Developmental Origins of Diabetes: The Role of Epigenetics

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Abstract The "thrifty phenotype" hypothesis proposes that the fetus adapts to an adverse intrauterine milieu by optimizing the use of a reduced nutrient supply to ensure survival, but, by favoring the development of certain organs over that of others, this strategy leads to persistent alterations in the growth and function of developing tissues. This concept has been somewhat controversial, however, as recent epidemiological, clinical, and animal studies provide support for the developmental origins of disease hypothesis. Underlying mechanisms include reprogramming of the hypothalamic-pituitary-adrenal axis, islet development, and insulin signaling pathways. Emerging data suggest that oxidative stress and mitochondrial dysfunction may also play critical roles in the pathogenesis of type 2 diabetes in individuals who were growth retarded at birth. Epigenetic modifications may be one mechanism by which exposure to an altered intrauterine milieu or metabolic perturbation may influence the phenotype of the organism much later in life. Epigenetic modifications of the genome provide a mechanism that allows the stable propagation of gene expression from one generation of cells to the next. This review highlights our current knowledge of epigenetic gene regulation and the evidence that chromatin remodeling and histone modifications play key roles in adipogenesis and the development of obesity. Epigenetic modifications affecting processes important to glucose regulation and insulin secretion have been described in the pancreatic β-cells and muscle of the intrauterine growth retarded (IUGR) offspring, characteristics essential to the pathophysiology of type 2 diabetes (T2DM). Epigenetic regulation of gene expression contributes to both adipocyte determination and differentiation in in vitro models. The contributions of histone acetylation, histone methylation, and DNA methylation to the process of adipogenesis in vivo remain to be evaluated.

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J.R. Seckl and Y. Christen (eds.), *Hormones, Intrauterine Health and Programming*, Research and Perspectives in Endocrine Interactions 12,
DOI 10.1007/978-3-319-02591-9_10, © Springer International Publishing Switzerland 2014

Introduction

It is becoming increasingly apparent that the in utero environment in which a fetus grows and develops may have long-term effects on subsequent health and survival (Hales and Barker 1992; Kermack 1934). The landmark cohort study of 300,000 men by Ravelli and colleagues (1976) showed that fetal exposure to the Dutch famine of 1944–1945 during the first half of pregnancy resulted in significantly higher obesity rates at age 19. Subsequent studies demonstrated a relationship between low birth weight and the later development of cardiovascular disease (Barker et al. 1989) and impaired glucose tolerance (Hales et al. 1991; Phipps et al. 1993; Fall et al. 1995) in men in England. Those men who were smallest at birth (2.5 kg) were nearly seven times more likely to have impaired glucose tolerance or type 2 diabetes than were those who were heaviest at birth. The investigators found a similar relationship between lower birth weight and higher systolic blood pressure and triglyceride levels (Barker et al. 1993). Subsequent studies in diverse populations through the world have demonstrated a significant correlation between low birth weight and the later development of type 2 diabetes (Hales and Barker 2001; Valdez et al. 1994; Curhan et al. 1996; Lithell et al. 1996; McKeigue et al. 1998; Leger et al. 1997; Jaquet et al. 2000; Egeland et al. 2000; Forsen et al. 2000; Rich-Edwards et al. 1999). More recent studies controlling for the confounding factors of socioeconomic status and lifestyle factors have further strengthened the association between low birth weight and increased risk of coronary heart disease, stroke, and type 2 diabetes (Curhan et al. 1996; Rich-Edwards et al. 1999). In 1976, the Nurses' Health Study was initiated and a large cohort of U.S. women born between 1921 and 1946 was established. The associations with low birth weight and increased risk of coronary heart disease, stroke, and type 2 diabetes remain strong even after adjusting for lifestyle factors such as smoking, physical activity, occupation, income, dietary habits, and childhood socioeconomic status and occur independently of the current level of obesity or exercise (Rich-Edwards et al. 1999). In a study of 22,000 American men, those born lighter than 5.5 lb had a significantly higher incidence of adult hypertension and type 2 diabetes compared with average birth weight adults (Curhan et al. 1996). Similar to the Nurses' Health Study, the association between birth weight and later disease is largely independent of the lifestyle risk factors (Curhan et al. 1996).

