, making VPA a relatively poor candidate to modulate the β epigenome in diabetes (79).. Lysine methylation of histones has been shown to either activate or deactivate gene transcription depending on which lysine residue of which histone isoform becomes methylated (125). Therefore lysine demethylase enzymes, such as LSD-1, could serve as attractive drug targets. Monoamine oxidase inhibitors (MAOI) are very old drugs approved for the treatment of depression. Given their complicated side-effect profile and the emergence of newer classes of antidepressants, MAOIs are now used infrequently. Interestingly, MAOIs have been shown to protect against alloxan-induced diabetes in rodent models (126). Moreover, MAOIs lower blood glucose in diabetic as well as non-diabetic individuals (127, 128). While these effects raise the possibility that LSD-1 inhibition may have anti-inflammatory actions and effects to promote insulin secretion, it remains to be tested whether newer MAOIs with more favorable side effect profiles, or other targeted LSD-1 inhibitors might be safe and efficacious in the treatment of T1D or T2D.

While the potential benefit of small molecule modulation of HDACi activity remains unknown, diagnostic strategies based on the detection of circulating methylated or unmethylated genomic DNA is currently an area of intense research. T1D has a fairly long clinically silent phase as the onset of β cell destruction may occur months or years prior to the occurrence of frank hyperglycemia. In fact, by the time an individual is diagnosed with T1D, it is estimated that nearly 80% of the β cell mass has been destroyed (129).

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Theoretically, earlier detection of T1D could pave the way for earlier inventions with disease-modifying antiinflammatory or immunomodulatory drugs, potentially sparing β cells (130). The insulin gene promoter is highly methylated and silenced in virtually all cells except for the β cell, and when β cells are destroyed by the immune system, their contents including DNA fragments are released into the circulation. At present, at least three independent groups are pioneering the use of PCR-based assays that detect an increase in circulating levels of unmethylated insulin gene DNA, which is presumed to be of β cell origin (34, 35, 131). This approach seeks to reliably and non-invasively assess β cell death, which theoretically might serve to predict the transition towards T1D in certain individuals. This technique has been successfully applied in multiple mouse models of T1D as well as in humans treated with anti-CD3 monoclonal antibody (132). Similar diagnostic approaches are also currently being tested as a way to diagnose cancer (133) and in pre-natal screening strategies, where circulating fetal DNA can be amplified from the maternal serum (134).

CONCLUSIONS

DNA methylation and post-translational histone modifications cooperate to lend flexibility to a relatively inelastic genome. These processes allow an organism to heritably alter phenotype without altering genotype. However changes in epigenetic structure in response to environmental cues and conditions may also negatively impact cell function and identity. Increasingly, a role for alterations in the β cell epigenome has been described as part of the pathophysiology of diabetes mellitus, and this area currently remains a focus of intense investigation. In the future, modulation of epigenetic pathways may lead to improved methods to identify those at risk of developing DM as well as novel therapies for the treatment of this metabolic disorder.

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