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Review

MicroRNAs: potential targets and agents of endocrine disruption in female reproduction

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Abstract: MicroRNAs are short non-coding RNAs that have been widely recognized as key mediators in the epigenetic control of gene expression and which are present in virtually all cells and tissues studied. These regulatory molecules are generated in multiple steps in a process called microRNA biogenesis. Distinct microRNA expression patterns during the different stages of oocyte and embryo development suggest important regulatory roles for these small RNAs. Moreover, studies antagonizing specific microRNAs and enzymes in microRNA biogenesis pathways have demonstrated that interference with normal miRNA function leads to infertility and is associated with some reproductive abnormalities. Endocrine disrupting chemicals such as Bisphenol A (BPA) are synthetic hormone mimics that have been found to negatively impact reproductive health. In addition to their direct effects on gene expression, these chemicals are widely implicated in the disruption of epigenetic pathways, including the expression and activity of miRNAs, thereby altering gene expression. In this review, the roles of microRNAs during mammalian oocyte and embryo development are outlined and the different mechanisms by which endocrine disruptors such as BPA interfere with these epigenetic regulators to cause reproductive problems is explored. (DOI: 10.1293/tox.2019-0054; J Toxicol Pathol 2019; 32: 213–221)

Key words: microRNA, endocrine disruptor, mammal, reproduction

Introduction

Reproduction is a complex process involving intricate and interconnected cellular and molecular pathways that come together to give rise to healthy offspring. One essential component of successful reproduction is the formation of viable oocytes and embryos in female animals. From a toxicological perspective, the complex signaling events involved, combined with the dependency on hormonal pathways, renders the female reproductive process particularly susceptible to the actions of endocrine disrupting chemicals (EDCs) such as Bisphenol-A (BPA). Recent studies have suggested that many actions of endocrine disruptors on reproductive processes are mediated by disruption or alteration of epigenetic pathways involving small RNAs known as microRNAs. This review will provide a brief overview of the potential roles of small RNAs in the regulation of cellular pathways during the formation and maturation of oocytes and the early stages of embryo development and discuss how endocrine disrupting chemicals may affect these important regulatory mediators, negatively affecting female reproductive success.

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Mammalian Oogenesis and Folliculogenesis

The ovarian follicle consists of an oocyte and a network of surrounding cumulus cells and is the site of several essential functional process for female reproduction. The formation of eggs, in a process known as oogenesis and the growth of ovarian follicles, called folliculogenesis are two interrelated processes that are responsible for the formation, growth, and maintenance of the unique cells (oocytes) that ultimately develop, after fertilization, into new members of a species[1] (Fig. 1). In mammals, oogenesis occurs only during fetal development and stops around the time of birth[2]. Folliculogenesis is the process that begins once the animal has reached sexual maturity, and involves the cyclical, coordinated growth and differentiation of granulosa and thecal cells that surround the oocyte and facilitate its maturation prior to ovulation[2]. The process involves the combined actions of peptide and steroid hormones acting on follicular cells. Multiple follicles are usually stimulated to grow and develop by hormones in any single estrous cycle, but a more limited number mature fully and undergo ovulation, while the other follicles from that cycle undergo atresia[3]. Once an

oocyte has matured and is ovulated, it becomes competent for fertilization, which normally occurs in the uterine tube.

Fertilization and Early Embryo Development

Fertilization of an oocyte by a spermatozoon, leads to resumption of meiosis, extrusion of a polar body and female pronucleus formation[4]. The male pronucleus from sperm then fuses with its female counterpart to form the diploid genome of the zygote, the first cell of an embryo. This embryo, termed a zygote then undergoes rapid mitotic proliferation and the cells double in number after each cleavage[4]. Ultimately, a blastocyst forms in successful pregnancies after 2–7 days in most mammals. Cleavage, compaction, the maternal to zygotic transition and embryonic genome activation are all important events that occur during this preimplantation developmental period. It is important to note that all of these embryonic developmental events after fertilization depend on proteins and RNA molecules that accumulate in the oocyte during folliculogenesis and maturation. As a result, factors which disrupt the normal events of folliculogenesis and oocyte maturation profoundly affect normal embryo development and overall female fertility. One major class of agents that affect both folliculogenesis and early embryonic development is known as EDCs, one of the most widely-studied examples of these is Bisphenol A.

