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Environmental factors, epigenetics, and developmental origin of reproductive disorders

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ABSTRACT

Sex-specific differentiation, development, and function of the reproductive system are largely dependent on steroid hormones. For this reason, developmental exposure to estrogenic and anti-androgenic endocrine disrupting chemicals (EDCs) is associated with reproductive dysfunction in adulthood. Human data in support of "Developmental Origins of Health and Disease" (DOHaD) comes from multigenerational studies on offspring of diethylstilbestrol-exposed mothers/grandmothers. Animal data indicate that ovarian reserve, female cycling, adult uterine abnormalities, sperm quality, prostate disease, and mating behavior are susceptible to DOHaD effects induced by EDCs such as bisphenol A, genistein, diethylstilbestrol, *p,p′*-dichlorodiphenyl-dichloroethylene, phthalates, and polyaromatic hydrocarbons. Mechanisms underlying these EDC effects include direct mimicry of sex steroids or morphogens and interference with epigenomic sculpting during cell and tissue differentiation. Exposure to EDCs is associated with abnormal DNA methylation and other epigenetic modifications, as well as altered expression of genes important for development and function of reproductive tissues. Here we review the literature exploring the connections between developmental exposure to EDCs and adult reproductive dysfunction, and the mechanisms underlying these effects.

1. Introduction

In recent years, significant insights have been gained in our understanding of the critical roles of steroid hormones and other morphogens in orchestrating development, differentiation, and

Abbreviations: BPA, bisphenol A; CpG, 5′-C-phosphate-G-3′; DDT, dichlorodiphenyltrichloroethane; DEET, *N,N*-diethyl-*meta*-toluamide; DEHP, bis(2-ethylhexyl)phthalate; DES, diethylstilbestrol; DNMT, DNA methyltransferase; DOHaD, Developmental Origins of Health and Disease; E, embryonic day; EDC, endocrine disrupting chemical; Endo-siRNA, endogenous small interfering RNA; ER, estrogen receptor; EZH2, enhancer of zeste 2; F, filial; GD, gestational day; GPER1, G-protein coupled estrogen receptor 1; HDAC, histone deacetylase; Hmgn5, high mobility group nucleosome biding protein 5; HOX, homeobox; H3K4me3, trimethylated histone 3 lysine 4; H3K9ac, acetylated histone 3 at lysine 9; H3K9me2, dimethylated histone 3 at lysine 9; H3K27me3, trimethylated histone 3 at lysine 27; H4K5ac, acetylated histone 4 at lysine 5; miR/miRNA, microRNA; NIEHS, National Institute of Environmental Health Sciences; Nsbp1, nucleosome binding protein 1; PAH, polycyclic aromatic hydrocarbon; PBDE, polybrominated dibenzoether; PBE, polybrominated diphenylether; PCB, polychlorinated biphenyl; PCDF, polychlorinated dibenzofuran; piRNA, PIWI-interacting RNA; PND, postnatal day; pp′-DDE, *p,p′*-dichlorodiphenyl-dichloroethylene; SAM, S-adenosyl methionine; Six1, sineoculis homeobox homolog 1; sncRNA, small noncoding RNA; TCDD, 2,3,7,8-tetrachlorodibenzodioxin; TET, ten-eleven translocation; WNT, wingless type MMTV integration site family.

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maturation of the reproductive system. These findings explain the exquisite sensitivity of the reproductive system to disruption by molecules that either mimic or disrupt steroid hormone actions. What remains to be uncovered are the long-term consequences of "environment by cell" and "environment by genome" interactions during critical developmental windows of the male and female reproductive systems and the mechanisms that govern these changes.

Here we discuss the concept of windows of sensitivity to developmental disruption and the "Developmental Origins of Health and Disease" (DOHaD) hypothesis. We next review evidence from both human and animal studies that demonstrate developmental origins of adult reproductive dysfunction, and include detailed tabular summaries of this information. Examples of studies documenting mechanisms by which environmental exposures can lead to different types of epigenetic modifications to mediate DOHaD effects are provided. Finally, we review reports exploring the concept of transgenerational epigenetic inheritance of environmental exposures, and point out areas of research ripe for future exploration.

1.1. Timing of reproductive system development

Development of the mammalian reproductive system begins in early pregnancy with specification and migration of germ cells, followed by morphogenesis of the gonads, reproductive tract structures, and external genitalia. As the reproductive tissues form, they differentiate under the influence of numerous molecules including growth factors, transcription factors, and steroid hormones. Gross morphogenesis of reproductive tissues is largely complete before birth, but slow growth and regional and cellular differentiation continue through the onset of puberty. During puberty, a rapid phase of growth and additional structural and cellular reorganization occurs, regulated in large part by steroid hormones.

