Environmental epigenetics: a role in endocrine disease?

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Abstract

Endocrine disrupting chemicals that are structurally similar to steroid or amine hormones have the potential to mimic endocrine endpoints at the receptor level. However, more recently, epigenetic-induced alteration in gene expression has emerged as an alternative way in which environmental compounds may exert endocrine effects. We review concepts related to environmental epigenetics and relevance for endocrinology through three broad examples: 1) effect of early-life nutritional exposures on future obesity and insulin resistance, 2) effect of lifetime environmental exposures such as ionizing radiation on endocrine cancer risk, and 3) potential for compounds previously classified as endocrine disrupting to additionally or alternatively exert effects through epigenetic mechanisms. The field of environmental epigenetics is still nascent, and additional studies are needed to confirm and reinforce data derived from animal models and preliminary human studies. Current evidence suggests that environmental exposures may significantly impact expression of endocrine-related genes and thereby affect clinical endocrine outcomes.

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Introduction

In the 1970s, diethylstilbestrol (DES), an estrogen agonist used to prevent miscarriages, was associated with vaginal clear-cell adenocarcinoma in adult female offspring exposed in utero (Robboy et al. 1977). Mothers who took DES, however, did not appear to be at increased risk for this adenocarcinoma, suggesting that in utero programming may be a mechanism for this observation. Since then, interest in studying the endocrine effects of exogenous agents, and their role in epigenetic programming, has increased dramatically.

Exogenous compounds that alter ‘hormonal and homeostatic systems’ (i.e. endocrine endpoints) have been termed ‘endocrine disrupting chemicals’ and were officially recognized in an Endocrine Society statement in 2009 (Diamanti-Kandarakis et al. 2009). The majority of currently identified endocrine disruptors are synthetic industrial chemicals rather than drugs. In the USA, although the Toxic Substances Control Act (TSCA) of 1976 provides a legislative framework to limit the spread of toxic chemicals, companies are not required to perform monitoring for adverse health effects of product chemicals. Toxicity is typically discovered only after the product is widely used. For this reason, many chemicals with suspected or even confirmed endocrine disruption properties are in current daily use.

Many endocrine disrupting chemicals have structural similarity to steroid or amine hormones. Acting as ligands, they can either activate or antagonize the hormone’s receptor, leading to altered endocrine endpoints (Diamanti-Kandarakis et al. 2009). However, more recently, the classification of endocrine disrupting chemicals has expanded. Agents such as heavy metals that do not act directly on steroid receptors have been shown to alter hormone metabolism via epigenetic alterations. Also, compounds such as DES that are structurally similar to hormones (Fig. 1) may induce epigenetic changes through interaction with hormone receptors.

What is epigenetics?

Epigenetics is defined as changes in gene expression that occur without changes in DNA sequence (Wolffe & Guscin 2000) and can be transmitted through mitosis and/or meiosis. Given that all cells in the human body contain the same DNA sequence, epigenetics can be thought of as those processes that regulate gene expression in a given cell leading to its cellular phenotype, a definition first proposed by
DNA methylation

DNA methylation is the addition of a methyl group to a cytosine (C) nucleotide at position 5 and typically occurs when a cytosine is positioned next to a guanine (G). Phosphates (P) link nucleosides in DNA, and, thus, this particular arrangement is termed a CpG dinucleotide. Regions of the genome dense in CpG dinucleotides are termed CpG islands. Although CpG islands have the potential for methylation and are frequently overrepresented at gene promoters, the majority of the time, they are not methylated (Jirtle & Skinner 2007). Regions with lower CpG density bordering the CpG islands are termed CpG shores and have been proposed to occur with increased frequency in regulatory sites involved in tissue differentiation (Doi et al. 2009).

Tissue-specific methylation of CpG dinucleotides by DNA methyltransferases (DNMTs) can lead to gene silencing (Wolffe & Matzke 1999, Orphanides & Reinberg 2002). The simplest way to understand DNA methylation is to think of DNA as not as a strand but as a coil wrapped tightly around a histone. Methylation leads to gene silencing because it alters DNA's three-dimensional structure such that the coil becomes tighter at the locus corresponding to the promoter region. Within this tightened coil, transcription factors can no longer be recruited to their binding sites. Also, methyl binding proteins (MBPs) can interact with methylated CpGs and actively repress gene transcription (Bird & Wolffe 1999).