Bisphenol A (BPA)

Bisphenol A (BPA: 4,4'-(propane-2,2-diyl)diphenol) is an organic phenol compound that is commonly used as an industrial plasticizer[5]. The most common route of exposure is ingestion and one recent study reported BPA in over 50 food groups with concentrations ranging from 0.2–106 ng/g[6]. Free forms of BPA are considered most harmful because they are lipophilic, hard to excrete, and possess significant estrogenic activity[7]. Biochemical changes including glucuronidation, sulfonation, sulfation, nitration, and alkylation can result in forms that are either estrogenically active or inactive[7]. BPA has been detected in serum, ovarian follicular fluid, urine, tissues, and umbilical cord blood[8]. The estrogenic activity of BPA is one of the primary concerns and its ability to mimic hormonal activity accounts for its classification as an endocrine disrupting chemical (EDC)[9]. This is consistent with the hypothesis that BPA generally affects ovarian and reproductive function more than other organs[10]. Countless genes that are involved in oocyte development are downstream of estrogen receptor (ER) signaling[10]. Two main types, ERα and ERβ are well recognized and crucial receptors that respond to estrogen and estrogen mimics to regulate gene expression, particularly during folliculogenesis[10].

BPA has been detected in human follicular fluid and is correlated with negative impacts on fertility. A study in 2018 by Cao and colleagues measured the levels of BPA concentrations in follicular fluid of women undergoing IVF treatment[11] and determined that BPA levels were comparable to estrogen levels in follicular fluids. An earlier study, also in humans, reported much higher concentrations of 1–2 ng/mL in premenopausal women[12]. BPA levels in women undergoing infertility treatment show a negative relationship with both number and quality of eggs retrieved[13]. *In vivo* studies show that BPA negatively impacts several aspects of oogenesis and has detrimental consequences in the oocyte[14]. In porcine oocytes, *in vitro* treatment with BPA modified important epigenetic regulators[15].

Epigenetics

The term epigenetics describes molecular pathways with the potential to alter the phenotype of an organism without changes in the genotype (DNA sequence)[16]. Epigenetic mechanisms are often categorized into three mechanistic groups: DNA methylation leading to transcriptional repression[16], histone and chromatin modifications that alter gene expression[16] and non-coding RNAs that modulate gene expression post-transcriptionally[16]. The first two mechanisms are highly important in early embryo development but lie outside the scope of this review and will not be described further. In this review, we have focused on specific non-coding RNAs known as microRNAs (miRNAs) and how the oocyte and embryonic pathways in which they participate are affected by endocrine disruptors.

Noncoding RNAs

Noncoding RNAs (ncRNA) encompass a wide range of long and short RNA molecules that serve different biological roles in essentially every cell type through the post-transcriptional regulation of gene expression. Types of ncRNAs include long non-coding RNAs (lncRNAs), piwi-interacting RNAs (piRNAs), short interfering RNAs (siRNAs), and microRNAs (miRNAs)[17] which are the focus of this review.

MicroRNAs

MicroRNAs are short endogenous RNA molecules ranging from 19–24 nucleotides in length that act post-transcriptionally to suppress gene expression through complementary binding to mRNA targets, usually in the 3'-untranslated region (UTR)[18]. First discovered in 1993 in nematodes (*Caenorhabditis elegans*) by Ambrose and colleagues[19], and subsequently implicated in RNA silencing[20], their importance has grown rapidly and steadily, and they are now recognized as important regulators in virtually every species and cellular system studied. Today, more than 38,000 miRNAs have been identified in over 250 species and are annotated in the miRBase version 22 database[21] that houses most miRNA sequences as well as predicted target information[18]. A single miRNA is capable of targeting hundreds of genes suggesting that small changes in specific miRNA levels can have multiple downstream effects on cellular functions. Many studies have reported specific miRNA expression and predicted targets and cellular con-