Recent observations have shown that impaired growth in infancy and rapid childhood weight gain exacerbate the effects of impaired prenatal growth. The highest risk for the development of type 2 diabetes is among adults who were born small and become overweight during childhood (Forsen et al. 2000; Eriksson et al. 2000; Bavdekar et al. 1999, 2004).

The mechanisms underlying the association between size at birth and impaired glucose tolerance or type 2 diabetes are unclear. A number of studies in children and adults have shown that non- or pre-diabetic subjects with low birth weight are insulin resistant and thus predisposed to developing type 2 diabetes (Hales and

Barker 2001; Lithell et al. 1996; McKeigue et al. 1998; Leger et al. 1997; Jaquet et al. 2000; Bavdekar et al. 1999, 2004; Hoffman et al. 1997; Li et al. 2001; Yajnik et al. 1995; Clausen et al. 1997; Flanagan et al. 2000; Phillips et al. 1994). Intrauterine growth retardation (IUGR) is known to alter the fetal development of adipose tissue, which is closely linked to the development of insulin resistance (Jaquet et al. 2000; Widdowson et al. 1979; Lapillonne et al. 1997). Other studies have shown that the adverse effect of intrauterine growth retardation on glucose homeostasis was mediated through programming of the fetal endocrine pancreas (Hales and Barker 1992; Van Assche et al. 1977; Jensen et al. 2002). Jensen and colleagues (2002) measured insulin secretion and insulin sensitivity in a well-matched Caucasian population of 19-year-old, glucose-tolerant men with birth weights of either below the 10th percentile (small for gestational age; SGA) or between the 50th and 75th percentile (controls). To eliminate the major confounders, such as "diabetes genes," none of the participants had a family history of diabetes, hypertension, or ischemic heart disease. There was no difference between the groups with regard to current weight, body mass index (BMI), body composition, or lipid profile. When controlled for insulin sensitivity, insulin secretion was reduced by 30 %. However, insulin sensitivity was normal in the SGA subjects. The investigators hypothesized that defects in insulin secretion may precede defects in insulin action and that, once SGA individuals accumulate body fat, they will develop insulin resistance (Jensen et al. 2002).

What Animal Models Can Tell Us

Animal models have a normal genetic background upon which environmental effects during gestation or early postnatal life can be tested for their role in inducing diabetes. For a comprehensive survey of the numerous animal models of fetal growth retardation, the reader is referred to two excellent reviews (Fowden and Forhead 2004; McMillen and Robinson 2005). The most commonly used animal models are caloric or protein restriction, glucocorticoid administration, or induction of uteroplacental insufficiency in the pregnant rodent. In the rat, maternal dietary protein restriction (approximately 40–50 % of normal intake) throughout gestation and lactation has been reported to alter glucose homeostasis and hypertension in the adult offspring (Dahri et al. 1991; Snoeck et al. 1990; Ozanne et al. 1996; Berney et al. 1997; Wilson and Hughes 1997; Burns et al. 1997). Offspring are significantly growth retarded, remain growth retarded throughout life and, in some cases, develop mild β-cell secretory abnormalities (Dahri et al. 1991; Snoeck et al. 1990; Ozanne et al. 1996; Berney et al. 1997; Wilson and Hughes 1997) and, in others, insulin resistance (Ozanne et al. 1996; Burns et al. 1997). Aged rats develop hyperglycemia characterized by defects in insulin signaling in muscle, adipocytes, and liver (Burns et al. 1997; Ozanne et al. 2003, 2005; Petry et al. 2001; Fernandez-Twinn et al. 2005).

Fetal overexposure to glucocorticoids either via maternal administration or by inhibition of placental 11beta-hydroxysteroid Dehydrogenase Type 2 (11ßHSD2) in the rat induces hypertension, glucose intolerance and abnormalities in hypothalamic-pituitary-adrenal (HPA) function after birth (Benediktsson et al. 1993; Lindsay et al. 1996a, b; Niyirenda and Seckl 1998).

