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Environmental induction of the fetal epigenome

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Abstract

The healthy adult is the result of successful interaction between the maternal environment and the developing fetal epigenome. The Barker hypothesis first suggested that *in utero* exposure to the maternal environment impacts adult health and disease. Since the origin of this theory, numerous studies have lent further support. Epigenomic alteration involves DNA methylation and histone modifications. Pregnancy, when the epigenome is typically actively programmed, is a vulnerable time, when exposures may have the most profound epigenetic effect. Recent advances have allowed an understanding of the extent and mechanism by which environmental exposures alter the epigenome of the fetus. Healthcare providers who treat and counsel reproductive-age women are in a unique position to protect against these epigenetic alterations and therefore prevent adverse impact on the developing fetus that may manifest throughout life.

Keywords

Barker hypothesis; bisphenol-A; diethylstilbestrol; epigenetics; folic acid; lead exposure; prenatal counseling; tobacco use

The theory of adult disease resulting secondary to *in utero* environmental exposure was proposed by Barker over 20 years ago [1]. Three key studies by Barker and colleagues led them to propose the ‘developmental origins of health and disease’. The first observation that adult disease may be linked to events *in utero* was the correlation of mortality rates from coronary heart disease in various regions of England and Wales, and mortality rates among newborn babies in similar regions [2]. Barker *et al.* followed this observation with a study in 1989 showing an inverse relationship between size at birth and death rates due to ischemic heart disease independent of gestational age at birth [3]. The third landmark study by Barker *et al.* in 1993 suggested that malnutrition during gestation alters development in the fetus, leading to the development of cardiovascular disease as an adult [4]. These studies form the basis of the current field that attempts to determine the developmental origins of disease. Since the development of the ‘Barker hypothesis’ on the developmental origins of adult disease, numerous studies have been presented that reinforce this theory that environmental stimuli result in permanent changes in metabolism and disease susceptibility [5]. Many of these changes are secondary to epigenetic changes that occur during fetal development [5].

Fetal programming has evolved as an adaptive response. For example, in times of food shortage, metabolic adaptations that increase energy storage may be beneficial. However, if

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the adult environment does not meet the environment perceived by the fetus, the effects of fetal programming may be detrimental. The increased energy conservation and metabolic alterations seen in the nutrient-deprived fetus can lead to obesity and all of the associated medical complications if this fetus is subsequently placed in a nutrient-rich environment.

Epigenetics

One mechanism that regulates fetal adaptation to the environment is epigenetics. The term ‘epi-genetic’ was first defined in the early 1940s by developmental biologist Conrad Waddingtion to explain “the interactions of genes with their environment, which bring the phenotype into being” [6,7]. From this initial definition, both the field and the definition of epigenetics have been molded into the study of phenotypes resulting from changes in the chromosome without alterations in the DNA sequence [8].

In the 1970s, Holliday and Pugh first proposed covalent chemical DNA modifications, including methylation of cytosine–phosphate–guanine (CpG) dinucleotides, as the molecular mechanism to explain Waddington's hypothesis [9]. Since this discovery additional mechanisms of epigenetic changes have been identified, including post-translational modifications of histone tails (acetylation, methylation, phosphorylation and so on), DNA methylation and higher-order packaging of DNA around nucleosomes [7]. The primary epigenetic mechanisms widely accepted and currently studied in mammals are the methylation of cytosine at the carbon-5 position in CpG dinucleotides and chromatin packaging of DNA by post-translational histone modification [7]. These two processes have critical roles in development and human disease.

Epigenetic regulation: DNA methylation & chromatin packaging

DNA methylation is critical for embryonic development [10]. DNA methylation occurs after DNA synthesis and involves the cytosine of the dinucleotide sequence CpG. Methylation of DNA occurs via the enzymatic addition of a methyl group from S-adenosylmethionine to the carbon-5 positon of the cytosine in the dinucleotide sequence CpG [11]. This reaction is catalyzed by DNA methyltransferases [11]. In mammals there are periods of development in which reprogramming of methylation patterns occur *in vivo*.

