HHS Public Access

Author manuscript

Curr Diab Rep. Author manuscript; available in PMC 2020 June 27.

Published in final edited form as:

Curr Diab Rep. ; 19(8): 47. doi:10.1007/s11892-019-1168-8.

Epigenetics and Type 2 Diabetes Risk

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Abstract

Purpose of review: The influence of environmental factors on Type 2 diabetes (T2D) risk is now well recognized and highlights the contribution of epigenetic mechanisms. This review will focus on the role of epigenetic factors in the risk and pathogenesis of T2D.

Recent findings: Epigenetic dysregulation has emerged as a key mechanism underpinning the pathogenesis of T2D and its complications. Environmental variations, including alterations in lifestyle, nutrition, and metabolic demands during prenatal and postnatal life, can induce epigenetic changes that may impact glucose homeostasis and the function of different metabolic organs. Accumulating data continues to uncover the specific pathways that are epigenetically dysregulated in T2D, providing an opportunity for therapeutic targeting.

Summary: Environmental changes can disrupt specific epigenetic mechanisms underlying metabolic homeostasis, thus contributing to T2D pathogenesis. Such epigenetic changes can be transmitted to the next generation, contributing to the inheritance of T2D risk. Recent advances in epigenome wide association studies and epigenetic editing tools presents the attractive possibility of identifying epimutations associated with T2D, correcting specific epigenetic alterations, and designing novel epigenetic biomarkers and interventions for T2D.

Keywords

Epigenetics; type 2 diabetes; glucose homeostasis; diabetes complications; biomarkers; epigenetic therapies

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Conflict of Interest

Sangeeta Dhawan declares no conflict of interest.

Rama Natarajan reports a pending patent on inhibitors of epigenetically modified targets.

Human and Animal Rights and Informed Consent

All the studies noted in this review that were performed by the authors involving animals were done in compliance with appropriate institutional committees for animal research (IACUC) and all institutional guidelines for the care and use of animals were followed. Studies quoted in this article undertaken by the authors involving human subjects were either carried out on retrospective de-identified samples obtained from appropriate repositories, in compliance with institutional review boards (IRB), or all the procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional review boards (IRB), and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Introduction

Type 2 diabetes (T2D) is defined by hyperglycemia, and results from metabolic syndrome and inadequate insulin availability in response to relative insulin resistance. T2D is fast turning into a global pandemic [1]. T2D and chronic hyperglycemia can also lead to significantly increased rates of multiple micro- and macrovascular complications such as retinopathy, nephropathy and neuropathy, as well as atherosclerosis [2, 3].

T2D pathogenesis has a strong hereditary component, such that family history of disease confers a much higher risk of developing T2D [4, 5]. This recognition has led to an intense search for genetic factors responsible for T2D pathogenesis. While genome-wide association studies have identified multiple loci associated with T2D risk [6], genetic factors account for only a small fraction of diabetes associated with family history [7]. Furthermore, adult-onset diabetes was recently recognized to be a heterogeneous disease with five subgroups differing in disease progression and complications risk [8]. The incidence of T2D has increased drastically in the past few decades, coincident with the increase in food availability, sedentary lifestyle, and obesity [9]. However, this time span is unlikely to cause significant changes in the human genome. Altogether, this points to a strong influence of environmental factors and gene-environment interactions on obesity and T2D risk [10].

The effect of environmental factors including diet, physical activity, circadian rhythms, stress, temperature etc. on gene expression can be mediated by epigenetic mechanisms, which dictate how cells respond and adapt to their environment [11]. Epigenetic changes refer to mitotically or meiotically heritable changes in gene function without alterations in the underlying DNA sequence. Epigenetic regulation of gene expression occurs through changes in chromatin accessibility, mediated by the individual or combinatorial involvement of multiple mechanisms such as DNA cytosine methylation, histone post-translational modifications, and noncoding RNAs [3, 12]. Environmental changes can drive transient or persistent changes in the epigenome, which may alter gene expression and cellular phenotypes. For example, metabolic variations can directly alter the epigenome, given that many enzymatic regulators of epigenetic modifications require metabolic intermediates as cofactors [13]. Epigenetic mechanisms play a critical role in governing the expression of key genes involved in the development and homeostasis of metabolic organs, such as the pancreatic insulin-producing beta cells [14]. An altered metabolic state can thus affect the epigenome and phenotype of different organs, and contribute to the development of T2D and its multiple peripheral complications [3]. The present review focuses on the epigenetic basis of glucose homeostasis in health and diabetes, and potential implications for epigenetic biomarkers and therapies.

Developmental origins of T2D risk: the contribution of epigenetics

A strong case for the involvement of epigenetic factors in T2D is made by studies on the effect of maternal and intrauterine nutrition and growth retardation on diabetes development in multiple species [15]. Studies on the Dutch Hunger Winter famine have shown that intrauterine malnutrition and low birth weight leads to an increased likelihood for developing diabetes in subsequent generations [16]. This phenomenon, referred to as the

"thrifty phenotype hypothesis", proposes that under-nutrition during development leads to permanent changes in glucose homeostasis [17]. Similarly, maternal over-nutrition (such as a high-fat diet) and gestational diabetes can also adversely affect the metabolic health of the offspring [15]. Impaired glucose homeostasis in the parent has been shown to alter the metabolic program in the offspring coincident with very specific epigenetic changes, suggesting an epigenetic basis for the transmission of metabolic disease risk [16, 18, 19]. Thus, factors such as poor maternal health, as well as over- and under-nutrition during the fetal and postnatal growth phase can impact the development and function of key metabolic organs, and predispose the offspring to metabolic syndrome and diabetes in early or later life [11, 15]. Environmentally induced epigenetic alterations can also occur in the germline, and may therefore be potentially transmitted to subsequent generations, contributing to the (epigenetic) inheritance of diabetes risk (reviewed in [20]).