To extend these experimental studies of growth retardation, we developed a model of uteroplacental insufficiency (IUGR) induced by bilateral uterine artery ligation at day 18 of gestation (term is 22 days) in the rat that restricts fetal growth (Simmons et al. 2001; Boloker et al. 2002). Growth-retarded fetal rats have critical features of a metabolic profile characteristic of growth-retarded human fetuses: decreased levels of glucose, insulin, insulin-like-growth factor 1 (IGF-I), amino acids, and oxygen (Ogata et al. 1986; Simmons et al. 1991; Unterman et al. 1990). By 6 months of age, IUGR rats develop diabetes with a phenotype remarkably similar to that observed in the human with type 2 diabetes: progressive dysfunction in insulin secretion and insulin action. Thus, the studies in various animal models support the hypothesis that an abnormal intrauterine milieu can induce permanent changes in glucose homeostasis after birth and lead to type 2 diabetes in adulthood.

Cellular Mechanisms: Mitochondrial Dysfunction and Oxidative Stress

The intrauterine environment influences development of the fetus by modifying gene expression in both pluripotential cells and 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. The fetus also adapts to an inadequate supply of substrates (such as glucose, amino acids, fatty acids, and oxygen) by metabolic changes, redistribution of blood flow, and changes in the production of the fetal and placental hormones that control growth.

The fetus' immediate metabolic response to placental insufficiency is catabolism: it consumes its own substrates to provide energy. A more prolonged reduction in availability of substrates leads to a slowing in growth, which enhances the fetus' ability to survive by reducing the use of substrates and lowering the metabolic rate. Slowing of growth in late gestation leads to disproportion in organ size, since organs and tissues that are growing rapidly at the time are affected the most.

Uteroplacental insufficiency, caused by such disorders as preeclampsia, maternal smoking and abnormalities of uteroplacental development, is one of the most common causes of fetal growth retardation. The resultant abnormal intrauterine milieu restricts the supply of crucial nutrients to the fetus, thereby limiting fetal growth. Multiple studies have now shown that intrauterine growth retardation is associated with increased oxidative stress in the human fetus (Myatt et al. 1997; Karowicz-Bilinska et al. 2002; Ejima et al. 1999; Kato et al. 1997; Bowen

et al. 2001; Wang and Walsh 1998, 2001). A major consequence of limited nutrient availability is an alteration in the redox state in susceptible fetal tissues, leading to oxidative stress. In particular, low levels of oxygen, evident in growth-retarded fetuses, will decrease the activity of complexes of the electron transport chain, which will generate increased levels of reactive oxygen species (ROS; Esposti and McLennan 1998; Chandel et al. 1996; Gorgias et al. 1996). Overproduction of ROS initiates many oxidative reactions that lead to oxidative damage not only in the mitochondria but also in cellular proteins, lipids, and nucleic acids. Increased ROS levels inactivate the iron-sulfur centers of the electron transport chain complexes and tricarboxylic acid cycle aconitase, resulting in shutdown of mitochondrial energy production.

A key adaptation enabling the fetus to survive in a limited energy environment may be the reprogramming of mitochondrial function (Gorgias et al. 1996; Peterside et al. 2003; Selak et al. 2003). However, these alterations in mitochondrial function can have deleterious effects, especially in cells that have a high energy requirement, such as the β-cell. The β-cell depends upon the normal production of ATP for nutrient-induced insulin secretion (Panten et al. 1984; Newgard and McGarry 1995; Schuit 1997; Mertz et al. 1996; Ortsater et al. 2002; Antinozzi et al. 2002; Malaisse et al. 1980; Lenzen et al. 1986) and proliferation (Noda et al. 2002). Thus, an interruption of mitochondrial function can have profound consequences for the β-cell.

Mitochondrial dysfunction can also lead to increased production of ROS, which will lead to oxidative stress if the defense mechanisms of the cell are overwhelmed. β-cells are especially vulnerable to attacks by ROS because expression of antioxidant enzymes in pancreatic islets is very low (Lenzen et al. 1996; Tiedge et al. 1997), and β-cells have a high oxidative energy requirement. Increased ROS impair glucose-stimulated insulin secretion (Noda et al. 2002; Maechler et al. 1999; Sakai et al. 2003), decrease gene expression of key β-cell genes (Kaneto et al. 1999, 2001, 2002a, b; Jonas et al. 1999, 2001; Efanova et al. 1998), and induce cell death (Moran et al. 2000; Donath et al. 1999; Silva et al. 2000).