DNA methylation patterns are stable in somatic cells but are susceptible to reprogramming during gametogenesis and in early preimplantation embryos [12]. During gametogenesis, demethylation of the haploid genome occurs with development of the primordial germ cells [13]. The mammalian genome is progressively demethylated during the preimplantation period in order to restore the genome to a pluripotent state prior to implantation [13]. After implantation of the embryo, the genome is remethylated and cell-specific DNA methylation patterns are established very early in development as cells differentiate [13,14].

Much of what is known about the roles and importance of genomic methylation has come from studies in mammals on DNA methyltransferases and phenotypes resulting from mutations in the genes encoding these enzymes [15]. The DNA methyltransferase (DNMT)-1 serves to maintain methylation patterns [16], whereas *de novo* methylation is performed by the DNA methylation enzymes DNMT3A and DNMT3B [17]. DNMT3A and DNMT3B target different sites for methylation depending on the stage of development and the cell type [17].

DNA methylation generally functions to repress transcription of methylated gene sequences [18]. DNA methylation directly interferes with transcription by obstructing the binding of transcriptional machinery that requires contact with the cytosine in the major groove of the double helix [18]. Methylation of CpG can also inactivate transcription by direct exclusion

of transcriptional machinery from the methylated promoter DNA [18]. Alternatively, in some circumstances methylation can prevent the binding of a transcriptional repressor to DNA and lead to gene activation.

In addition to methylation, the packaging of genomic DNA through association with histone proteins plays a critical role in epigenetics [19]. The basic repeat element of chromatin is the nucleosome, consisting of 146 base pairs of DNA wrapped around an octameric histone core and interconnected by sections of linking DNA. This histone core consists of two copies each of histones H2A, H2B, H3 and H4 [20]. The organization of chromatin restricts physical access of nuclear factors to the DNA and alters regulation of gene expression [21]. The majority of nucleosomes are composed of the aforementioned types of histones; however, variation in these structures is created by post-translational modifications, such as acetylation and methylation of the histones [22]. These modifications are reversible and are often associated with regulated expression of individual genes [22].

Finally, noncoding RNA can interfere with gene translation or RNA stability. These processes result in altered phenotype without changes in DNA sequence. These molecules primarily affect RNA rather than DNA and are short-lived. For noncoding RNA to have a lasting effect, their transcription must be altered as well. It is likely that fetal developmental programming involves some of the mechanisms described earlier that regulate their expression.

Epigenetics & the environment

Nutritional and environmental exposures *in utero* are thought to play an important role in developmental variation and disease susceptibility through epigenetic mechanisms [23]. Considerable epidemiologic data on humans and animal models have demonstrated that *in utero* nutrition and environmental exposure influence adult susceptibility to chronic diseases such as cardiovascular disease, Type 2 diabetes and hypertension [24]. As more information about these adverse environmental interactions with the fetus comes to light, more responsibility will be placed on obstetricians and gynecologists to serve as a ‘guardian’ of the fetal epigenome. We will present current evidence on these epigenetic modifiers and discuss the role of healthcare providers in epigenetic disease prevention.

Endocrine-disrupting chemicals

The term ‘endocrine-disrupting chemicals’ (EDCs) is used to describe compounds found in the environment that mimic actions of endogenous hormones, both *in vitro* and *in vivo* [25]. Humans are commonly exposed to EDCs, which have been coupled to the disruption of normal developmental processes [26]. In particular, exposure to EDCs during critical stages of differentiation can interfere with hormonal signaling, thus altering development and resulting in aberrant gene expression. Diethylstilbestrol (DES) and bisphenol-A (BPA) are EDCs whose effects on the developing fetus have been extensively studied.

