DNA methylation in metabolic disorders 1–4

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ABSTRACT

DNA methylation is a major epigenetic modification that controls gene expression in physiologic and pathologic states. Metabolic diseases such as diabetes and obesity are associated with profound alterations in gene expression that are caused by genetic and environmental factors. Recent reports have provided evidence that environmental factors at all ages could modify DNA methylation in somatic tissues, which suggests that DNA methylation is a more dynamic process than previously appreciated. Because of the importance of lifestyle factors in metabolic disorders, DNA methylation provides a mechanism by which environmental factors, including diet and exercise, can modify genetic predisposition to disease. This article considers the current evidence that defines a role for DNA methylation in metabolic disorders. Am J Clin Nutr 2011;93(suppl):897S–900S.

INTRODUCTION

Although the genetic code is identical in essentially all cells of an organism (except for lymphocytes), each individual cell type possesses its own gene-expression pattern. The tissue-specific phenotype in any physiologic state is established by DNA methylation. Methylation of the 5′ position of cytosine, which is the most abundant base modification in the genome of eukaryotic cells, is a major epigenetic modification (1). In the most common examples, DNA methylation suppresses gene expression by modulating the access of the transcription machinery to the chromatin or by recruiting methyl-binding proteins (2). In turn, these proteins recruit chromatin-remodeling proteins that can modify histones, thereby forming a compact, inactive chromatin (2).

DNA methylation is involved in the control of genomic imprinting, which is an epigenetic form of gene regulation whereby a gene or genomic domain can be biochemically marked with information about its potential origin. Changes in DNA-methylation profiles can occur during aging and in pathologic states, such as cancer and metabolic diseases (3–6). Metabolic disorders such as obesity and type 2 diabetes (T2D) have reached epidemic rates in most developed and developing countries (7, 8), but little is known of the role of DNA methylation in diabetes pathogenesis. Several lines of evidence support a role for non-genetic factors in the development of insulin-resistance and indicate that epigenetic factors, possibly through DNA methylation, may play a role in diabetes pathogenesis. For example, skeletal muscle cultures from T2D patients retained an insulin-resistant phenotype after cell division. The insulin resistant phenotype observed in cell cultures established from diabetic subjects was preserved as shown by impairments in insulin-mediated glycogen synthase activity and glucose transport (9, 10). The property of cultured cells to preserve the diabetic phenotype in vitro was not associated with a genetic polymorphism of the donor, which suggested that environmental factors were involved. Furthermore, in human primary myocytes, the messenger RNA concentration of peroxisome proliferator-activated receptor γ, coactivator 1α (PGC-1α), which is a master regulator of mitochondrial function, is negatively associated with the plasma free fatty acids concentration of the donor (11). Collectively, these results strongly implicate a role for epigenetic modifications through DNA methylation in the memory of metabolic impairments. The purpose of this article was to consider the role of DNA methylation in the development of insulin resistance in metabolic diseases (Figure 1).

DNA METHYLATION AND ENVIRONMENTAL FACTORS

DNA methylation is mitotically stable, and hence the assumption has been that environmental factors were unlikely to induce significant and sustained changes in DNA methylation patterns in normal adult tissues. However, twin studies have shown that DNA methylation profiles were more divergent in older twins than in infant twin pairs, which validated the influence of environmental factors on the epigenome over time (12). De novo methylation in somatic tissue can occur in cells exposed to environmental toxins such as heavy metals (13). Graded methylation-driven silencing of the retrotransposon intracisternal A particle C element that regulates the agouti phenotype has been shown in the offspring of mice fed diets with different amounts of the methyl donor folic acid (14). Oestrogenic and antiandrogenic toxins that decrease male fertility alter DNA methylation, and these changes are inherited by subsequent

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generations (15). A remarkable example of an environmental effect on the epigenome is the modification of glucocorticoid receptor methylation seen in the hippocampus of rat pups in response to maternal grooming (16).

Dietary modification can have a profound effect on DNA methylation and genomic imprinting. A deficiency of the methyl donors folic acid and methionine modified the DNA methylation imprint of insulin-like growth factor 2 (17). In mammals, a deprivation of folic acid altered DNA methylation levels in the liver (18, 19). Methylation levels were restored upon the administration of a normal diet, which suggested that the DNA methylation was reversible (18, 19). Since this discovery, evidence has accumulated for dynamic DNA modifications in somatic cells. DNA methylation was originally considered stable and irreversible because the cytosine methylation is a covalent modification, and evidence of demethylase activity was lacking. The treatment of breast cancer cell lines cells with deacetylase inhibitors induced the methylation of the trefoil factor 1 promoter (20). Additional evidence for the rapid reversibility of DNA methylation has emerged as shown by a rapid cycling of DNA methylation of the trefoil factor 1 gene promoter upon activation by estrogens (21, 22). These findings opened a novel area of research related to the influence of environmental factors on the regulation of DNA methylation in mammals. In one of the initial reports on methylation, butyrate, which is a short-chain fatty acid, induced global DNA methylation (23). Recently, we reported that the acute exposure of the free fatty acids palmitate or oleate increased the promoter methylation of genes involved in mitochondrial function (24).