We have found that uteroplacental insufficiency induces oxidative stress and marked mitochondrial dysfunction in the fetal β-cell (Simmons et al. 2005). ATP production is impaired and continues to deteriorate with age. The activities of complexes I and III of the electron transport chain progressively decline in IUGR islets. Mitochondrial DNA point mutations accumulate with age and are associated with decreased mtDNA content and reduced expression of mitochondrial-encoded genes in IUGR islets. Mitochondrial dysfunction results in impaired insulin secretion. These results demonstrate that IUGR induces mitochondrial dysfunction in the fetal β-cell, leading to increased production of ROS, which in turn damage mtDNA (Simmons et al. 2005). A self-reinforcing cycle of progressive deterioration in mitochondrial function leads to a corresponding decline in β-cell function. Finally, a threshold in mitochondrial dysfunction and ROS production is reached and diabetes ensues.

Mitochondrial dysfunction is not limited to the β-cell in the IUGR animal. IUGR animals exhibit marked insulin resistance early in life (prior to the onset of

hyperglycemia), characterized by blunted whole body glucose disposal in response to insulin and impaired insulin suppression of hepatic glucose output (Vuguin et al. 2004). Basal hepatic glucose production is also increased (Vuguin et al. 2004). Oxidation rates of pyruvate, glutamate, succinate, and α-ketoglutarate are significantly blunted in isolated hepatic mitochondria from IUGR pups (prior to the onset of diabetes; Peterside et al. 2003). Rotenone-sensitive NADH-O_2 oxidoreductase activity is similar in control and IUGR mitochondria, showing that the defect responsible for decreased pyruvate, glutamate and α-ketoglutarate oxidation in IUGR liver precedes the electron transport chain and involves pyruvate and α-ketoglutarate dehydrogenases. Increased levels of manganese superoxide dismutase (MnSOD) suggest that an antioxidant response has been mounted, and 4-hydroxynonenal (HNE) modification of pyruvate dehydrogenase E2 catalytic and E3 binding protein subunits suggests that HNE-induced inactivation of this key enzyme may play a role in the mechanism of injury. These results indicate that uteroplacental insufficiency impairs mitochondrial oxidative phosphorylation in the liver and this derangement predisposes the IUGR rat to increased hepatic glucose production by suppressing pyruvate oxidation and increasing gluconeogenesis (Peterside et al. 2003).

Mitochondria in muscle of IUGR young adult rats, prior to the onset of hyperglycemia, exhibit significantly decreased rates of state 3 oxygen consumption with pyruvate, glutamate, α-ketoglutarate and succinate (Selak et al. 2003). Decreased pyruvate oxidation in IUGR mitochondria is associated with decreased ATP production, decreased pyruvate dehydrogenase activity and increased expression of pyruvate dehydrogenase kinase 4 (PDK4). Such a defect in IUGR mitochondria leads to a chronic reduction in the supply of ATP available from oxidative phosphorylation. Impaired ATP synthesis in muscle compromises energy-dependent GLUT4 recruitment to the cell surface, glucose transport and glycogen synthesis, which contributes to insulin resistance and hyperglycemia of type 2 diabetes (Selak et al. 2003).

Other animal models of fetal growth retardation also show mitochondrial abnormalities. Mitochondrial DNA content is reduced in liver, pancreas and skeletal muscle of male offspring of dams fed a low-protein diet during pregnancy and lactation (Park et al. 2003, 2004). This was associated with reduced expression of mitochondrial DNA-encoded genes (Park et al. 2003).

A number of recent studies in humans further suggest that mitochondrial dysfunction may contribute to type 2 diabetes. Studies using ^{13}C and ^{31}P magnetic resonance spectroscopy (MRS) have shown decreases in mitochondrial activity and increases in intramyocellular fat content in young insulin-resistant offspring of parents with type 2 diabetes, a group that has a strong tendency to develop diabetes later in life (Petersen et al. 2004). Expression of genes involved in oxidative phosphorylation is reduced among patients with type 2 diabetes mellitus and insulin resistance (Mootha et al. 2003), although this may be an effect rather than a cause of diabetes.

Chromatin Structure, DNA Methylation and Gene Expression

Epigenetic modifications of the genome provide a mechanism that allows the stable propagation of gene expression from one generation of cells to the next. Epigenetic states can be modified by environmental factors, which may contribute to the development of abnormal phenotypes. There are at least two distinct mechanisms through which epigenetic information can be inherited: histone modifications and DNA methylation (Berger 2007; Reik 2007).