Diethylstilbestrol, a synthetic estrogen, is known to be a perinatal carcinogen in humans and experimental animals. DES was widely prescribed from the 1940s to 1970s to women with the belief that it would prevent spontaneous abortion [27]. In 1971, a study was published linking vaginal clear cell adenocarcinoma to DES exposure [28]. The association of DES exposure *in utero* and an increased incidence of genital tract malformations in adult women resulting in impaired reproductive perfomance was later discovered [29].

One of the known targets of DES action is the Homeobox (*HOX*) gene family [29,30]. The *HOX* gene family is a group of genes that are essential for regulating development [31]. More specifically, *HOX* genes direct the development of the reproductive tract from the

undifferentiated Müllerian and Wolffian ducts [32–35]. *HOXA10*, a component of the *HOX* gene family, directs embryonic development of the uterus and is expressed in the adult endometrium, where it plays an essential role in embryo implantation [36–38]. *HOXA10* is expressed specifically in the uterine epithelial and stromal cells [31], and its expression is regulated by sex steroids [39]. The transcriptional regulation of *HOXA10* expression is also altered by DES [29] through aberrant methylation of the promoter and intron [30]. Prolonged exposure to DES *in utero* induces a permanent epigenetic effect on *HOX* gene expression through DNA methylation, as well as upregulation of DNMTs persisting beyond fetal development and into adulthood [30].

Another EDC commonly found in the environment is BPA. BPA is a key monomer in the production of epoxy resins and in the most common form of polycarbonate plastic. Polycarbonate plastic is used to make a variety of products such as baby bottles, food containers and dental fillings [40]. BPA has been found in 95% of urine samples in a human reference population, suggesting widespread exposure and a ubiquitous presence in the environment [41]. Release of BPA and thus exposure occurs as bottles and other containers are repeatedly reheated or sterilized [42,43]. BPA leaches from the sealants used in almost all canned goods. Exposure also occurs as BPA is released from dental sealants and composites during the period immediately following placement [44].

Bisphenol-A is a weakly estrogenic compound [45]. It is through this estrogenic activity that we presume BPA affects rodent reproductive tract development; examples include advanced puberty [45], altered mammary development, higher risk of breast and prostate cancer [46] and altered reproductive function [47]. Moreover, BPA enters the placenta and accumulates in rodent fetuses after maternal exposure [48]. In humans, exposure to BPA has been linked to recurrent miscarriage [49] and decreased BPA serum levels are associated with complex endometrial hyperplasia [50]. Aside from reproductive effects, BPA exposure correlates with obesity, diabetes and heart disease in humans [51].

Bisphenol-A is similar to DES in that it is an estrogenic compound and alters expression of *HOXA10* after *in utero* exposure. While *in utero* treatment with DES decreases adult *HOXA10* expression, BPA increases it. The mechanism underlying this altered expression that persists long after exposure is epigenetic. BPA exposure results in decreased methylation, while DES exposure leads to increased methylation compared with controls [52]. *In utero* exposure to BPA results in aberrant methylation in both the promoter and intron of *Hoxa10* [53]. Exposure of adults, however, failed to result in altered methylation of *Hoxa10*, suggesting that epigenetic modifications from BPA exposure occur only during a critical developmental window [53]. The paradoxical methylation pattern after exposure to DES and BPA explains variable develop mental consequences of estrogenic compounds and suggests that the epigenetic alterations are independent of estrogenicity.

Areas of altered methylation include the *Hoxa10* estrogen response element (ERE), and thereby affect estrogen response of the adult. DES exposure increased ERE methylation, while BPA exposure decreased ERE methylation. Hypomethylation of the *Hoxa10* ERE results in enhanced binding of estrogen receptor and increased transcription, subsequently leading to a hypersensitivity to estrogens as adults [53]. Conversely, hypermethylation of the ERE by DES reduced estrogen receptor binding and estrogen-driven transcription. Response as an adult depended on estrogen exposure of that animal as a fetus. Fetal exposure to a strong estrogen such as DES results in a limited estrogen response, while exposure to a weak estrogen such as BPA results in an increased estrogen response in the adult. As described by Barker, the fetus modifies its DNA to respond to the environment as an adaptive response. The DES-exposed fetus perceives an environment with excessive estrogen and dampens its

response to estrogen exposure throughout life. The opposite is true of BPA; the fetus perceives a low-estrogen environment and heightens its subsequent sensitivity to estrogens.