sequences through computational analysis. In addition, it has been widely suggested that miRNAs in biofluids have strong potential as biomarkers of specific disease states[22]. Fewer studies have addressed the actual roles of miRNAs in oocyte and early embryo biology and how environmental agents or toxicants may perturb these pathways. In order to better understand the potential effects in these systems a description of the relevant pathways for miRNA biogenesis and activity is warranted.

Generation of miRNA Precursors: Pri-miRNAs

The most common mechanism for the generation of miRNAs is through processing of longer precursor miRNAs known as primary miRNAs (pri-miRNAs) that are transcribed similarly to coding mRNAs by RNA polymerase II (Pol II) from genomic DNA[23]. The genes encoding pri-miRNAs can be intronic, meaning they are located within introns of coding genes, or intergenic, with structure similar to other genes but lacking an open reading frame. In either case, transcription of the pri-miRNA can be regulated by the promoter of the "host" coding gene, or independently through pri-miRNA specific promoters[23]. Interestingly, pri-miRNA encoding genes often cluster and are transcribed together giving rise to a single pri-miRNA containing multiple stem loop structures[24]. These genes are polycistronic and can be differentially processed to generate multiple mature miRNAs[24]. Others are monocistronic and are processed into only one mature miRNA. Studies suggest that miRNAs that originate from the same transcript potentially have similar targets and play similar functional roles; however, several downstream processing events occur between transcription and the final synthesis of a mature miRNA[24].

MicroRNA Processing

Newly synthesized pri-miRNAs are processed in the nucleus, then in the cytoplasm to generate functional miRNAs (Fig. 2). The canonical pathway is the most common biogenesis pathway and is dependent on several processing enzymes; pri-miRNAs are first processed by the "microprocessor" containing two proteins[25]. The first protein, DiGeorge Syndrome Critical Region 8 (DGCR8), acts as a guide that recognizes specific sequences within the pri-miRNA and recruits the second protein with ribonuclease III activity, Drosha[25], which cleaves both the 3' and 5' strands of pri-miRNAs producing a precursor miRNA (pre-miRNA) with a 3' overhang. This overhang facilitates subsequent processing steps[26].

After Drosha-mediated cleavage, a specific transport protein known as Exportin-5 plays a key role in miRNA biogenesis by shuttling pre-miRNAs to the cytoplasm via nuclear pores[27]. Once in the cytoplasm, pre-miRNAs are cleaved at hairpin structures by another RNAse III known as Dicer to form a mature double-stranded miRNAs[28]. Double stranded miRNAs are then loaded onto an Argonaute protein (AGO), which, with other accessory proteins forms an RNA induced silencing complex (RISC)[25]. The functional activity of RISC requires only one miRNA strand to act as a guide, so one strand is unwound and degraded using other cellular machinery[25]. Several other proteins act as regulators to these processing enzymes, such as TRA-RNA binding protein (TRBP), a protein activator (PACT), SMADs, p53, and even ERα[29, 30].

The non-canonical pathway of miRNA biogenesis involves different cooperative activities of the processing enzymes. Most commonly, non-canonical miRNAs are synthesized in a Drosha/DGCR8- independent but Dicer-dependent manner. These miRNAs can originate from several precursors including small nucleolar RNA (snoRNAs), short hairpin RNA (shRNAs), and tRNAs[31–33]. These 'pre-miRNA like' transcripts are readily exported to the cytoplasm by Exportin proteins and subsequently cleaved by Dicer into mature functional miRNAs.

