Developmental Origins of β-Cell Failure in Type 2 Diabetes: The Role of Epigenetic Mechanisms

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ABSTRACT: Intrauterine growth retardation (IUGR) has been linked to later development of type 2 diabetes in adulthood. An abnormal metabolic intrauterine milieu affects the development of the fetus by permanently modifying gene expression of susceptible cells. Altered gene expression persists after birth, suggesting that an epigenetic mechanism may be responsible for changes in transcription. Uteroplacental insufficiency (IUGR) is associated with hypomethylation and hyperacetylation of genomic DNA in brain and liver of IUGR fetal and juvenile rats. These findings are associated with zinc deficiency that often accompanies fetal growth retardation. Studies in the IUGR rat also demonstrate that an abnormal intrauterine environment induces epigenetic modifications of key genes regulating β-cell development and experiments directly link chromatin remodeling to suppression of transcription. Dietary protein restriction of pregnant rats causes fetal growth retardation and is associated with hypomethylation of the glucocorticoid receptor (GR) and PPARγ genes in liver of the offspring. It is postulated that these epigenetic changes result in the observed increase in gene expression of GR and PPARγ. Future research will be directed at elucidating the mechanisms underlying epigenetic modifications in offspring. (*Pediatr Res* **61: 64R–67R, 2007**)

An adverse intrauterine milieu impacts the development of the fetus by modifying gene expression in both pluripotential cells or terminally differentiated, poorly replicating cells. The long-range effects on the offspring (into adulthood) depend upon the cells undergoing differentiation, proliferation, and/or functional maturation at the time of the disturbance in maternal fuel economy. Permanent alterations to the phenotype of the offspring suggest that fetal growth retardation is associated with stable changes in gene expression. In this article, a general review of epigenetics will be provided and the possible causal role of chromatin remodeling in the development of type 2 diabetes will be discussed.

CHROMATIN STRUCTURE, DNA METHYLATION, AND GENE EXPRESSION

Epigenetic modifications of the genome provide a mechanism that allows the stable propagation of gene activity states from one generation of cells to the next. Excellent reviews on this topic appear frequently, reflecting the rapid advances of knowledge in the field (1–4). Epigenetic states can be modified by environmental factors, which may contribute to the development of abnormal phenotypes. There are at least two distinct classes of epigenetic information that can be inherited with chromosomes. One class of epigenetic control of gene expression involves changes in chromatin proteins, usually involving modifications of histone tails. In eukaryotes, DNA is assembled with histones to form the nucleosome, in which DNA is wrapped approximately two turns around an octameric complex composed of two molecules of each of the four histones H2A, H2B, H3, and H4. The amino termini of histones can be modified by acetylation, methylation, sumoylation, phosphorylation, glycosylation, and ADP ribosylation. The most common modifications involve acetylation and methylation of lysine residues in the amino termini of H3 and H4. Increased acetylation induces transcription activation, whereas decreased acetylation usually induces transcription repression. Methylation of histones is associated with both transcription repression and activation. Moreover, lysine residues can be mono-, di-, or trimethylated *in vivo*, thus providing an additional mechanism of regulation. Trimethylation of lysine residues is only found at active genes, whereas dimethylation occurs in both active and inactive chromatin. Several chromatin modification states are mutually reinforcing. For example, methylation of lysine 9 on histone H3 can promote DNA methylation, and CpG methylation (see below) stimulates methylation of lysine 9 on histone H3 (5). Thus, chromatin modifications induced by adverse stimuli are self-reinforcing and can propagate.