The impact that EDCs can have on development and disease in humans is exemplified by the effect of DES on female reproduction and cancers. DES is a profound example of how physicians must take care in introducing new exposures during fetal development, as there could be consequences to future adult health. This can constitute an enormous obstacle for obstetricians and gynecologists, as there are numerous challenges in associating EDC involvement with a particular disorder. Environmental and lifestyle exposures may regulate fetal epigenetic modification; however, data are still limited. However, as associations with EDCs and epigenetic effects come to light, we must be vigilant and vigorously attempt to protect the developing fetal epigenome from harm.

Maternal nutrition & epigenetic changes

In addition to EDCs, early nutrition can have a profound impact on the developing fetal epigenome. A classic example on how diet can alter epigenetics is the Dutch winter famine of World War II [54]. A German embargo of The Netherlands in 1944 resulted in an abrupt decrease in daily rations to below 1000 calories. At the height of the famine from December 1944 to April 1945, the official daily rations varied between 400 and 800 calories [54]. After removal of the embargo in May 1945, an abundance of food resulted in an abrupt rise of daily rations to almost 2000 calories per day [54]. Fortunately, during the famine midwives and doctors continued to offer obstetric care and kept detailed records on 2414 births [54]. Studies on this population have shown that fetuses exposed to famine early during gestation subsequently experienced an increase incidence of coronary heart disease and obesity as adults [55]. The individuals who underwent fetal development during the famine compared with same-sex siblings born after this time had hypomethylated growth-regulatory regions in the genome [56]. Numerous more recent studies have correlated coronary heart disease risk factors with birthweight. Furthermore, numerous controlled animal models of altered nutrition in pregnancy support the same conclusions as human correlative studies. Taken together, these findings further support the Barker hypothesis and the developmental origins of disease.

The fetal epigenome is not only shaped by the maternal caloric intake but also by dietary contents. In order for DNA methylation to occur, dietary methyl groups are needed for methylation reactions catalyzed by DNA methyltransferases [57]. Therefore, dietary methyl donors and cofactors must be available during critical developmental periods when they can influence DNA methylation patterns [58]. Food micronutrients such as choline, vitamin B_{12}, folic acid and betaine are used as methyl donors for the enzymatic methyl ation reactions [40]. Additional nutrients likely have a significant role in regulating epigenetic modifications beyond simply serving as methyl donors. This is currently an active field of investigation.

One of the most widely investigated nutritional supplements with a profound epigenetic impact on fetal development is folic acid. Daily consumption of 400 μg of folic acid prior to conception and during early pregnancy has been shown to significantly reduce the incidence of neural tube defects (NTDs) [59]. Folic acid deficiency causes hypomethylation of DNA secondary to the need for 5′-methyltetrahydrofolate to produce *S*-adenosylmethionine and, consequently, the methylation of DNA [60]. Methylation is also necessary for the synthesis of myelin basic protein and neurotransmitters, as well as the establishment of gene expression during fetal development [61]. Maternal consumption of the recommended amount of folate has been demonstrated to result in lasting methylation change after birth [62]. The US Preventive Services Task Force recommends that women of reproductive age take a supplement containing 400–800 mg of folic acid every day to reduce their risk of

having a child with a NTD [63,64]. The recommended daily intake for women with a previous pregnancy with a NTD is currently 4 mg per day [65].