NON-CpG METHYLATION

DNA methylation in mammals has been primarily described in cytosines that precede a guanine (CpG methylation). Whether cytosine methylation occurs exclusively on CpG nucleotides or on non-CpG nucleotides has been disputed. Non-CpG methylation, which is defined as the methylation of cytosines within the CpC, CpT, and CpA sequences, is abundant in plants (24) and mammalian embryonic stem cells (25, 26). Non-CpG methylation was first described in mammalian replication origins (27), but this was subsequently disputed (28). Other authors provided unequivocal evidence for CpA, CpT, and CpC methylation in mammalian DNA by using the nearest-neighbor technique (26, 29). Very recently, a genome-wide, single-base-resolution mapping of DNA methylation identified non-CpG methylation in human cell lines (30). Unlike the conventional CpG methylation, the non-CpG methylation density was decreased toward the transcription start site. Previously, we provided evidence that non-CpG exists at substantial levels in tissue from human origin and is induced by environmental factors (3). Thus, non-CpG methylation may play a particular role in the regulation of gene activity by environmental factors. The understanding of the exact function of non-CpG methylation requires further investigation.

DNA METHYLATION AND METABOLIC DISEASES

Several lines of evidence support a role for epigenetic processes in the regulation of metabolic disease and indicate a strong link between genes and the environment. In particular, changes in DNA-methylation levels in humans were associated with alterations in the expression of genes involved in mitochondrial function, including cytochrome c oxidase subunit VIIa polypeptide 1 (COX7A1), NADH dehydrogenase (ubiquinone) 1 β subcomplex 6 (NDUFB6), and PGC-1α (6, 31). From our methylated DNA immunoprecipitation–array screen of skeletal muscle biopsies, we retrieved numerous genes with a differential methylation status in skeletal muscle from T2D compared with normal glucose-tolerant volunteers, including subsets of genes involved in primary metabolic processes and mitochondrial function (3).

DNA methylation plays a role in the regulation of key genes involved in the regulation of glucose homeostasis. The promoter of the insulin (INS) gene is demethylated in insulin-producing β-pancreatic cells as well as upon the differentiation of mouse embryonic stem cells into insulin-producing cells (32). The methylation of the Ins2 promoter also leads to the binding of methyl CpG binding protein 2, which further suggests a mechanism for silencing of the insulin gene (32). Glucose transporter 4 (GLUT4), which translocates to the plasma membrane in response to insulin in adipose tissue and skeletal muscle, is a critical player in glucose homeostasis. The GLUT4 promoter is highly demethylated upon adipocyte differentiation, and methylation on specific CpG sites can inhibit nuclear factor binding to the promoter (33). Similarly, the promoter of the peroxisome proliferator-activated receptor γ (PPARγ) gene is progressively demethylated on adipocyte differentiation (34), which provides evidence that DNA methylation sets the adipocyte-specific gene expression. In T2D, a decreased expression of GLUT4 was reported in adipose tissue (35) but not in skeletal muscle (36). Whether DNA methylation is implicated in the down-regulation of GLUT4 in metabolic diseases is unknown.

The methylation status is altered in the adipose tissue of inborn and diet-induced obese mice such that hypermethylation of the
permethylation of the hypothalamic proopiomelanocortin (POMC) gene on the nuclear transcription factor aB binding site (37). This is of a physiologic relevance because POMC mediates the leptin-induced anorexigenic effect on the central nervous system. On the basis of the positive correlation between POMC methylation and hyperglycemia, a direct role for glucose in the methylation of the POMC promoter was evoked. Other studies have linked behavioral factors with DNA methylation in the central nervous system. Therefore, one cannot exclude the influence of behavioral factors in the development of POMC methylation.

Low birth weight (LBW) is associated with a higher prevalence of T2D. Methylation of the PGC-1α promoter was shown to be elevated in individuals born with LBW (38). Short-term overfeeding altered PGC-1α promoter methylation in matched normal-weight individuals but was without an effect in the LBW group (38). Differences in the degree of CpG methylation and gene expression of the CCAAT/enhancer binding protein z and neuronatin, which are 2 genes involved in adipocyte differentiation and insulin secretion, respectively, are observed in children known to have an LBW who are conceived naturally in comparison with those who are conceived with assisted reproduction (39). These findings would be consistent with a role of environmental factors in the development of metabolic disorders. However, whether the alterations observed are due to in vitro conception or are a hallmark of the patients who use this procedure has not been addressed.

### CONCLUSIONS

Although a genetic predisposition can contribute to the development of T2D, diet and physical activity are environmental factors that can also have a positive effect on insulin sensitivity. Reports that show that DNA methylation can be influenced by environmental factors in somatic tissues are multiplying and, thus, open new avenues for treatment strategies for insulin resistance in target tissues. Most important, emerging evidence for a possible epigenetic inheritance may change the understanding of the temporal delimitation of the cause of metabolic disorders. In our view, important challenges remain in the identification of the molecular carriers of epigenetic inheritance in the germ line. Moreover, the specific factors in the environment that are responsible for their appearance remain to be determined.

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