Mechanisms of miRNA Targeting and Control of Gene Expression

Once pre-miRNAs are incorporated into the RISC complex, they bind to target messenger RNAs with either complete or partial complementarity, suppressing expression of the encoded protein by either inhibiting the translation or by destabilizing the mRNA target leading to decay[34]. miRNAs bind to specific complementary sequences on their targets known as seed sequences (usually 8 nucleotides long). These sequences form the basis of a number of bioinformatic target prediction assays; any mRNA that possesses a seed sequence complementary to a miRNA has the potential to be regulated by that miRNA and is therefore a putative target[35]. However, seed sequence alone is not the only factor necessary for mRNA regulation and potential targets are typically biologically validated using functional assays such as RNA interference (RNAi) techniques[35].

Important elements that influence miRNA activity on specific targets include abundance of miRNA, proximity, location and number of seed sequences, and the presence of other regulatory molecules such as RNA binding proteins (HuR, TTP). For example, higher levels of miRNAs against specific targets increase the repression of those transcripts[36, 37]. With regard to the seed sequences, microarray and site conservation analysis on miRNA-transfected HeLa cells has revealed little to no suppression for genes with only one seed sequence and an incremental increase in suppression linked to an increase in the number of seed sites[38]. The majority of genes targeted by miRNAs have seed sites located in highly conserved sequences within the 3' untranslated regions (3'UTR) of the mRNA although some have also been found in the 5'UTR and even occasionally in the open reading frame (ORF)[39]. Studies have suggested that binding to the 3'UTR is more efficient at repressing translation[38, 40] which may be attributable to the shorter distance between miRNA binding sites and mRNA translational machinery[38].

Several additional molecular factors are essential components of both miRNA mediated translational repression

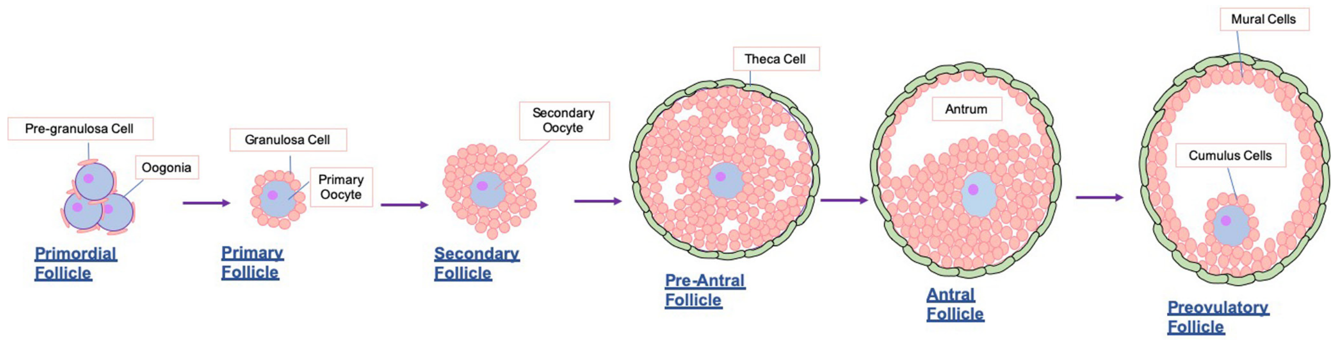

Fig. 1. Stages of follicular growth. Primordial germ cells recruited for oocyte differentiation grow and develop along with their associated somatic cells, the pre granulosa cells. Dominant follicles enter the antral stage where a fluid filled cavities form with the follicle and is linked to granulosa cell differentiation into mural cells that line the basement of the follicle membrane and cumulus cells that directly contact the oocyte. Modified after reference 2.