The medical community has enormous potential to alter the epigenetic outcomes of the developing fetus through adequate counseling on nutrition prior to and during pregnancy. Nutrition counseling is an integral part of perinatal care and should focus on well-balanced dietary intake supplemented with folic acid-containing prenatal vitamins. Since the US Public Health Service mandated the fortification of foods with folic acid and recommended folic acid supplementation in all reproductive-age females [65], the incidence of NTDs has decreased significantly [66]. A 27% decline has been noted; however, the overall goal set forth by the US Public Health Service is a 50% reduction [66]. In order to reach this manageable goal, medical personnel caring for pregnant women must increase efforts to initiate folic acid supplementation in all women capable of reproduction. According to a Gallup poll published in early 2008, both knowledge and consumption of folic acid supplements is inadequate [67]. As late as 2007, only 6% of females between 18 and 24 years of age knew of the need for folic acid supplementation during pregnancy and only 40% of all women surveyed (18–45 years of age) reported folic acid supplementation [67]. Perhaps more concerning is the finding that only 33% of women who were aware of folic acid reported that they had received counseling from their healthcare provider regarding the need for folic acid [67]. These data suggest the need for more aggressive counseling regarding folic acid supplementation and provides an opportunity for all physicians involved in women's healthcare to protect the fetal epigenome.

Additional nutrients such as choline, vitamin B_{12} and betaine are needed for optimal methylation. Lower risk of NTDs have been associated with increased availability of these compounds. Further research will likely raise awareness of the full spectrum of agents needed for optimal methylation. We anticipate that future dietary recommendations during pregnancy will stress the need for multiple supplements to ensure correct epigenetic programming of the fetus.

Environmental toxins & occupational hazards, & epigenetics

In addition to EDCs and nutrition, numerous studies have shown a link between epigenetic changes and environmental metals. Lead is a known toxicant [68]. Fetal lead exposure can occur through concurrent maternal exposure or through the increased mobilization of maternal bone lead stores during pregnancy [69]. Lead diffuses across the placenta [70] and is associated with spontaneous abortions [71], low birthweight [72] and abnormal development of the CNS [70]. Pilsner *et al.* presented evidence of global DNA hypomethylation in mothers with elevated bone lead stores [68]. Metal-induced oxidative stress is thought to be the mechanism accounting for altered methylation [73]. Metals can cause oxidative DNA damage resulting in interference with the ability of methyltransferases to interact with DNA, thus causing altered methylation of cytosine residues [74].

Overexposure to lead continues to be an important health problem worldwide [75]. The overall exposure to lead has decreased tremendously in the USA since the 1990s following efforts by industry and government to prevent toxic lead exposure [75]. Secondary to this marked decline in lead exposure in the USA, many healthcare providers have neglected or minimized screening for lead exposure in the female reproductive population [76]; however, certain populations are still at risk for exposure. Lead paint was commonly used prior to 1955 and is still present in up to 70% of houses constructed prior to 1960 [77]. Deterioration can release lead into the surrounding air and soil. According to a study carried out between 2000 and 2001, more than 57% of public school buildings in Philadelphia (PA, USA) had water lead levels exceeding the Environmental Protection Agency's recommended level [78]. Lead exposure has also been documented to occur from pica, the eating of nonfood

substances such as clay or starch [79]. Pica in pregnancy occurs most often in women in low socioeconomic groups and in rural areas [78]. Given the persistent presence of lead in the environment, physicians should counsel patients to avoid exposure to certain environments and monitor risks for lead exposure, in order to prevent lead-induced damage of the fetal epigenome.