In eukaryotes, the nucleosome is formed when DNA is wrapped around an octameric complex 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 histone 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, on the other hand, is associated with both transcription repression and activation (Berger 2007; Reik 2007). Moreover, lysine residues can be mono-, di-, or trimethylated in vivo, providing an additional mechanism of regulation (Berger 2007; Reik 2007).

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 (Reik 2007). 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 they serve as promoters for their associated genes. Approximately half of mammalian genes have CpG islands (Reik 2007). 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. In normal cells, most CpG islands remain unmethylated; however, under circumstances such as cancer (Yoshida et al. 2006; So et al. 2006; Takahashi et al. 2006) and oxidative stress, 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.

DNA methylation is commonly associated with gene silencing and contributes to X-chromosomal inactivation and genomic imprinting, as well as transcriptional regulation of tissue-specific genes during cellular differentiation (reviewed in Cedar and Bergman 2009; Schübeler et al. 2000; Gopalakrishnan et al. 2008). It is not known why some genes are able to undergo aberrant DNA methylation; however, a study by Feltus et al. (2003) suggests that there is a “DNA sequence signature associated with aberrant methylation.” Of major significance to T2D is

their finding that *Pdx1*, a pancreatic homeobox transcription factor, was one of only 15 genes (of 1,749 examined) with CpG islands within the promoter that were methylation-susceptible (which was induced by over-expression of a DNA methyltransferase). This study demonstrates that genes essential to pancreatic development, like *Pdx1*, are susceptible to epigenetic modifications, which could ultimately affect gene expression.

Histone methylation can influence DNA methylation patterns and vice versa (Cedar and Bergman 2009). For example, methylation of lysine 9 on histone 3 (H3) promotes DNA methylation, whereas CpG methylation stimulates methylation of lysine 9 on H3 (Schübeler et al. 2000). Recent evidence indicates that this dual relationship between histone methylation and DNA methylation might be accomplished by direct interactions between histone and DNA methyltransferases (Cedar and Bergman 2009). Thus, chromatin modifications induced by adverse stimuli are self-reinforcing and can propagate.

Epigenetic Regulation of Gene Expression in Fetal Growth Retardation

A number of studies suggest that uteroplacental insufficiency, a common cause of IUGR, induces epigenetic modifications in offspring (MacLennan et al. 2004; Fu et al. 2004; Park et al. 2008; Raychaudhuri et al. 2008). Epigenetic modifications affecting processes important to glucose regulation and insulin secretion, characteristics essential to the pathophysiology of T2D, have been described in the IUGR liver, pancreatic β cells and muscle (MacLennan et al. 2004; Fu et al. 2004; Park et al. 2008; Raychaudhuri et al. 2008).

Chromatin Remodeling in the β-Cell of IUGR Rat

Pdx-1 is a homeodomain-containing transcription factor that plays a critical role in the early development of both the endocrine and exocrine pancreas and in the later differentiation and function of the β-cell. As early as 24 h after the onset of growth retardation, *Pdx1* mRNA levels are reduced by more than 50 % in IUGR fetal rats. Suppression of *Pdx1* expression persists after birth and progressively declines in the IUGR animal, implicating an epigenetic mechanism.

Changes in histone acetylation are the first epigenetic modifications found in β-cells of IUGR animals. Islets isolated from IUGR fetuses show a significant decrease in H3 and H4 acetylation at the proximal promoter of *Pdx1* (Park et al. 2008). These changes in H3 and H4 acetylation are associated with a loss of binding of USF-1 to the proximal promoter of *Pdx1* (105). USF-1 is a critical activator of *Pdx1* transcription, and its decreased binding markedly decreases *Pdx1*

transcription (Qian et al. 1999; Sharma et al. 1996). After birth, histone deacetylation progresses and is followed by a marked decrease in H3K4 trimethylation and a significant increase in dimethylation of H3K9 in IUGR islets (Park et al. 2008). H3K4 trimethylation is usually associated with active gene transcription whereas H3K9 dimethylation is usually a repressive chromatin mark. Progression of these histone modifications parallels the progressive decrease in *Pdx1* expression that manifests as a deterioration in glucose homeostasis and increased oxidative stress in the aging IUGR animals (Park et al. 2008). Nevertheless, at 2 weeks of age, the silencing histone modifications in the IUGR pup are responsible for suppression of Pdx1 expression, since there is no appreciable methylation of CpG islands in mice at this age (Park et al. 2008). Reversal of histone deacetylation in IUGR islets at 2 weeks of age is sufficient to nearly normalize Pdx1 mRNA levels permanently, perhaps due to active β-cell replication present in the neonatal rodent (Park et al. 2008).