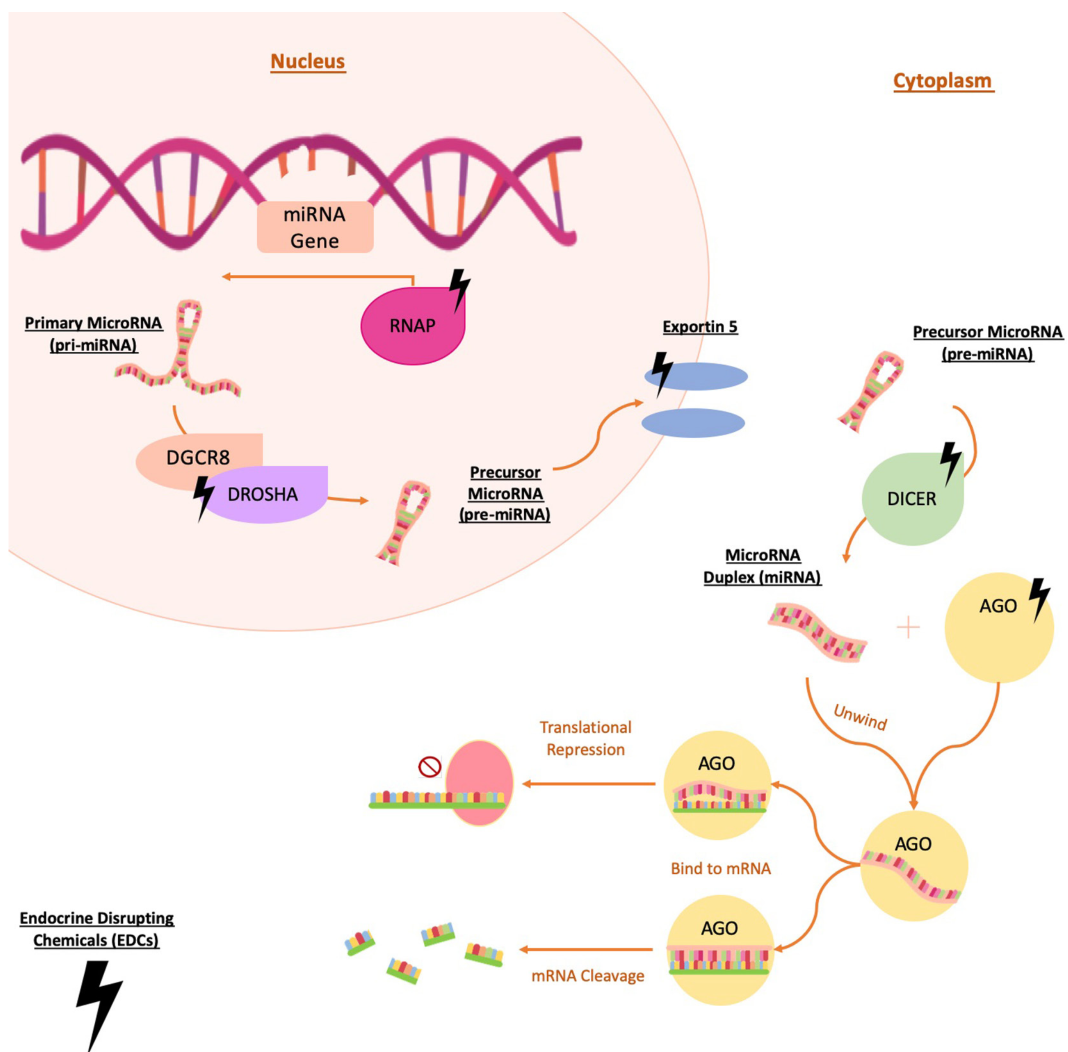

Fig. 2. MicroRNA biogenesis and function. miRNAs are transcribed into primary miRNAs (Pri-miRNA) that are processed by the enzyme Drosha to form the precursor miRNA (pre-miRNA) that is taken to the nucleus via an exportin 5 protein. The pre-miRNA is cleaved by Dicer and loaded onto a silencing complex (RISC/AGO complex). This complex binds with perfect or imperfect complementary target transcripts and either degrade transcripts or repress translation. Endocrine disrupting chemicals (EDC) may alter miRNA expression. EDCs can potentially interfere with the activity of RNA polymerase II, Drosha, Exportin 5, Dicer, and Argonaute proteins, thereby interfering with miRNA biogenesis as well as function.

and mRNA degradation. These proteins include coactivators of miRNAs, deadenylases, decapping enzymes (DCP1 and 2), exonucleases (XRN), and others[41]. Destabilization is initiated by deadenylation, followed by 5' decapping and decay by the exoribonuclease, XRN1[41]. This typically occurs in processing bodies known as GW bodies. AGO protein binds to these bodies thereby regulating miRNA activation, repression, and target destabilization[41]. Disrupted expression of any of these regulatory enzymes results in aberrant miRNA function.