There is evidence that physiological DNMT activity is under hormonal control. For example, DNMT1 and MBP levels vary with menstrual cycle phase and with estrogen and progesterone secretion in endometrial explant tissues (van Kaam et al. 2011). Thus, based on their analogy to steroid hormones, some endocrine disrupting compounds have the potential to affect DNMT activity and consequently affect epigenetic marks.

Histone modifications

Histones are globular proteins around which DNA is packaged to make chromatin. Enzyme modifications such as acetylation and methylation of lysine residues in the amino terminus lead to a histone conformational change. Acetylation leads to increased DNA accessibility, and methylation can either increase or decrease DNA accessibility depending upon the specific type of methylation and histone affected (Yan & Boyd 2006). DNA methylation and histone modification often work in tandem, as MBPs recruited by DNA methylation may exert their effects through recruitment of histone deacetylases resulting in chromatin condensation and transcriptional inactivation (Jones et al. 1998, Nan et al. 1998).

Epigenetic reprogramming and inheritance

Epigenetic patterns undergo erasure and reprogramming two times during the human life cycle. One important result of epigenetic reprogramming is the correct establishment of imprinting at sites with allele-specific methylation. Independent of gender, human adult somatic cells contain one haploid set of chromosomes inherited from the mother that carry female-specific imprints, and a second haploid set of chromosomes inherited from the father that carry male-specific imprints. The first phase of erasure and reprogramming occurs during gametogenesis. In the primordial germline, DNA methylation – including methylation at imprinted loci – is erased and later reestablished. At imprinted loci, DNA methylation is reprogrammed so that it will take female-specific
imprints in oocytes and male-specific imprints in spermatozoa. The second phase of epigenetic erasure and reprogramming occurs during pre-implantation when the genome, with the possible exception of imprinted genes and some retrotransposons, becomes demethylated. After implantation, DNA methylation is restored de novo and rapidly acquires cell lineage-specific patterns to drive cell differentiation. This is the basis for the tissue-specific gene methylation pattern seen after birth and through adulthood. (Reik et al. 2001, Shi & Wu 2009, Perera & Herbstman 2011). Folate and vitamin B12 serve as exogenous methyl donors, and their influence on DNA methylation during the in utero time period will be discussed in more detail later in this manuscript.

The prenatal erasure and reprogramming of DNA methylation patterns makes the in utero time period a window of potential vulnerability for epigenetic dysregulation from environmental exposures. This is particularly relevant in endocrinology where there is burgeoning evidence that the fetal environment may program adult outcomes such as obesity and type 2 diabetes mellitus (Law et al. 1992, Stocker et al. 2005).

Other potential vulnerable windows for epigenetic dysregulation that might affect endocrine systems include puberty, during which time there is an overall rapid increase in DNA turnover and cell growth, as well as old age, which has been associated with progressive age-related changes in DNA methylation (Bjornsson et al. 2008; Fig. 2).

Epigene–environment interaction

Epigenetic dysregulation can result from environmental exposures including dietary factors, physical activity, social stressors, and environmental toxicants (Mathers et al. 2010, Alegria-Torres et al. 2011). However, there is a paucity of human studies that document the causal pathway from environmental exposure to epigenetic modification to clinical outcome (Fig. 3).

We have chosen to demonstrate these themes by reviewing three broad examples where environmental epigenetics has impacted endocrinology. These include 1) effect of early-life nutritional exposures on future obesity and insulin resistance, 2) effect of lifetime environmental exposures such as ionizing radiation on endocrine cancer risk, and 3) potential for endocrine disrupting compounds to affect endocrine endpoints through epigenetic modifications. We review available data and suggest avenues for future research.

