Endocrine disrupters, microRNAs, and primordial germ cells: a dangerous cocktail

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Endocrine-disrupting chemicals (EDCs) are environmental pollutants that may change the homeostasis of the endocrine system, altering the differentiation of germ cells with consequences for reproduction. In mammals, germ cell differentiation begins with primordial germ cells (PGCs) during embryogenesis. Primordial germ cell development and gametogenesis are genetically regulated processes, in which the posttranscriptional gene regulation could be mediated by small noncoding RNAs (sncRNAs) such as microRNAs (miRNAs). Here, we review the deleterious effects of exposure during fetal life to EDCs mediated by deregulation of ncRNAs, and specifically miRNAs on PGC differentiation. Moreover, the environmental stress induced by exposure to some EDCs during the embryonic window of development could trigger reproductive dysfunctions transgenerationally transmitted by epigenetic mechanisms with the involvement of miRNAs expressed in germ line cells. (Fertil Steril® 2016;106:871–9. ©2016 by American Society for Reproductive Medicine.)
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ENDOCRINE DISRUPTORS

Every day human and animal populations are environmentally exposed to a great and diverse number of external substances. The exposure to these natural or synthetic compounds could alter many physiologic processes during development and/or adult life. Specifically, exposure to such compounds may impair the reproductive system, which negatively impacts fertility. A particular group of substances are called endocrine-disrupting chemicals (EDCs). EDCs are defined as chemicals that may interfere with the body's endocrine system inducing adverse developmental, reproductive, neurologic, and immune effects in both humans and wildlife animals (1). To date, hundreds of compounds are considered EDCs, and thousands of others suspected to have similar properties have been identified. EDCs include a very broad repertoire of natural and man-made substances found in daily products, such as plastic food bottles, food cans, detergents, flame retardants, toys, cosmetics, and pesticides. Studies in human populations are limited, but there have been a plethora of studies performed in cells and animal models that suggest the potential adverse effects of EDCs in human health (2–4). EDCs have broad effects on the endocrine system, but specifically the exposure of EDCs leads to reduced fertility, increased risk of obesity, diabetes, endometriosis, and some types of cancer (2, 5, 6).

We are particularly interested in the effect of EDCs in reproduction. Reproductive homeostasis depends on a highly regulated interaction between organs, timing, stage of development, and hormone doses. In this sense, EDCs could act as either agonists or antagonists of the steroidal sex hormones, estrogens and androgens, and this action could be at any age including (and particularly importantly) embryonic stages (7, 8). Fetal and adult gonads are targets of EDC action (5).

Abundant reports showing the effects of EDCs on the testis and its effects along different generations have pointed out the relevance of these compounds and their deleterious action upon fertility and cancer (2, 9). In 1996, Toppari et al. (10) reported a significant decline in the quality and quantity of semen of different human populations when considering the previous 5 decades. This work also

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reported a 2% to 4% annual increase in testicular cancer that was diagnosed in men less than 50 years of age in developed countries. The impact of EDCs, however, is still unclear due to the wide range of possible mechanisms for EDC action, levels of EDC exposure, mixture of chemicals potentially acting as EDCs, and the genetic sensitivity of individuals or populations to the compounds. The effects from EDCs on germ cells may not only affect the exposed individual but can also be inherited and potentially influence the phenotype of subsequent generations by epigenetic mechanisms. In this review we analyze the effects of EDCs in primordial germ cells (PGCs) through small noncoding RNAs (sncRNAs), with a focus on miRNAs and the potential transgenerational epigenetic effects.

PRIMORDIAL GERM CELLS

In mammals, PGCs are the embryonic precursors of the germ cell lineage, which are restricted to form spermatozoa and oocytes after their specification from pluripotent cell stages (11). PGCs colonize the embryonic gonads through active migration where they start their differentiation into a male or female pathway (12). In the mouse, PGCs first become identifiable as a cluster of approximately 40 cells at the base of the incipient allantois at around embryonic day 7.25 (E7.25). PGCs migrate to the developing hindgut endoderm at E7.75, into the mesentery at E9.5, and colonize the genital ridges at E10.5 (12). Primordial germ cell specification, migration, and division are controlled by a series of genes, of which the most relevant are *Blimp1*, *Prdm14*, and *Tcfap2c* (13). *Blimp1* (B-lymphocyte-induced maturation protein 1) is a transcriptional repressor that participates in the initial specification of PGCs repressing their somatic program (14, 15). *Blimp1* interacts with many distinct epigenetic regulators, including several histone deacetylases (16). *Prdm14* is transiently expressed in the inner cell mass (ICM) and later only in the germline until E13.5 (17). *Prdm14* permits the reactivation of pluripotency and also controls the methylation status of histones. The third factor is *Tcfap2c* (*AP2γ*), which is expressed in PGCs from E6.75 up to E12.5–E13.5 (18, 19). *Tcfap2c* permits the migration and maintenance of the PGC population. Thus, *Blimp1*, *Prdm14*, and *AP2γ* contribute to PGC specification, both individually and combinatorially (13).