The second class of epigenetic regulation is DNA methylation, in which a cytosine base is modified by a DNA methyltransferase at the C5 position of cytosine, a reaction that is carried out by various members of a single family of enzymes. Approximately 70% of CpG dinucleotides in human DNA are constitutively methylated, whereas most of the unmethylated CpGs are located in CpG islands. CpG islands are CG-rich sequences located near coding sequences and serve as promoters for the associated genes. Approximately half of mammalian genes have CpG islands. Methylation of CpG sites is also maintained by DNA methyltransferases. DNA methylation is commonly associated with gene silencing and contributes to X-chromosomal inactivation, genomic imprinting, and transcriptional regulation of tissue-specific genes during

Abbreviations: HDAC1, histone deacetylase 1; **IUGR,** intrauterine growth retardation

cellular differentiation (6–8). The methylation status of CpG islands within promoter sequences works as an essential regulatory element by modifying the binding affinity of transcription factors to DNA binding sites.

Most CpG islands remain unmethylated in normal cells, however, under some circumstances such as cancer (9–14) and oxidative stress (see below), they can become methylated *de novo*. This aberrant methylation is accompanied by local changes in histone modification and chromatin structure, such that the CpG island and its embedded promoter take on a repressed conformation that is incompatible with gene transcription. It is not known why particular CpG islands are susceptible to aberrant methylation. A recent study by Feltus *et al.* (15) suggests that there is a "sequence signature associated with aberrant methylation." Of major significance to type 2 diabetes is their finding that *Pdx*-1, a pancreatic homeobox transcription factor, was one of only 15 CpG genes (a total of 1749 genes with CpG islands were examined) that were methylation susceptible under conditions of increased methylation induced by over-expression of a DNA methyl transferase.

NUTRITIONAL STATUS AND EPIGENETIC MODIFICATIONS

The metabolic or nutritional state of the organism directly influence epigenetic modifications and the process of chromatin remodeling depends upon a number of products derived from intermediary metabolism such as S-adenosyl methionine (SAM), acetyl CoA, and nicotinamide adenine dinucleotide (NAD^+).

A role for environmental regulation of epigenetic phenomena has been established by experiments performed in agouti mice (16,17). In this animal, an endogenous retrovirus-like transposon sequence is inserted close to the gene coding for the Agouti protein. An unmethylated retrotransposon promoter overrides the agouti promoter, resulting in ectopic agouti expression and a yellow coat. A methylated retrotransposon cannot do this, resulting in a wild-type agouti coat. Yellow mothers produce more yellow offspring than agouti mothers, even when all the mice are genetically identical. These mice have yellow hair, obesity, hyperinsulinemia, diabetes, increased somatic growth, and increased susceptibility to hyperplasia and tumorigenesis (18). Wolff *et al.* (16) have investigated whether maternal diet can alter the phenotype of the A^{IAP} mice. They found that when pregnant females are fed a diet supplemented with methyl donors, a larger proportion of offspring have a wild-type coat color as compared with the offspring of mothers fed a standard diet. These results suggest that an environmental stimulus early in life can change the stable expression of genes and affect the phenotype of the adult.

CHROMATIN REMODELING AND DISEASE STATES

Homeobox genes are frequently down-regulated in association with aberrant methylation in human cancer cells (10) and the HOX gene clusters are a hotspot of *de novo* methylation in lung cancers (11). In addition to targeted DNA methylation changes in response to external stimuli, random DNA methylation changes have been shown to occur during aging of organisms in several tissue types (12,13).

Hypermethylation of specific genes has also been observed in tissues of aging individuals (13,19). Type 2 diabetes is strongly age-related, as its incidence is increased in older populations, and the metabolic profile of individual patients deteriorates over time. DNA methylation errors that accumulate with increasing age could provide one explanation, and this may be related to oxidative stress.

Reactive oxygen species can also lead to alterations in DNA methylation without changing the DNA base sequence (20). Such changes in DNA methylation patterns have been shown to affect the expression of multiple genes (20). Replacement of guanine with the oxygen radical adduct 8-hydroxyguanine profoundly alters methylation of adjacent cytosines (20). Histones, because of their abundant lysine residues, are also very susceptible to oxidative stress (21–23).