Some temporal aspects of reproductive system differentiation are distinct in females and males. For example, female germ cells enter meiosis prenatally and complete the initial phases of meiosis before birth, whereas male germ cells only begin to enter meiosis postnatally and continuously do so throughout adulthood. The protracted time period of reproductive system formation, growth, and differentiation creates a wide window of susceptibility to disruption by environmental factors, and because of differences in timing of specific developmental events, this window differs in some aspects between females and males.

1.2. Developmental origin of adult diseases – windows of susceptibility

The DOHaD hypothesis proposes that the environment an individual experiences during early development, can affect their sensitivity to, or risk of developing, disease later in life [1]. During development, dynamic interplay between the genome, epigenome, and stochastic and environmental factors contributes to the fate of individual cells to form functional organ systems in a "developed" adult state with stably differentiated tissues. That these tissue systems are stably, rather than terminally differentiated, allows for continual maintenance of a critical balance between cell death and proliferation, regeneration, and repair [2]. Most cells or organs have various degrees of phenotypic plasticity, whereby the phenotype expressed by a genotype is dependent on environmental influences [3]. The principle that the nutritional, hormonal, and metabolic environment afforded by the mother may permanently program the structure and physiology of her offspring was established long ago [4]. The DOHaD theory has now advanced to extend the critical developmental temporal windows of tissue reprogramming beyond *in utero* development to include preconception, perinatal, neonatal, postnatal, and pubertal development [5] (Fig. 1). These adaptive traits are usually beneficial to the health of the individual. However, exceptions arise when an individual who is developmentally adapted to one environment is exposed to a contradictory environment [6]. Such exposures include the introduction of new chemicals and pollutants, which may increase the risk of developing disease later in life.

A prime example is the strong correlation observed between gestational exposure to diethylstilbestrol (DES) and increased female and male reproductive tract structural anomalies including a rare form of cancer, an increased infertility rate and poor pregnancy outcomes in female offspring, and an increased incidence of genital abnormalities and possibly urological cancers in male offspring [7–9]. Fetal exposure to environmental chemicals with estrogenic or anti-androgenic action can disrupt testosterone synthesis and sexual differentiation, leading to adult testis dysfunction and infertility [10–13]. In addition, exposure to endocrine disrupting chemicals (EDCs) during fetal life disrupts female reproductive tract development by altering expression of genes encoding secreted signaling proteins critical for directing this process [14]; these effects have permanent consequences for reproductive tract morphology and function in both rodents and humans [15,16].

In summary, many of the developmental differentiation events critical for reproductive function, are dependent at least in part on steroid hormone signaling [14,17–20]. For this reason, exposure to environmental EDCs, during this critical window of reprogramming, may induce profound changes in regulatory signaling pathways, and have a significant impact on development in ways that affect later reproductive health [21]. This concept of DOHaD could easily be extended to other windows of susceptibility, although evidence from epidemiological, clinical, and experimental studies remains sparse for these windows.

1.3. Epigenetics – as a mechanism shaping DOHaD

Epigenetic modifications are defined as heritable changes in gene function that occur without a change in the nucleotide sequence [6,22–24]. In the context of DOHaD, epigenetics can be viewed as an important "biostat" that allows an organism or a tissue to switch on or off anticipatory gene transcription programs in response to environmental changes, leading to adaptive phenotypic alterations to enhance survival. Gene transcriptional programs are changed in both a functional and temporal context as immediate and long-term responses to environmental cues. DNA methylation, histone modifications, transcription of new micro- and long non-coding RNAs, and other higher order chromatin remodeling events establish new adaptive traits for the tissue or organism. These epigenetic modifications are generated, maintained, and removed by a class of proteins known as "chromatin modifying enzymes". The expression of these enzymes is exquisitely sensitive to specific environmental changes. Conversely, undesirable inherited or sporadic epimutations [25], or dysregulation of the epigenome in a tissue by harmful environmental disruption, could lead to disease development.

The most well studied epigenetic modification to DNA is methylation of cytosine residues in the context of a CpG (5′-C-phosphate-G-3′) dinucleotide. Methylation of CpG rich regions of DNA generally confers relatively stable silencing of gene expression, whereas unmethylated CpG regions are more accessible to transcription factor binding, which leads to gene transcription [26]. DNA methyltransferases (DNMTs) are primarily responsible for placing methyl groups on CpG dinucleotides, whereas the ten-eleven translocation (TET) family proteins remove methyl groups. DNMT1 is primarily responsible for maintaining CpG methylation once these marks have been established. DNMT3A and DNMT3B carry out *de novo* DNA methylation, which is important in embryo and tissue development as well as differentiation [27,28].

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