MicroRNAs in Reproduction

MicroRNAs play important roles in virtually every biological system examined, including the female reproductive system; they have been implicated in key processes in ovaries, oocytes, cumulus cells, and preimplantation embryos[42–46]. Using both conventional multiple detection methods, significant differential expression of specific miRNAs has been detected in the ovary compared to other organs has been detected in several species[42–46]. These miRNAs also showed differential expression at different developmental stages of ovarian and follicular growth, implicating them in regulatory processes. In one example, knockdown of the key miRNA processing enzyme Dicer in the ovary adversely impacts folliculogenesis, oocyte maturation, and every aspect of ovarian biology[47]. Importantly, these effects are associated with infertility. miRNAs identified play various roles in the ovary, oocyte, and early embryo, including hormone production, cell cycle progression, cell fate determination, folliculogenesis, oocyte maturation, fertilization, and embryo development[48–50].

One important consequence of Dicer suppression is the failure of normal oocyte maturation due to meiotic malfunction, aberrant chromosome segregation, high spindle abnormalities and overall reduced maturation[51]. Knockout of Dicer in primordial germ cells (PGCs) has detrimental effects on the oocytes, including meiosis arrest due to chromosome misalignments and spindle abnormalities[52]. Identical results have been observed in mouse oocytes when AGO protein expression was suppressed instead of Dicer[53]. Progression female germ cells from PGCs to immature oocytes to mature oocyte cooincides with, and depends on, the generation of miRNAs. In the cow, there are several miRNAs with distinctive expression between immature and MII oocytes[53]. These results highlight the intricacy of miRNA regulation.

Several miRNAs and their functional roles in female reproduction have been previously reviewed: miR-494 and miR-143 are both involved in primordial follicle recruitment but have different routes of regulation. miR-494 targets phosphatase and tensin protein (PTEN) in human oocytes[54]. PTEN is essential for folliculogenesis; it inhibits the PI3K signaling pathway which in turn prevents recruitment of primordial follicles from the ovarian reserve. PTEN deficient mice cannot inhibit this pathway and all primordial follicles become activated resulting in premature ovarian failure[54]. miR-143, which increases at the same time primordial follicles are formed in mice, is also involved in follicle recruitment. Primordial follicle formation involves proliferation of pre granulosa cells which relies on signaling between cycle proteins (cyclins) and protein kinases. miR-143 transfection into mouse pre-granulosa cells suppresses these genes, thereby regulating recruitment and preventing premature follicle depletion[55].

MicroRNA-21 is an antiapoptotic miRNA abundantly expressed in both granulosa cells and oocytes in rats, pigs, cattle, and humans[56]. It enhances cell survival by targeting apoptotic genes such as PDCD4, PRC1, and CDC25a[56]. Studies in mice and pigs reveal granulosa cell apoptosis, meiosis disruptions, and repression of embryo development at the 4–8 cell stage in animals with suppressed miR-21[57]. In other studies, miR-29 and miR-203 were identified as epigenetic regulators during oocyte development that target DNMT3A and DNMT3B[58]. Overexpression of these miRNAs in mice suppressed these DNMTs, altered methylation patterns, and reduced oocyte quality[58]. This is one example of epigenetic mechanisms working cooperatively to control gene expression. Other examples of important microRNAs in human oocytes include miR-190b, miR-514, miR-144, and miR-513a-3p[51] which target the TGFβ pathway that controls normal folliculogenesis[51]. Importantly, these miRNAs are abnormally expressed in women with polycystic ovarian syndrome (PCOS)[59]. Regulatory functions have been identified for many additional miRNAs in granulosa cells. Specifically, miR-378 regulates synthesis of estrogen and aromatase and reduces oocyte maturation when overexpressed in granulosa cells within porcine cumulus oocyte complexes[60]. miR-125 is an important regulator of cumulus cell migration during preovulatory stages through targeting a Src family kinase (FYN) that is crucial for granulosa cell reorganization during folliculogenesis[61].