Nutritional status and epigenetic changes

Poor nutrition during pregnancy has been associated with DNA hypomethylation in offspring, which may result from a decrease in dietary sources of methyl group donors such as folate, methionine, and choline in conjunction with decreased availability of B vitamins (B2, B6, and B12; Mathers et al. 2010). Plasma homocysteine level, an inverse marker of folate
supplementation, has been associated with LINE-1 hypomethylation, gene-specific CpG island methylation patterns, and lower birthweight percentile (Fryer et al. 2009, 2011). Also, offspring of humans exposed to famine in early gestation had hypomethylation of the imprinted IGF2 gene compared to unexposed siblings (Heijmans et al. 2008). Parental overfeeding has also been associated with epigenetic modifications in offspring. In humans, paternal insulin resistance, presumably a result of overeating and obesity, was associated with low infant birth weight and increased risk of diabetes in offspring (Lindsay et al. 2000, Hypponen et al. 2003). Similarly, in rodents, female offspring of high-fat diet (HFD) fathers had lower birth weight and developed glucose intolerance, impaired insulin secretion, and decreased pancreatic islet and β-cell mass compared with control offspring. Il13ra2, part of the Jak–Stat signaling pathway, was hypomethylated and upregulated in HFD offspring, suggesting that a HFD may have affected the epigenetic profile of the paternal germ cells (Ng et al. 2010).

More recently, in a related study in humans, hypermethylation of the RXRA gene in umbilical cord tissue was associated with lower maternal carbohydrate intake during early pregnancy and later childhood adiposity. RXRA may be involved in insulin sensitivity, adipogenesis, and fat metabolism based on its interaction with PPARγ when serving as a transcription factor (Godfrey et al. 2011). These rodent and human studies suggest that parental nutritional status affects the DNA methylation profile and subsequent obesity and insulin resistance in offspring.

**Epigenetics and endocrine cancers**

Abundant literature exists linking environmentally induced epigenetic modifications to tumor formation and progression in non-endocrine cancers through altered expression of tumor suppressor genes and proto-oncogenes. For example, benzene exposure has been associated with increased risk of acute myelogenous leukemia (AML; 2005). Individuals with higher than average benzene exposure had a significant reduction in methylation of selected genomic repetitive elements, in conjunction with hypermethylation of tumor suppressor gene p15 (CDKN2B) and hypomethylation of the MAGE1 (MAGEA1) gene, suggesting epigenetic modification as a possible pathogenic mechanism (Bollati et al. 2007).

With regard to endocrine-related cancers, epigenetic modifications have also been implicated in thyroid carcinoma via hypermethylation and inactivation of tumor suppressor genes including cyclin-dependent kinase inhibitor p16INK4A (CDKN2A), microtubule stabilizer RASSF1A, GTPase-activating protein RAP1GAP, and PI3K/Akt pathway modulator PTEN (Russo et al. 2011). The PTEN promoter is

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**Figure 3** There are multiple potential causal pathways through which an environmental exposure may lead to epigenetic modifications, which may, in turn, impact clinical outcomes. Additional human studies that extend over the entire causal pathway are needed to establish cause-and-effect relationships between exposures and outcomes. EDC, endocrine disrupting compound.
hypermethylated in 50% of papillary carcinomas and almost 100% of follicular carcinomas, which is notable because a deleterious mutation in this gene causes PTEN hamartoma tumor syndrome leading to increased risk of papillary and thyroid carcinomas (Hobert & Eng 2009). As far as a potential environmental trigger for this epigenetic profile, ionizing radiation exposure has traditionally been thought to increase risk of thyroid cancer as a result of DNA mutagenesis, for example, through RET/PTC rearrangements (Caudill et al. 2005, Christodouleas et al. 2011). However, ionizing radiation has also been associated with global DNA hypomethylation that may be genotype specific (Giotopoulos et al. 2006) and that even occurs in cells not directly irradiated (Tamminga et al. 2008). Prior ionizing radiation has also been associated with gene-specific hypermethylation in cancer patients (Figueroa et al. 2009, Bennett et al. 2010, Mathers et al. 2010). Additional research is needed to establish whether ionizing radiation leads to specific epigenetic changes seen in thyroid carcinoma and subsequent thyroid carcinoma development.