In IUGR, *Pdx1* is first silenced due to recruitment of co-repressors, including histone deacetylase 1 (HDAC1) and mSin3A (Park et al. 2008). These repressors catalyze histone deacetylation. Binding of these deacetylases facilitates loss of trimethylation of H3K4, further repressing *Pdx1* expression (Park et al. 2008). We found that inhibition of HDAC activity by trichostatin A (TSA) treatment normalizes H3K4me3 levels at *Pdx1* in IUGR islets (Park et al. 2008). These data suggest that the association of HDAC1 at *Pdx1* in IUGR islets likely serves as a platform for the recruitment of a demethylase, which catalyzes demethylation of H3K4.

The molecular mechanism responsible for DNA methylation in IUGR islets is likely dependent on the methylation status of lysine 9 on H3 (H3K9). Previous studies have shown that changes in methylation of H3K9 precede changes in DNA methylation (Li et al. 2006; Bachman et al. 2003; Kouzarides 2002). It has also been suggested that DNA methyltransferases may act only on chromatin that is methylated at H3K9 (Bachman et al. 2003). Histone methyltransferases specifically DNA methyltransferase 3A (DNMT3A) and DNA methyltransferase 3B (DNMT3B), bind to DNA methylases, thereby initiating DNA methylation (Li et al. 2006).

These results demonstrate that IUGR induces a self-propagating epigenetic cycle in which the mSin3A/HDAC complex is first recruited to the *Pdx1* promoter, histone tails are subjected to deacetylation and *Pdx1* transcription is repressed. At the neonatal stage, this epigenetic process is reversible and may define an important developmental window for therapeutic approaches. However, as dimethylated H3K9 accumulates, DNMT3A is recruited to the promoter and initiates de novo DNA methylation, which locks in the silenced state in the IUGR adult pancreas, resulting in diabetes.

How do these epigenetic events lead to diabetes? Targeted homozygous disruption of *Pdx1* in mice results in pancreatic agenesis, and homozygous mutations yield a similar phenotype in humans (reviewed in Bernardo et al. 2008). Milder reductions in *Pdx1* protein levels, as occurs in the *Pdx*+/- mice, allow for the development of a normal mass of ß cells but result in the impairment of several events in glucose-stimulated insulin secretion (Bernardo et al. 2008). These results

indicate that *Pdx1* plays a critical role in the normal function of ß cells in addition to its role in ß cell lineage development, which may be the reason that humans with heterozygous missense mutations in *Pdx1* exhibit early and late onset forms of T2D (Bernardo et al. 2008).

The discovery of a critical developmental stage during which aberrant epigenetic modifications may be reversed represents a therapeutic window for the use of novel agents that could prevent common diseases with late-onset phenotypes. T2D is one such disease, where predisposed individuals could be treated with agents that normalize the epigenetic programming of key genes, thus providing protection against development of the adult diabetic phenotype.

Genome-Wide DNA Methylation Is Disrupted in IUGR Islets

Epigenetic modifications are not confined to the *Pdx1* locus in the IUGR rat. We mapped DNA methylation across approximately 1,000,000 loci using the HELP assay (Thompson et al. 2010). Comparison of IUGR with normal rats at 7 weeks of age, prior to the onset of diabetes, revealed changes in DNA methylation at a number of novel loci, not limited to canonical CpG islands or promoters. We found that IUGR in the rat caused consistent and non-random changes in cytosine methylation, affecting $<1\ \%$ of HpaII sites in the genome in the islet. The majority of these changes took place not at promoters but at intergenic sequences, many of which are evolutionarily conserved. Furthermore, some of these loci were in proximity to genes manifesting concordant changes in gene expression and were enriched near genes that regulate processes that are markedly impaired in IUGR islets (e.g., vascularization, proliferation, insulin secretion, and cell death).