MicroRNAs in Preimplantation Embryos

After ovulation and fertilization, miRNAs are also involved in regulating processes within the early developing embryo as demonstrated with Drosha and Dicer knockout studies. Drosha suppression in both the male and female pronuclei reduces embryo development; whereas, Drosha deficient sperm pronuclei can still fertilize a wild type oocyte and produce a viable embryo[62]. This suggests that embryos may rely on maternally derived miRNAs more than those derived from the sperm. Dicer knockdown is lethal in mouse embryos, suggesting development relies on canonical miRNAs or endo-siRNAs that are also generated by this enzyme[63]. One miRNA specifically implicated in successful embryo development is miR-199. Using genetic knockdown and overexpression assays, miR-199 alterations were associated with an increase in developmental disruptions and embryo death[64]. Interestingly, comparisons of miRNA expression profiles between *in vivo* and *in vitro* produced embryos showed consistently lower expression of this miRNA in *in vitro* produced embryos[64]. Other studies have investigated miR-320[65], miR-125[66], miR-155 and miR-34c[67], implicating

them in different aspects of development.

Based on their widespread expression and importance in the developing embryo and gametes, miRNAs have clear potential as both targets of toxic injury and as participants in the pathways of toxic injury to the female reproductive system. This is particularly important in the context of embryo development, where complex and varied interactions of miRNAs appear to play key roles in developmental success, and minor changes in miRNA levels in the early embryo could ultimately compromise development.

Dysregulation of microRNA Expression by Environmental Factors

Multiple factors may interfere with or alter miRNA expression leading to changes in the regulatory control of gene expression. These include disruption of pri-miRNA transcription, miRNA processing pathways, endogenous factors (hormones), and exogenous factors (xenobiotics) (Fig. 2). In particular, hormone signalling is a major pathway involved in the regulation of miRNA expression. Estrogens, androgens, thyroid hormones, and corticosteroids are examples of relevant hormones in the reproductive system that can also modulate miRNA expression[68]. With respect to exogenous factors, environmental exposure to xenobiotics such as polyphenols, benzenes, and dioxin, have been shown to alter the expression of a number of important miRNAs[68, 69].

The observation that miRNA levels change in stressful, toxic, and disease conditions support their potential utility as biomarkers of toxic injury to cells. MicroRNA biomarkers typically consist of specific extracellular miRNAs found in bodily fluids including blood, plasma, serum, urine, and saliva. These miRNAs are typically carried in exosomal vesicles (EV) which contributes to a relatively high stability. Circulating miRNAs have been shown to have differential expression after toxic injury[70, 71]. While miRNA biomarkers of toxic injury may be useful for damage to liver, kidney and other somatic tissues, their relevance to the female reproductive system has not been tested and may be difficult to determine based on the limited availability of suitable tissue and fluids to be sampled. However, mechanisms of toxic injury to the reproductive system that involve changes in miRNAs and their downstream targets are promising areas of investigation. The remainder of this review will focus on potential pathways of toxic injury to female gametes and early developing embryos by the EDC BPA and how they may act through altering normal levels and activities of miRNAs.