EPIGENETIC REGULATION OF GENE EXPRESSION IN FETAL GROWTH RETARDATION

A number of studies have suggested that uteroplacental insufficiency, the most common cause of intrauterine growth retardation in the developed world, induces epigenetic modifications in the offspring (24–26). Fetal growth retardation is induced by bilateral uterine artery ligation in the pregnant rat (27). The unique feature of this model is its ability to induce diabetes in adult animals at approximately 15–26 wk of age with underlying β-cell secretory defects and insulin resistance, the salient features of most forms of type 2 diabetes in the human (27,28). Genome-wide DNA hypomethylation has been found in postnatal IUGR liver and was associated with an increase in total H3 acetylation (24). Acetylation of histone H3 and acetylation of H3 lysine-9 (H3/K9), lysine-14 (H3/K14), and lysine-18 (H3/K18) was increased at the promoters of PGC-1 and CPTI, respectively, in IUGR liver (26). At d 21 of life, the neonatal pattern of H3 hyperacetylation persisted only in the IUGR males. Whether hyperacetylation at these sites actually causes increased transcription of PGC-1 or CPT1 and how these findings relate to a phenotype in the offspring remains to be determined.

CHROMATIN REMODELING IN THE β-CELL OF IUGR RATS

Studies in the IUGR rat also demonstrate that fetal growth retardation induces epigenetic modifications of key genes regulating β-cell development (28a). *Pdx*-1 is a homeodomain-containing transcription factor that plays a critical role in the early development of both endocrine and exocrine pancreas, and then in the later differentiation and function of the β cell. As early as 24 h after the onset of growth retardation, *Pdx-1* mRNA levels are reduced by more than 50% in IUGR fetal rats. Suppression of *Pdx*-1 expression persists after birth and progressively declines in the IUGR animal, implicating an epigenetic mechanism.

The proximal promoter of *Pdx-1* is obligate for transcription of the gene, and the histones H3 and H4 are heavily acetylated in normal β-cells (29). However, in islets of IUGR animals, H3 and H4 in this region of the *Pdx-1* promoter are deacetylated. Histone deacetylation is catalyzed by HDAC, and HDAC1 is strongly associated with the proximal *Pdx-1* promoter in IUGR β-cells. Reversal of deacetylation by an HDAC inhibitor normalizes *Pdx*-1 expression in islets of IUGR animals, demonstrating that histone deacetylation contributes to the observed *Pdx*-1 transcription suppression.

Unlike acetylation, histone H3 methylation can be equally associated with either transcriptional activation or repression. Methylation of the lysine residue Lys4 H3 (H3-K4) correlates with activation of gene expression, whereas H3Lys9 (H3-K9) methylation is involved in the establishment and maintenance of silent heterochromatin regions. Lysine methylation is catalyzed by the action of histone methyltransferases (SET7/9), which demonstrate a high degree of specificity for H3–K4. There is a loss of binding of SET7/9 to the proximal promoter of *Pdx-1* in β-cells from IUGR animals, which results in a marked reduction of methylation of H3K4 in this region of *Pdx-1*. These observations demonstrate that the level of H3 acetylation is linked to the degree of H3K4 methylation.