After specification PGCs migrate, and it is at this particular time of development that PGC fate is regulated by a variety of growth factors and cytokines including BMPs (bone morphogenic proteins), Kit ligand (Kitl), and Sdf1 (stromal cell derived-1) (20). In addition to *Blimp1*, *Prmd14*, and *Tcfap2c*, the development of PGCs also requires the RNA-binding factor Lin28, which binds to a specific microRNA (miRNA) precursor: the *let-7* pri-miRNA thus preventing its processing into mature forms of *let-7* miRNAs. In the absence of Lin28, *let-7* miRNAs are overexpressed in PGCs and bind to the 3′UTR (untranslated region) of the Blimp1 mRNA, which blocks its translation and inhibits PGC development (21).

A key epigenetic event during PGC proliferation is a genomewide DNA demethylation that starts around E7.5 and reaches the lowest levels of methylation at E13.5 (22–25). After the repression of DNA methylation, the loss of 5-methylcytocine (5mC) along the genome occurs through replication-coupled dilution (23, 24) and by a conversion of 5mC to hydroxymethylcytosine (5hmC) by TET enzymes (26–28). At the same time other important epigenetic changes occur such as the depletion of H3K9me2, enrichment of H3K27me3, and X-chromosome reactivation (11, 29, 30). However, a limited number of genes escape this process (21), suggesting that changes in those genes could be transmitted to the next generation. Recently, studies from the Surani, Clark, and Qiao laboratories have analyzed the methylation changes in human PGCs. The results from their studies indicate that some elements in human PGCs, such as retroelements and loci associated to metabolic and neurologic disorders, are resistant to DNA demethylation (26, 31, 32).

Taken together, we posit that to have a healthy population of PGCs, the embryo needs not only to maintain a balance between the correct timing and expression of factors such as *Blimp1*, *Prdm14*, and *Tcfap2c*, but also to have the correct loss of epigenetic marks in addition to a balance in the expression of *Lin28* and the microRNA *let-7*. The complexity of the processes involved in PGC development make this period of embryonic-fetal development a critical window for the disruption of the epigenetic profile by environmental substances such as EDCs.

MicroRNAs AND OTHER NONCODING RNAs

Our knowledge of the role of ncRNAs acting as posttranscriptional gene expression regulators is growing in complexity (33), including in germ cells and the reproductive system (3). Two classes of RNAs that lack protein-coding potential have been identified: long (lncRNAs) and small (sncRNAs). Among lncRNAs (usually over 200 nucleotides) the best known is Xist (X-inactive specific transcript), which acts in the silencing of the X chromosome by modifying the structure of the chromatin and the factors interacting in chromosome X of mammalian females during development (26, 34, 35). The sncRNAs class (approximately 18–35 nucleotides) includes microRNAs (miRNAs), Piwi-interacting RNAs (piRNAs), endogenous-small interfering RNAs (endo-siRNAs), as well as other types of small noncoding RNAs derived from tRNAs, rRNAs, and small nucleolar RNAs (snoRNAs) (36). However, the functional and biogenesis boundaries between the different types of ncRNAs are not fully defined. To date, the best known players in these regulatory complex mechanisms are the miRNAs (37).

The miRNAs (approximately 21–23 nucleotides), acting by RNA interference as posttranscriptional regulators of gene expression, play important roles in most developmental and cellular processes of eukaryotic organisms. Negative regulation of gene expression is performed by miRNAs via base-pairing with complementary mRNA sequences, usually at the 3′UTR of mRNAs. Interaction of miRNAs with their targets may inhibit translation and/or induce mRNA degradation. The high level of conservation of each functional miRNA across species (38) points out how important miRNAs