MicroRNAs and Endocrine Disruptors

There is mounting evidence to suggest that estrogen signalling is a strong modulator of miRNA biogenesis[72]. Estrogen can not only modulate the expression of miRNA generation and processing factors such as RNA polymerase II and Drosha, but it can also directly modulate specific miRNAs. Some pri-miRNA genes contain estrogen response elements (EREs) in their promoters and can be regulated by the activity of estrogen receptors (ERα and ERβ). miR-21, -155, and -124 are only a few examples of many miRNAs with the potential to be modulated by estrogen[72]. Since BPA acts as an estrogen mimic, it is likely to interfere with the expression of multiple miRNAs. However, specific evidence of miRNA-mediated effects of BPA in reproductive models incorporating oocytes and embryos has not yet been widely described.

One informative study by Veiga-Lopez and colleagues treated sheep with BPA *in vivo* and analyzed the effects in ovarian genes known to be regulated by miRNAs[73]. They found decreased expression of 45 different miRNAs in the fetal ovary, as well as abnormal expression of associated target genes. For example, members of the let-7 family were shown to be repressed, leading to increased expression of the enzyme CYP19, a let-7 target that is required for estrogen synthesis. This suggests that a decrease in let-7 decreases CYP19 repression leading to an overproduction of steroids that is characteristic of BPA exposure. Interestingly, this same study reported no significant effect of BPA on specific miRNA processing enzymes (Drosha, Dicer, DGCR8, and AGO) suggesting that the effect of BPA on miRNAs in this case occurs at the transcriptional level prior to processing.

Another miRNA affected by BPA is miR-224, important for cumulus cell expansion and oocyte maturation. It was found to be dysregulated in rat granulosa cells treated with BPA *in vivo*. miR-224 is known to regulate crucial enzymes in hormone production including aromatase[74]. The simultaneous observation of aberrant expression of aromatase, estradiol, and FSH demonstrated that BPA-mediated interference with oocyte development may be in part due to induced changes in specific miRNAs.

As stated earlier, studies on the effects of BPA on microRNAs in the context of the ovary and oocyte development are limited and require more investigation, examples in other systems including other components of the female reproductive axis remain highly relevant on a comparative basis, as some of the miRNAs involved are also important in ovaries and oocytes. In human breast cancer cells, BPA induces ERα signaling that results in reducing miR-21, Let-7, miR-15b expression while enhancing miR-638, miR-663, and miR-1915 expression[75]. In human placental cells, miRNAs showed altered expression after BPA treatment[76]. miR-146a was notably increased and associated with slow proliferation and higher susceptibility to toxic effects of harmful agents[76]. miR-146a plays an important role in bovine oocyte maturation, as stated earlier, so BPA may also affect this miRNA within the oocyte environment. In human endometrial cells, BPA was shown to affect several miRNAs including miR-149 and miR-107 which were significantly suppressed and increased respectively[77]. miR-149 is predicted to control both DNA repair and cell cycle arrest genes, which were concurrently regulated by BPA and are among predicted targets. This may reveal an epigenetic mechanism of BPA that contributes to increased susceptibility to reproductive cancers.

The mechanisms by which EDCs affect gene expression in oocytes and embryos are likely not limited to microRNAs that directly target important genes but may also involve microRNAs that participate in epigenetic control[78]. One example can be found in the changes of miR-29 expression in testicular tissue treated with estradiol benzoate and a concomitant decrease in important DNMTs crucial for DNA methylation[78]. miR-29 was previously mentioned as a key regulator involved in human oocyte quality[59]; therefore, its dysregulation could have implications on oocyte competence. Like all estrogen-dependent pathways, BPA treatment could readily disrupt this axis and disturb downstream changes in genes that control oocyte competence.

Summary and Future Directions

The regulation of oocyte and embryo development are crucial hormone dependent events that are uniquely susceptible to toxic insult by EDCs that can inappropriately activate hormone pathways. While many of the resulting detrimental effects on fertility and development are probably the result of changes in canonical gene expression pathways, evidence is emerging to suggest that small RNA pathways involving microRNAs may also be key participants in the response to EDCs that contribute to their toxic effects. Further investigation and experimentation in this field will be crucial to identify the extent to which these pathways are important for EDC effects and whether active strategies to antagonize miRNA function might be employed.

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