Epigenetic Landscape in Human Islets

Recently, Kaestner and colleagues (Bhandare et al. 2010) used chromatin immunoprecipitation with massively parallel sequencing (ChIP-seq) technology to create a genome-wide map of histone modifications associated with gene activation or repression in human pancreatic islets. They mapped the genome-wide enrichment and location of four histone marks: three associated with gene activation—H3K4me1, H3K4me2, and H3K4me3—and one associated with gene repression, H3K27me3. H3K4me1, H3K4me2, and H3K4me3 are frequently found near active gene promoters, whereas H3K4me1 is also often associated with enhancers. Interestingly, there was little enrichment of H3K4me2 and H3K4me3 at the promoters of the highly transcribed insulin and glucagon genes. In contrast, there was robust enrichment of H3K27me3 at the NEUROG3 promoter, a regulator of fetal islet development that is repressed during adult life. They found 16.5 % of the H3K4me3 loci were >5 kbp from the nearest gene, indicating a large number of potentially

novel transcriptional start sites active in islets. A larger fraction of H3K4me1 (24.8 %) and H3K4me2 (24.3 %) loci was intergenic and may represent potential regulatory regions. The insulin and nearby genes in an extended 80-kb region are a part of a large, human islet-specific, open chromatin domain and share a common control mechanism. The presence of intergenic transcription in this region has been proposed to play a role in the maintenance of open chromatin structure, suggesting that a locus-specific control mechanism might be responsible for constitutive insulin gene expression in humans. The data in the Kaestner study also indicate a region (chr1:2,100,000–2,200,000 mm[8]) of high levels of H3K4me1, a mark associated with regulatory regions covering the insulin gene locus. Thus the pattern of histone modifications in the islet is complex.

The epigenetic landscape in the human β-cell also appears to be markedly altered. Fuks and colleagues (Volkmar et al. 2012) carried out a comprehensive DNA methylation profiling of human T2D pancreatic islets using the Infinium 27 k Methylation Assay. This assay interrogates the methylation status of 27,578 CpG sites corresponding to 14,475 consensus coding sequences and well-known cancer genes. They identified 276 differentially methylated CpG sites that were affiliated with 254 genes. Interestingly, they found predominantly promoter hypomethylation in T2D islets that was frequently associated with increased gene expression. However, for a significant proportion of differentially methylated genes, there was no significant differential expression. Thus, for many genes the link between differential methylation and gene activity is complex. Of major importance was the finding that the methylation changes were not present in blood cells from T2D individuals; neither were they experimentally induced in non-diabetic islets by exposure to high glucose, further underscoring the cell-specificity of DNA methylation patterns.

Summary

The combined epidemiological, clinical, and animal studies clearly demonstrate that the intrauterine environment influences both growth and development of the fetus and the subsequent development of adult diseases. There are critical, specific windows during development, often coincident with periods of rapid cell division, during which a stimulus or insult may have long-lasting consequences on tissue or organ function postnatally. Birthweight is only one marker of an adverse fetal environment, and confining studies to this population only may lead to erroneous conclusions regarding etiology. Studies using animal models of uteroplacental insufficiency suggest that mitochondrial dysfunction and oxidative stress play an important role in the pathogenesis of the fetal origins of adult disease. Environmental effects can induce epigenetic alterations, ultimately affecting expression of key genes linked to the development of T2D, including genes critical for pancreatic development and β-cell function, peripheral glucose uptake and insulin resistance and atherosclerosis. Understanding the role of developmental programming of

genes crucial to the development of T2D may unveil a critical window during which epigenetic therapeutic agents could be used as a means to prevent the later development of a disease. Prior to the use of such therapeutic agents there remains much to be learned about the programming of the epigenetic code, especially on a genome-wide scale. 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 now 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 methylation may then be used as a biomarker for disease. The genome-wide mapping of histone modifications by ChIP-chip and ChIP-seq 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 and in mammalian cells (Lieb et al. 2006; Kim et al. 2005). Although Bisulfite-seq (anlaysis of genome-wide DNA methylation) and Chip-Seq (analysis of genome-wdie histone modifcations) experiments are currently being performed in human tissue, obstacles such as intrinsic human epigenetic variability (including age-related changes) and tissue-specific epigenetic variability must be characterized and mapped in the healthy, non-diseased state before this information can be applied to diseases such as T2D. Eventually genome-wide epigenetic characterization will lead to specific therapies with epigenetic targets and also will allow monitoring of genome-wide epigenetic consequences of these therapies once they are applied.

Acknowledgments Rebecca Simmons has no conflict of interest. This work was supported by NIH grants: DK55704, DK078761.

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