While the contribution of maternal health to disease risk in the offspring is well recognized (reviewed in [15]), recent data suggest that the epigenome of male germ cells is also altered by nutritional imbalance during intrauterine life [21], and can influence gene regulation during the development of the offspring. Paternal diet has been shown to influence cholesterol and lipid metabolism in the offspring [18]. Over- and under-nutrition, as well obesity in the paternal generation leads to reprogramming of the sperm epigenome, resulting in a trans-generational influence on metabolic homeostasis in the offspring [22, 23]. These studies suggest that parental metabolic environment and lifestyle can induce transgenerational changes in epigenome and metabolic fitness. Disturbances in the epigenetic regulation of imprinted genes (genes with differential allelic regulation based on parental origin) can further dictate the pattern of inheritance of diabetes risk [24]. Epigenetic factors may therefore not only mediate the effect of environmental factors on the development of T2D and its various complications, but also contribute to the transmission of disease risk to subsequent generations (Figure 1).

Epigenetic mechanisms of beta cell homeostasis and failure

Failure of beta cells to compensate for insulin resistance is central to the pathogenesis of T2D, and involves the progressive impairment of beta-cell identity, function, and survival. These aspects of beta cell homeostasis are governed by epigenetic mechanisms (reviewed in [14]), suggesting that epigenetic changes driven by adverse metabolic environment can potentially induce beta cell failure. A large body of evidence shows that stage-specific patterning of DNA methylation, histone modifications, and chromatin architecture is essential for pancreas lineage specification and endocrine differentiation [25–28]. Studies using human embryonic stem-cell differentiation have shown that epigenetic priming of lineage-specific enhancers dictates the stage-specific developmental competence and response to inductive signals throughout pancreatic differentiation [29].

Epigenetic regulation also plays a pivotal role in the establishment and maintenance of cellular identity and functional maturity of beta cells. DNA methylation patterning regulates the alpha-versus beta-cell fate choice by repressing the expression of the alpha-cell lineage determining transcription factor Arx in beta cells [30]. The DNA methyltransferase Dnmt1 maintains the methylated and repressed state of *Arx* locus during beta cell replication.

Accordingly, loss of Dnmt1 in beta cells leads to induction of *Arx* expression due to promoter de-methylation, driving the trans-differentiation of beta-to alpha-cells [31]. DNA methylation also serves to establish the metabolic program that allows the establishment of glucose-stimulated insulin secretion (GSIS) in postnatal beta cells towards a functionally mature beta-cell phenotype [32]. A comparison of human alpha- and beta-cell DNA methylation profiles shows that differential methylation patterns are largely concentrated in enhancer regions, indicating putative roles of these regions in regulating cell identity [33]. Epigenetic regulation via micro RNAs (miRNAs) and long noncoding RNAs (lncRNAs) has also been implicated in islet development and functional maturation [34–36]. Mice lacking the miRNA processing enzyme Dicer in the pancreatic, endocrine, or beta cell lineages display severe beta cell deficits [37, 38]. In addition, changes in the beta-cell miRNA landscape in response to postnatal nutrient shifts are essential for beta cell functional maturation [39]. Similarly, the lncRNA *blinc1* regulates beta-cell differentiation and function through its effect on specific islet transcription factors located in its genomic neighborhood [40]. Epigenetic mechanisms also control beta cell replication and expansion during postnatal growth, adaptation, and aging via the regulation of cell-cycle inhibitors such as $p27^{Kip1}$ and $p16^{Ink4a}$, and pro-replication imprinted genes such as the maternally imprinted lncRNA *H19* [41–44]. The replicative and adaptive capacity of beta cells declines with age. Epigenetic regulation of $p16^{Ink4a}$ expression is also central to the Platelet Derived Growth Factor (PDGF) and Transforming Growth Factor-beta (TGF-beta) dependent control of age-related changes in beta cell replication [45, 46]. Furthermore, aging induces profound beta cell specific changes in the epigenetic states of genes involved in beta cell replication and function, such as *Cdkn1a*, *Ccnd3*, *Plk1*, *Abcc8*, and *Kcnj11* [47]. Aging is a well-known risk factor for T2D, and it is likely that the age-dependent epigenetic changes in beta cell homeostasis play an instrumental role in this process.