Transcriptional repression is also facilitated by methyl-CpG binding proteins that bind to promoter-proximal methylated DNA sequences, thereby maintaining the condensed nucleosome structure (30). However, one methyl-CpG-binding domain protein-MeCP2 also mediates transcription repression through histone deacetylation (31–33). MeCP2 contains a transcriptional repression domain that functions by recruitment of the co-repressor Sin3A, a histone deacetylase (31–33), and a histone 3 lysine 9 methyltransferase (Suv39h) (34,35). MeCP2 binding is seen in IUGR fetal pancreas as early as 24 h after uterine artery ligation. Association of MeCP2 with the proximal promoter of *Pdx-1* precipitates Sin3A binding at d 1 of life in IUGR islets. The repressor complex consisting of MeCP2, Sin3A, HDAC1, and Suv39h induce H3 deacetylation and methylation of H3K9. Thus, a cascade of epigenetic events is triggered by IUGR, resulting in permanent suppression of Pdx-1 expression. The sequence of epigenetic events (Fig. 1) that occurs in IUGR islets leading to suppression of *Pdx-1* transcription appears to be the following: MeCP2 binds to methylated DNA in the CpG island at the *Pdx-1* promoter. This results in recruitment of a repressor complex, which catalyzes the deacetylation of H3 and methylation of H3K9, respectively. Deacetylation of H3 in turn promotes the loss of H3K4 methylation, further suppressing *Pdx-1* transcription. As the IUGR animals age, DNA methylation of the CpG island progresses, thereby locking in the silencing of *Pdx-1* expression.

How do these events lead to diabetes? Targeted homozygous disruption of *Pdx*-1 in mice results in pancreatic agenesis (36), and homozygous mutations yield a similar phenotype in humans (37). Milder reductions in *Pdx*-1 protein levels, as occurs in the *Pdx+/–* mice, allow for the development of a normal mass of β cells (38), but result in the impairment of several events in glucose-stimulated insulin secretion (39). These results indicate that *Pdx*-1 plays a critical role, distinct
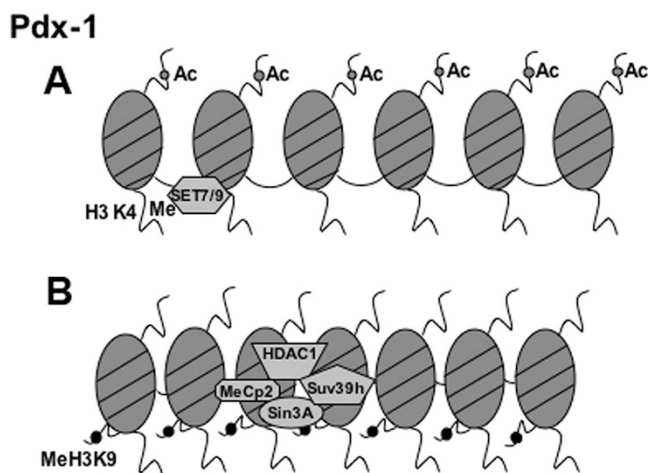
Figure 1. Schematic of histone acetylation and methylation of the proximal promoter of Pdx-1. (*A*) H3 is heavily acetylated and H3K4 is methylated in islets from control animals. H3K4 methylation is catalyzed by SET7/9. (*B*) Acetylation of H3 and methylation of H3K4 of Pdx-1 are lost in IUGR islets.

from its developmental role, in the normal function of β cells (40). This may be the reason that humans with heterozygous missense mutations in *Pdx*-1 exhibit early and late onset forms of type 2 diabetes (40,41).

SUMMARY

The studies described above clearly show that environmental effects can induce epigenetic alterations. Much of the recent progress in understanding epigenetic phenomena is directly attributable to technologies that allow researchers to pinpoint the genomic location of proteins that package and regulate access to the DNA. The advent of DNA microarrays and inexpensive DNA sequencing has allowed many of those technologies to be applied to the whole genome. It is possible that epigenetic profiling of CpG islands in the human genome can be used as a tool to identify genomic loci that are susceptible to DNA methylation. Aberrant hypermethylation may be then be used as a biomarker for disease (42–45).

The genome-wide mapping of histone modifications by ChIP-chip has led to important insights regarding the mechanism of transcriptional and epigenetic memory, and how different chromatin states are propagated through the genome in yeast (45). To date, there is only one published report of a genome-wide, high-resolution ChIP-chip study in mammalian cells (46). In the near future, it is likely that technologies will be developed that will allow genome-wide epigenetics studies, especially applied to the limited numbers of cells that can be isolated to a high degree of purity by techniques such as laser capture microscopy.

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