While exposure to some environmental toxins, such as lead, is inadvertent, certain toxins are presented to the developing epi genome intentionally. For instance, tobacco use is one of the most important modifiable risk factors associated with adverse pregnancy outcome. Tobacco use has been associated with 30% of small-for-gestational-age infants, 5% of infant deaths and 10% of preterm infants [80]. Low-birthweight infants in general are at increased risk for experiencing chronic disease as adults [3]. Therefore, tobacco use has the potential for a profound adverse health outcome. One proposed mechanism for the long-term effects of tobacco use is DNA methylation. Breton *et al.* showed global and gene-specific DNA hypomethylation in those exposed to cigarette smoke *in utero* [81]. Efforts to prevent smoking and increase cessation prior to pregnancy have been unsuccessful [75]. Pregnancy is a critical time to encourage smoking cessation, and counseling and smoking cessation programs have been beneficial in promoting smoking cessation during pregnancy [82]. Studies of prenatal care providers indicate that only 30–60% provide interventions or referrals to these programs for women smoking during pregnancy [81]. Healthcare providers caring for reproductive-age women need to increase efforts to prevent or cease the exposure of the developing fetus to cigarette smoke.

Conclusion

The fields of epigenetics and developmental origins of disease are relatively new. There is still much to learn in order to fully understand environmental interaction with the developing fetus. Each disruptor is likely to have differential and perhaps specific effects on the epigenome. Similarly, each developmental process is distinct and likely to be differentially affected. Furthermore, many of the medical treatments currently used in pregnancy may have epigenetic consequences; therefore, we must weigh the benefits of these treatments against any potential for future harm.

The study of epigenetics has brought tremendous potential for developing biological markers to identify those at risk for certain diseases and perhaps prevent them. For the time being, physicians must use available information to protect the develop ing fetus. Even a short exposure time during this critical time of epigenomic programming will have a lasting impact. We must continue to focus on modifying maternal behavior, including smoking cessation, avoiding environmental exposures and proper dietary intake, to ensure that the next generation will not suffer the consequences. As further advances are made in the field of epigenetics, we must take special care in screening for these exposures and counseling patients in order to protect the fetus from epigenetic harm that will be carried throughout life.

Expert commentary

Since the first suggestion of the developmental origins of disease, numerous interactions between the maternal environment during pregnancy and the developing fetal epigenome have been identified. Chemical and environmental exposures can have negative repercussions on epigenetic programming. Obstetricians and gynecologists have the potential to intervene on behalf of the fetus in order to prevent exposure to epigenetic disruptors that are disseminated in the environment. In this article, we suggest an increased

need for counseling and intervention by obstetricians and gynecologists in an attempt to prevent epigenetic changes in the fetus that will affect the offspring throughout life.

Five-year view

The full extent of maternal environment interactions and resulting epigenetic changes has yet to be established. Over the next 5 years, the field of environmental epigenetics and the developmental origins of disease will mature. Appropriately designed studies are needed to identify potential causes of epigenetic alterations and uncover therapeutic options to counteract adverse modifications of the epigenome. As this information becomes available, there will be increasing opportunities for intervention by obstetricians and gynecologists to prevent adverse epigenetic alterations and their consequences.

Key issues

- Nutritional and environmental exposures *in utero* are thought to play an important role in developmental variation and disease susceptibility through epigenetic mechanisms.
- Poor maternal nutrition during pregnancy has been linked to cardiovascular disease, Type 2 diabetes and hypertension in the adult life of the fetus.
- *In utero* exposure to endocrine-disrupting chemicals, such as bisphenol-A and diethylstilbestrol, is associated with hormone-related cancer, altered reproductive potential and abnormal reproductive development.
- Maternal nutritional supplements, such as folic acid, are essential for normal epigenetic development and obstetricians/gynecologists should counsel women on the need for these supplements.
- Environmental toxins are ubiquitous and can affect the fetal epigenome, and screening for exposure is particularly important in females of reproductive age.
- Tobacco use can result in epigenetic changes associated with small-for-gestational-age infants, preterm delivery and infant death; appropriate counseling on tobacco use by obstetricians and gynecologists can result in smoking cessation and improved pregnancy outcomes.
- Obstetricians and gynecologists have an enormous potential to influence the incidence of both childhood and adult disease with appropriate maternal screening and counseling.

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