The significance of epigenetic regulation of islet homeostasis is further highlighted by imprinting disorders such as the Beckwith-Wiedemann Syndrome (BWS) and Transient Neonatal Diabetes Mellitus (TNDM) (reviewed in [48]). In BWS, imprinting defects lead to lack of cell-cycle inhibitor CDKN1C ($p57^{Kip2}$), leading to unrestrained beta cell proliferation, and consequent excessive beta cell mass, hyperinsulinemia, and hypoglycemia. Similarly, in TNDM, imprinting defects lead to the overexpression of two genes, namely *ZAC* and *HYMAI*, leading to hypoinsulinemia in neonatal life, which resolves subsequently [48]. Variants of imprinted genes *GRB10* (regulates insulin signaling) and *KCNQ1* (K^+channel subunit, regulates insulin secretion) are also associated with increased T2D risk [49, 50], and islets from human subjects with T2D display differential methylation of *KCNQ1* [51]. Human islets from donors with T2D display altered imprinting of the *DLK1-MEG3* locus, which has important pathophysiological consequences. Hypermethylation of the *MEG3* promoter in T2D islets leads to downregulation of a cluster of miRNAs which regulate genes involved in beta cell function and survival [52]. Locus-specific changes in histone modifications in T2D islets de-repress Neuropeptide Y (NPY) in beta cells, leading to impaired function. NPY is abundant in neonatal beta-cells, and is epigenetically repressed in beta cells during their functional maturation. Epigenetic dysregulation of *NPY* in diabetic beta cells leads them to resemble the functionally immature fetal beta cells [53]. These data, combined with the role of epigenetic mechanisms in beta cell identity, suggest that

epigenetic dysregulation plays an important role in the loss of mature beta cell identity in diabetes, a phenomenon referred to as de-differentiation [54]. Recent work demonstrating the role of polycomb repressive complex 2 (PRC2)-dependent epigenetic regulation in beta cell identity, and the loss of PRC2-dependent gene repression in T2D islets further supports this idea [55].

A combination of sophisticated high-throughput sequencing techniques and powerful integrative data analysis approaches has led to a surge of epigenome-wide association studies (EWAS) in T2D cohorts to gain more insights into disease pathology [56–58]. Studies focusing on genome-wide profiling of DNA methylation in human islets from control and T2D donors show large-scale, but specific changes in the islet methylome in diabetes, translating into differential expression of loci critical for insulin secretion, adaptation, and survival [51, 59, 60]. Importantly, motifs for key islet transcription factors such as MAFA, PDX1, and RFX6, are enriched within the differentially methylated regions in T2D islets, suggesting dysregulation of islet transcriptional networks. These data indicate that epigenetic changes in diabetic islets lead are at least in part responsible for defects in beta cell function and survival in T2D (Table 1). Furthermore, environmentally induced epigenetic changes in the islets can be perpetuated trans-generationally, leading to increased T2D risk (reviewed in [15]). For example, the epigenetic landscape of genes related to beta-cell replication, function, and survival undergo profound changes in the progeny exposed to intrauterine growth retardation (IUGR) [61]. Altogether, these studies support the view that epigenetic alterations underlie beta cell defects in T2D, can be triggered by environmental factors, and transmitted to subsequent generations, contributing to T2D risk.

Epigenetics of insulin resistance: the effect of obesity and metabolic health

The postprandial release of insulin ensures metabolic homeostasis by promoting nutrient uptake and storage in several tissues. Insulin promotes muscle glucose uptake, hepatic glycogen synthesis, and triglyceride synthesis, and suppresses lipolysis in adipose tissue. Insulin resistance refers to the impairment of such peripheral cellular responses to insulin, and can result from obesity, metabolic syndrome and chronic over-nutrition [62]. Epigenome-wide profiling has been very informative in elucidating novel epigenetic mechanisms underlying insulin resistance across metabolic tissues, especially in the context of obesity (Table 1). Recent EWAS data show that body mass index (BMI; a key measure of adiposity), is associated with large-scale changes in DNA methylation patterns in lymphocytes [63]. Additional EWAS studies using blood genomic DNA from various cohorts have demonstrated key DNA methylated sites associated with T2D, fasting blood glucose and HbA1c levels [64].

DNA methylation profiling of adipose tissue shows that the differentially methylated regions associated with obesity mark genes involved in lipid and lipoprotein metabolism, nutrient transport, inflammation, and T2D risk, and such alterations in the DNA methylation patterns are predictive of future development of T2D [65–67]. High throughput analysis of DNA methylation in adipose samples from patients pre- and post-gastric bypass surgery identified obesity-related differentially methylated regions that overlapped with 27 genetic T2D risk loci, implicating a cross-talk between genetics and epigenetic risk factors [68]. These data

suggest that the epigenome is highly sensitive to body weight changes in either direction, and such epigenetic changes may be predictive of T2D risk. The importance of DNA methylation in metabolic homeostasis is further underscored by recent data implicating the DNA methyltransferase Dnmt3a in regulating insulin sensitivity in adipose tissue [69]. Epigenomic profiling of multiple histone modifications has also been instrumental in the identification of key enhancer elements and nuclear receptor pathways (glucocorticoid and vitamin D receptor) that drive insulin resistance in adipocytes, in response to cues such as steroid exposure and inflammation [70].

Studies using epigenetic and transcriptomic analysis of skeletal muscle in the context of newly diagnosed T2D and a family history of T2D show key differences in the muscle transcriptional program and insulin signaling, with some of the differentially regulated regions associated with T2D risk SNPs [71, 72]. Diet-induced obesity in the grand-paternal generation can lead to the transgenerational reprogramming of unfolded protein response (UPR) in skeletal muscle in the F2 (grand-child) generation [73].