Pituitary adenomas have also been associated with hypermethylation-related silencing of tumor suppressor genes such as RB1 and cyclin-dependent kinase inhibitors p15 and p16 (reviewed in Vandeve et al. (2010)). In some studies, GH-secreting adenomas have resulted in tissue-specific loss of imprinting at the paternal stimulatory G-protein allele, which leads to increased expression of GNAS and constitutive activation of adenylylcyclase (Hayward et al. 2001, reviewed in Mantovani et al. (2010)). As imprinting is an early embryonic process, in utero exposure may be responsible for dysregulated imprinting and subsequent development of GH-secreting adenomas; however, this has not yet been evaluated.

**Endocrine disruptors and epigenetics**

A growing number of animal models have linked prenatal endocrine disruptor exposure to offspring epigenetic modifications. Bisphenol A (BPA), a synthetic chemical and weak estrogen agonist found in food and beverage containers (Le et al. 2008), baby bottles (Nam et al. 2010), and dental materials (Fleisch et al. 2010), induced hypomethylation and increased expression of the Agouti gene in prenatally exposed mice leading to yellow rather than brown fur, as well as obesity, diabetes, and tumorigenesis (Dolinoy 2008). Furthermore, rodent mothers with the agouti phenotype were more likely to have offspring with that phenotype in the second generation. This important study serves as proof of principle that prenatal exposure to synthetic estrogen agonists such as BPA can affect the epigenome and thereby lead to endocrinological sequelae.

DES, an estrogen agonist found to cause vaginal clear-cell adenocarcinoma in the female offspring of users, has been associated with epigenetic modifications in offspring uterine tissues. Specifically, in mouse models of prenatal DES exposure and resultant genital tract neoplasia, hypomethylation occurred along with increased uterine expression of estrogen-sensitive LTF and proto-oncogenes including EGF and SRF (Nelson et al. 1994, Falck & Forsberg 1996, Li et al. 1997).

Exposure to vinclozolin, a fungicide and anti-androgen, during embryogenesis decreased adult sperm motility and concentration and increased the rate of kidney and prostate disease, immune system abnormalities, hypercholesterolemia, and tumorigenesis in rat first-generation male offspring. This phenotype was transferred through four generations (F1–F4) of male offspring and was associated with alterations in the sperm methylation profile of the F1–F3 generations (Anway et al. 2005). This is one of the first studies to suggest the potential for transgenerational inheritance of epigenetic marks presumably resulting, in this case, from incomplete erasure of epigenetic marks during gametogenesis. However, similar studies attempting to replicate the above findings in vinclozolin-exposed mice demonstrated changes in sperm methylation profile in the F1 and F2, but not the F3 generation (Schneider et al. 2008, Inawaka et al. 2009, Stouder & Paoloni-Giacobino 2010), suggesting that the phenotype of the F2 generation may be due to exposure of the F1 germline to the maternal environment rather than true transgenerational inheritance.

**Future directions**

Environmental epigenetics offers several clear research opportunities, particularly as it relates to endocrinology, including the following:

- Human studies of fetal epigenetic vulnerability to environmental exposures at physiologically relevant doses will confirm existing animal models.
- Human observational studies able to show the relationship from exposure to epigenetic modification to outcome over a life span will be required to differentiate causality from mere association.
- Additional endocrine tissue- and gene-specific studies of epigenetic modifications will further delineate causality.
- Finally, integrated teamwork including clinical endocrinologists, environmental toxicologists, and epidemiologists will strengthen study design and thereby lead to heightened understanding of the impact of environmental epigenetics on endocrinology.
Conclusions

Although the field of environmental epigenetics is just moving out of its infancy, it has already begun to demonstrate the breadth of potential impact of environmental exposures on the expression of endo-

crine-related genes. These results not only suggest the

need for increased biomonitoring of synthetic

compounds, but also point out specific windows of human susceptibility as well as potential mechanisms that could represent the substrate for future preventive interventions.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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