The liver epigenome is also sensitive to obesity and hyperglycemia, as shown by large-scale epigenetic profiling. Obesity and T2D are associated with methylation changes at regions associated with T2D risk, and reprogram the liver epigenome towards increased glycolysis and lipolysis, which may promote the development of insulin resistance [74].

Collectively, these studies point to epigenetic dysregulation across multiple tissues as an underlying phenomenon in insulin resistance and T2D. Furthermore, they suggest that loci affected by both genetic and epigenetic changes may have a higher association with disease risk, or that epigenetics may confer functionality/causality to disease-related SNPs.

Epigenetics as a mediator of environmental influences on T2D risk

Changes in diet, including the fat content and composition have a strong impact on the adipose and muscle epigenome, especially at regions associated with metabolism [75, 76]. Besides diet, other environmental factors such as seasonal variation, exercise, and sleep can also shape the epigenome and metabolic homeostasis. Variations in temperature, such as heat or coldexposure, have been shown to change the epigenome and phenotype of beige adipocytes, to allow metabolic adaptation to temperature changes [77]. Cold exposure also induces epigenetic reprogramming in the sperm, with the offspring showing improved adaptation to over-nutrition and hypothermia [78]. Lifestyle interventions such as acute and chronic exercise lead to reprogramming of the DNA methylome in subcutaneous white adipose tissue (sWAT) and skeletal muscle in sedentary humans, affecting several genes involved in regulating adipogenesis, mitochondrial function, contraction, and inflammation [71, 79, 80]. Circadian rhythm is another critical environmental factor that directly affects the epigenome, as exemplified by the inherent histone acetyltransferase (HAT) activity of CLOCK (a core molecular component of the circadian clock). There is a strong link between metabolic and nutrient shifts, circadian clock, and epigenome, such that the feeding-fasting behavior regulates circadian gene-expression patterns to adapt to the diurnal variations in nutrient availability (reviewed in [81]). For example, an RNA-binding protein NONO serves as a novel epigenetic regulator of genes involved in glucose and lipid metabolism in the liver

in response to nutrient availability [82]. The link between circadian clock and metabolism is further strengthened by data showing that circadian disruption is a major risk factor for T2D [83]. In line with this, time restricted feeding has been shown to prevent metabolic syndrome in mice harboring disruptions in the clock machinery [84]. Thus, circadian disruption can alter the cellular metabolic and epigenetic landscape, and consequently impair adaptation to nutrient availability, predisposing to an increased risk of T2D. Together, these studies show that adverse environmental and lifestyle changes can contribute to T2D pathogenesis as well as the inheritance of T2D risk [85] (Figure 1).

Epigenetic dysregulation as a mediator of diabetes complications

A significant number of patients with T2D develop serious secondary health problems that can severely impair quality of life, and increase morbidity and mortality. These include microvascular complications such as retinopathy, nephropathy and neuropathy, and macrovascular diseases such as atherosclerosis and hypertension [3]. Hyperglycemia and consequent metabolic dysregulation is one of the major triggers for vascular complications of diabetes, and can lead to vascular damage through multiple pathways, such as increased cellular stress, accumulation of advanced glycation end-products (AGEs), dysregulation of profibrotic and inflammatory pathways downstream of Transforming growth factor-beta (TGF-β, NF-κB, and angiotensin-II (AngII) [2]. These cellular alterations lead to upregulation of genes involved in growth, inflammation, apoptosis, and fibrosis resulting in endothelial dysfunction, vascular smooth muscle and renal cell growth and fibrosis, macrophage infiltration, inflammation and ultimately to multiple complications across different organs [2].

Epigenetic profiling studies have enhanced our understanding of the mechanisms underlying diabetes-related complications (reviewed in [3, 86], summarized in Table 2). A comparison of genome-wide DNA methylation data from renal tubules in humans with chronic kidney disease including diabetic nephropathy and control subjects shows significant differences in DNA methylation at loci involved in fibrosis [87], highlighting the significance of epigenetic dysregulation in diabetic nephropathy. Furthermore, EWAS of DNA methylation in human peripheral blood samples show specific and predictive changes in DNA methylation associated with the decline of renal function in diabetic nephropathy [88, 89]. TGF-β signaling plays a crucial pathologic role in diabetic nephropathy, and both DNA methylation and key histone modifications have been implicated in driving TGF-β dependent activation of genes associated with renal fibrosis (reviewed in [3, 86]). Enrichment of activating histone modifications at promoters of fibrotic genes associated with diabetic nephropathy are also observed *in* vivo in rodent models of diabetes [3]. A high glucose milieu has been shown to disrupt the DNA methylation patterns of key loci involved in endothelial and neuronal complications, in primary vascular cells and Schwann cells, respectively [90, 91]. Epigenetic dysregulation is also implicated in the disruption of redox homeostasis, extracellular matrix, and inflammation in retinal endothelial cells (RECs), in a model of diabetic retinopathy [92]. Genome-wide comparison of activating and repressive histone marks in monocytes cultured under high glucose conditions as well as monocytes from diabetic patients with controls further highlights large-scale changes in the epigenome in diabetes [93]. Similarly, in vascular smooth muscle cells (VSMCs), epigenetic changes in

key histone modifications mediate the upregulation of inflammatory gene expression in response to hyperglycemic conditions *in vitro* and in mouse models of T2D [94].

Non-coding RNAs (miRNAs and lncRNAs) have also been identified as key epigenetic players in the development of diabetes complications (reviewed in [86, 95–97]). For example, the *miR-216/miR-217* cluster promotes TGF-β dependent activation of Akt kinase and subsequent changes in extracellular matrix (ECM) gene expression and hypertrophy in mesangial cells by targeting PTEN (an inhibitor of Akt) [98]. Endoplasmic reticulum (ER)-stress induces *lnc-MGC* in mesangial cells treated with high glucose or TGF-β, as well as in the glomeruli of diabetic mice to mediate early events in diabetic nephropathy [99]. Similarly, upregulation of lncRNA *Dnm3os* in the macrophages of diabetic mice, as well as in monocytes from patients with T2D promotes inflammatory gene expression. Dnm3os interacts with the nucleolar protein, nucleolin, in macrophages and disruption of this interaction under diabetic conditions allows *Dnm3os* to enhance histone H3K9 acetylation at promoters of target inflammatory genes [100]. In VSMC, AngII, which is associated with numerous diabetic vascular complications, activates enhancers and super-enhancers associated with target genes, including lncRNAs, related to VSMC dysfunction [101]. In rat and human VSMCs, AngII also upregulates a novel lncRNA *Giver* which induces VSMC growth and oxidant stress [102]. Together these studies illustrate the emerging importance of lncRNAs in diabetic complications, as well the epigenetic cross talk between the non-coding RNA and chromatin layers.

The importance of epigenetic regulation in the pathogenesis of diabetes complications is also evident from the phenomenon of metabolic memory, which underlies the long-term protection from intensive glycemic control, or conversely the continued progression of diabetes complications even upon achieving glycemic control. Metabolic memory refers to the observation that cells somehow retain the memory of prior exposure to hyperglycemic milieu, even after normoglycemia is attained. This phenomenon has been observed in experimental models as well as in clinical trials such as the Diabetes Control and Complications Trial (DCCT), and the long-term follow-up observational Epidemiology of Diabetes Interventions and Complications (EDIC) study [3, 103]. Even though all subjects maintained similar intensive glycemic control (HbA1c) in the EDIC phase, those with prior history of conventional glycemic control during the DCCT phase had higher risk of developing diabetes complications compared to subjects who received intensive glycemic control throughout [103]. Studies in T2D patients have similarly demonstrated that the benefits of intensive glycemic control lasted long after the completion of such a regimen [104]. These data suggest that epigenetic alterations conferred by prolonged exposure to hyperglycemic milieu may be responsible for the metabolic memory of dysfunction in target tissues. Accordingly, epigenetic profiling of multiple histone modifications and DNA methylation in blood monocytes from the DCCT/EDIC cohorts demonstrates clear epigenetic differences at key genes involved in inflammation between subjects on conventional control vs. intensive control [3, 105]. Notably, DNA methylation profiling of whole blood genomic DNA collected at the end of DCCT (~1993) and monocyte DNA collected ~17 years later during EDIC from the same patient demonstrated a persistence of DNA methylation at key loci, including those associated with complications, supporting a close connection between epigenetics and metabolic memory [106]. In summary, changes in

the metabolic environment of a cell can drive changes in the epigenome possibly as an adaptive mechanism. However, such changes can epigenetically program the cells to sustain and continue to dictate the cellular response, even after the initial metabolic assault has ceased (Figure 2). A clearer understanding of epigenetic adaptive responses and the mechanisms that serve to maintain them will be essential to design therapeutic protocols that address the issue of metabolic memory.

Epigenetic biomarkers and therapies

Development of biomarkers for T2D is a challenging task, and demands practical, non-invasive methods, such as those using peripheral blood samples, that have minimal adverse impact on the patient. An ideal blood-based biomarker candidate would be a stable molecular species that can be reliably detected, and accurately reflect disease initiation/progression related molecular alterations in the affected tissue(s). Cell-free DNA is released into the bloodstream through cell death, necrosis, or active secretion in the body, and can mirror tissue changes during disease pathogenesis. Cell-free DNA is highly stable, and the epigenetic signatures of these DNA fragments faithfully mirror their tissue-of-origin [107, 108]. The epigenetic profiles of cell-free DNA in the peripheral blood can therefore be potentially used as biomarkers to detect tissue specific epigenetic changes in disease conditions. Several studies have demonstrated that beta-cell death can be detected by assaying for beta-cell specific DNA methylation patterns of genes such as *INS* in circulating DNA [109, 110]. While these approaches have primarily focused on type 1 diabetes (T1D), they may be useful in T2D, as well as highlighted by recent data demonstrating that the DNA methylation changes associated with T2D in beta cells and peripheral insulin sensitive tissues are reliably captured in circulating DNA [111, 112].

miRNAs represent another molecular species that can be found stably circulating in the serum, and their profiles undergo changes in response to pathological conditions, including T2D [97]. For example, the serum levels of miR-192 and miR-193b are increased in pre-diabetic human subjects [113], while levels of miR-155 are downregulated in T2D [114]. Circulating miRNAs are often present in exosomes, and shown to be involved in cell-cell communication in metabolic homeostasis, insulin sensitivity, and T2D pathogenesis [115]. Exosomes containing obesity-associated miRNAs can induce glucose intolerance in lean mice, highlighting their relevance to T2D [116]. Thus, disease-specific epigenetic mechanisms not only serve as a highly promising avenue for biomarker development, but also as potential therapeutic targets for T2D.

Approaches that target epigenetic marks such as DNA methylation and chromatin modifications systemically have been successfully used for cancer therapeutics, and are now beginning to be considered for diabetes. Among these, inhibitors of the bromodomain proteins (BRDs) have shown much promise for cancer and inflammatory disease therapeutics, and have been used in the context of autoimmune diabetes in mice [117]. Given the importance of BRD proteins in metabolic homeostasis [118], BRD inhibitors also hold promise for T2D therapy. Of relevance to beta-cell replacement strategies, BRD inhibitors have been shown to promote pancreatic endocrine differentiation from stem cells [119]. However, drugs such as BRD inhibitors which target a whole class of epigenetic regulators,

may not be the most optimal avenue for therapeutic use, given the potential for side effects. The development of more selective BRD inhibitors is warranted to address these concerns, but will have to await clearer understanding of how individual BRD proteins regulate different aspects of metabolic homeostasis.

Recent advances in gene-editing using CRISPR/Cas9 and TALEN systems have now made it possible to tailor the epigenetic patterns at specific genomic regions, and thus potentially correct disease-specific epigenetic changes. Such locus-specific epigenetic tailoring can be used to target DNA methylation or demethylation, as well as alter chromatin structure [120]. A recent study used this approach to drive human beta-cell proliferation by tailoring the DNA methylation pattern of an imprinted cell-cycle inhibitor gene *CDKN1C* [121]. As discussed earlier, hypo-methylation at the *CDKN1C* locus in patients with BWS leads to reduced levels of $p57^{Kip2}$, resulting in beta-cell hyperproliferation. By targeting the DNA demethylase TET1 to *CDKN1C* to tailor a locus-specific epigenetic milieu reminiscent of the BWS beta-cells, this study successfully induced replication of adult human beta-cells. Similarly, CRISPR/Cas9 based targeting of DNA methyltransferase Dnmt3a to drive the DNA methylation and repression of alpha-cell fate determinant gene *Arx* in the developing pancreatic progenitors has recently been used to promote beta-cell lineage [122]. In a slightly different approach, a CRISPR/Cas9 *trans* epigenetic remodeling system was employed to transcriptionally activate target genes *in vivo* by recruiting specific transcriptional machinery and modulating histone marks, rather than editing DNA sequences. This strategy was used to alter cell fates by inducing trans-differentiation factors, *e.g.* alter liver cells to an insulin expressing, “beta-cell like” phenotype by ectopically expressing Pdx1 [123].

Non-coding RNAs such as miRNAs and lncRNAs have also been widely studied as potential epigenetic therapeutic targets in T2D and its complications [86, 96]. For example, locked nucleic acid (LNA) modified oligonucleotide mediated inhibition of *miR-192* or *lnc-MGC* attenuates features of early diabetic nephropathy in mice [96, 99]. Similarly, CRISPR/Cas9 based targeting of key enhancers regulated by AngII has been shown to ameliorate angiotensin-dependent gene expression (including lncRNAs) in VSMCs related to hypertensive phenotypes [101]. These studies collectively illustrate the potential therapeutic benefits of targeting the epigenetic landscape of specific loci involved in metabolic tissues homeostasis or T2D pathogenesis.

Conclusions

Together these reports illustrate the contribution of epigenetic factors to the pathogenesis and complications of T2D, as well as the inheritance of T2D risk across generations. Comprehensive epigenetic profiling and EWAS show that T2D pathogenesis is marked by highly specific epigenetic changes in distinct gene categories involved in cell identity, function, inflammation etc. across target organs. Combining EWAS with GWAS candidates for T2D can significantly enhance the identification of putative causal variants for further experimental validation. It is likely that variations in the macro and micro-environmental factors such as light/dark cycle, temperature, diet, activity, metabolism, cellular-stress etc. initially induce epigenetic changes as a means of adaptation. How sustained exposure to

"adverse" environmental milieu leads to a failure of epigenetic regulation (Figure 1) and whether lifestyle changes such as exercise and improved diet can reverse pathological changes remains to be determined. Are there some regions of the genome that are more vulnerable to environmental changes? If so, what determines the epigenetic plasticity of any genomic region, and are such regions amenable to therapeutic interventions? Elucidation of the molecular basis of epigenetic dysregulation in T2D will not only inform our understanding of adaptive mechanisms in metabolic tissues, disease pathogenesis, and inheritance of disease risk, but will also guide the development of innovative epigenetic biomarkers and therapies. Modulation of the enzymatic regulators of epigenetic marks using small molecule drugs is being pursued with great interest for addressing different aspects of T2D pathology. Such approaches, however, often suffer from off-target effects, and require the development of more specific small molecule agents and tissue specific delivery methods to become therapeutically successful. Targeted epigenetic engineering of key genes that are dysregulated in T2D has emerged as a promising alternative avenue, and is likely to improve the efficacy of approaches such as beta-cell replacement. However, detailed studies are required to identify specific epigenetic regulators and changes that are cell/tissue-type specific in T2D, to develop novel and targeted therapeutic strategies that address diabetes pathogenesis and complications.

Acknowledgments

The authors gratefully acknowledge research funding from the National Institutes of Health (NIDDK and NHLBI), the Wanek Family Project to Cure Type 1 Diabetes at City of Hope, and the Juvenile Diabetes Research Foundation (to RN), and from the Wanek Family Project to Cure Type1 Diabetes at City of Hope, Human Islet Research Network (NIH) UC4 DK104162, and the National Institutes of Health (NIDDK; R01 grant DK120523) (to SD).

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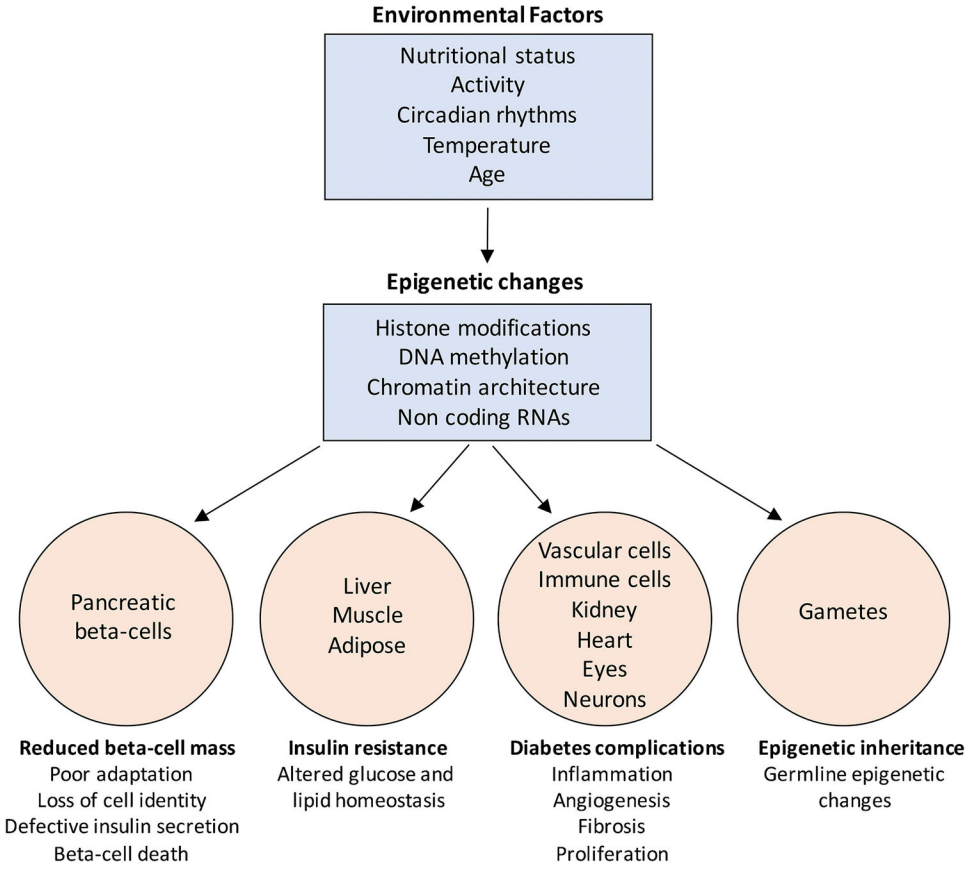

Figure 1.

Variations in environmental factors such as nutritional status (diet), activity (sedentary lifestyle), circadian rhythms (sleep disruption), seasonal changes in temperature, and even aging can alter the cellular epigenome. These changes may occur in the histone modifications, DNA methylation patterns, chromatin accessibility, as well as the expression of non-coding RNA species such as lncRNAs and miRNAs. The epigenetic dysregulation in response to adverse environmental exposure in turn drives transcriptional changes across several tissues such as the insulin producing beta-cells and insulin sensitive organs including liver, muscle, and adipose. This can eventually induce a deficit of functional beta-cell mass and impaired insulin secretion, as well as drive insulin resistance, thus disrupting glucose homeostasis towards the pathogenesis of T2D. In addition, epigenetic alterations in vascular cells, kidney, retina, neurons, and immune cells can lead to multiple micro- and macro-vascular complications of diabetes. Finally, epigenetic changes in response to adverse environment can also occur in the germline and be potentially transmitted to the offspring, contributing to the inheritance of T2D risk.

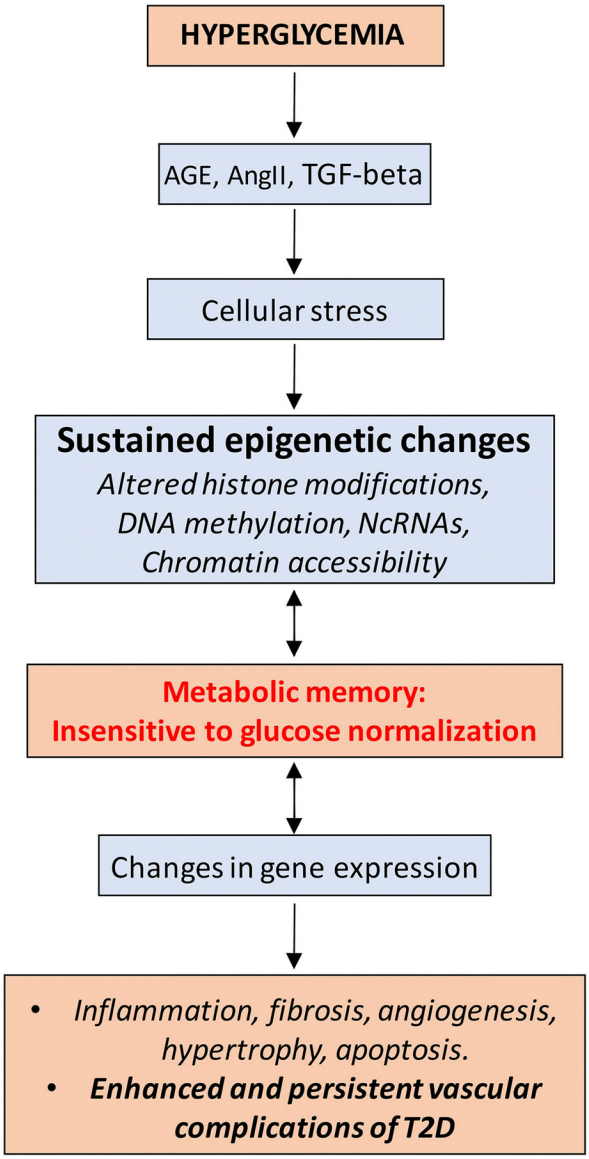

Figure 2.

Hyperglycemia in T2D can activate multiple pathways such as signaling via AGEs, AngII and TGF-beta, as well as induce a milieu of cellular-stress. This can lead to dysregulation of different epigenetic mechanisms such as histone modifications, DNA methylation, and ncRNAs, and consequently alter chromatin accessibility and gene expression profiles in multiple tissues, resulting in the development of diabetes complications. Such aberrant epigenetic patterns can persist and lead to metabolic memory, such that there is increased risk of developing diabetes complications even after achieving glycemic control.

Table 1.

Tissue specific epigenetic regulation, EWAS, and T2D risk in humans

Tissue	Context	Epigenetic mechanism	Key Finding	Reference
Islets	T2D	DNA methylation	Disrupted regulation of loci critical for islet function, adaptation, and survival.	[51, 59, 60]
Lymphocytes	Obesity	DNA methylation	Association of BMI with large scale DNA methylation changes.	[63]
Blood	T2D	DNA methylation	Identification of key DNA methylation sites associated with T2D, fasting blood glucose, and HbA1c levels.	[64]
Adipose	Obesity	DNA methylation	Obesity related changes in DNA methylation patterns may predict future development of T2D.	[65–67]
Adipose	Bariatric surgery, obesity	DNA methylation	Identification of key obesity related differentially methylated regions that overlap with specific genetic T2D risk loci.	[68]
Skeletal Muscle	T2D diagnosis and family history	DNA methylation	Epigenetic changes at loci related to insulin signaling, and association of some of these regions with T2D risk SNPs.	[71, 72]
Liver	Obesity, T2D	DNA methylation	DNA methylation changes at regions associated with T2D risk in the context of Obesity and T2D.	[74]
Adipose, skeletal muscle	Diet	DNA methylation	Epigenetic dysregulation of regions associated with metabolic pathways upon exposure to short-term high fat diet.	[75, 76]
Adipose, skeletal muscle	Exercise	DNA methylation	Both short- and long-term exercise reprograms the epigenetic landscape of genes involved in adipogenesis, and muscle contraction.	[71, 79, 80]

Table 2.

EWAS and diabetes complications in humans

Tissue	Context	Epigenetic mechanism	Key Finding	Reference
Human Renal Tubuli	Nephropathy	DNA methylation	Altered DNA methylation at loci involved in fibrosis in tubuli from humans with diabetic nephropathy and renal dysfunction.	[87]
Peripheral blood samples	Nephropathy	DNA methylation	Specific DNA methylation changes associated with eGFR identified, and a distinct subset of these also associated with kidney fibrosis and showed concordant DNA methylation changes in the kidney cortex biopsies from patients with chronic kidney disease.	[88]
Peripheral blood leukocytes	Nephropathy	DNA methylation	Key DNA methylation changes associated with decline of renal function (estimated glomerular filtration rate (eGFR)) identified in the context of diabetic nephropathy, in a cohort of Pima Indians with T2D.	[89]
Primary vascular endothelial cells	Vascular complications	Histone acetylation (activating), DNA methylation	Hyperglycemia mediated induction of genes associated with endothelial dysfunction occurs via histone acetylation, and is inversely correlated with DNA methylation.	[90]
Human monocytes (Primary and THP-1 cells)	Effect of hyperglycemia	Activating And repressive histone modifications	Chronic hyperglycemia can alter the chromatin states to drive changes in expression of key genes associated with inflammation.	[93]
Vascular smooth muscle cells from diabetic mice	Vascular complications, metabolic memory	Histone methylation (repressive)	Dysregulation of epigenetic states is a key mechanism underlying metabolic memory, as well as inflammation in vascular cells.	[94]
Human blood monocytes and lymphocytes	Metabolic memory	Histone modifications	Monocyte histone acetylation was associated with HbA1c level during the DCCT phase and the long-term (EDIC) follow-up, pointing to an epigenetic basis for metabolic memory.	[103]
Human whole blood and blood monocytes	Metabolic memory	DNA methylation	Several key genes associated with complications display sustained differential DNA methylation patterns in the same diabetic subjects over 16 years in association with HbA1c and an adverse diabetes complications outcome.	[104]