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Bisphenol A exposure modifies methylation of imprinted genes in mouse oocytes via the estrogen receptor signaling pathway

Hu-He Chao · Xi-Feng Zhang · Bo Chen · Bo Pan · Lian-Jun Zhang · Lan Li · Xiao-Feng Sun · Qing-Hua Shi · Wei Shen

Abstract Bisphenol A (BPA), a synthetic additive used to harden polycarbonate plastics and epoxy resin, is ubiquitous in our everyday environment. Many studies have indicated detrimental effects of BPA on the mammalian reproductive abilities. This study is aimed to test the potential effects of BPA on methylation of imprinted genes during oocyte growth and meiotic maturation in CD-1 mice. Our results demonstrated that BPA exposure resulted in hypomethylation of imprinted gene *Igf2r* and *Peg3* during oocyte growth, and enhanced estrogen receptor (ER) expression at the levels of mRNA and protein. The relationship between ER expression and imprinted gene hypomethylation was substantiated using an ER inhibitor, ICI182780. In addition, BPA promoted the primordial to primary follicle transition, thereby speeding up the depletion of the primordial follicle pool, and suppressed the meiotic maturation of oocytes because of abnormal spindle assembling in meiosis I. In conclusion, neonatal exposure to BPA inhibits methylation of imprinted genes during oogenesis via the ER signaling pathway in CD-1 mice.

H.-H. Chao · B. Chen · B. Pan · L.-J. Zhang · L. Li · X.-F. Sun · W. Shen (✉)
Laboratory of Germ Cell Biology, Key Laboratory of Animal Reproduction and Germplasm Enhancement in Universities of Shandong, Qingdao Agricultural University,
Qingdao 266109, China
e-mail: shenwei427@163.com

X.-F. Zhang
College of Biological and Pharmaceutical Engineering, Wuhan Polytechnic University, Wuhan 430023, China

Q.-H. Shi
Hefei National Laboratory for Physical Sciences at Microscale and School of Life Sciences, University of Science and Technology of China, Hefei 230027, China

Introduction

In recent years, there has been a growing concern about the effects of bisphenol A (BPA) on human health. BPA is a synthetic additive used to harden polycarbonate plastics and epoxy resin present in many consumer products; furthermore, it can be released from industrial products by various physical or chemical processes and be absorbed through the human skin or inhaled as dust (Brotons et al. 1995; Calafat et al. 2005; Ikezuki et al. 2002; Schonfelder et al. 2002; Vandenberg et al. 2007; Yamamoto et al. 2001). However, exposure to BPA-contaminated food and water is the common route in humans. BPA exposure results in many adverse reproductive effects such as decreased epididymal weight, increased prostate size and androgen receptor binding activity in laboratory rodents (Nagel et al. 1997). Some studies suggest that BPA, as an endocrine disruptor, can cause germ cell malfunction. Prenatal exposure to low doses of BPA (20 μg/kg daily) from embryonic day 0.5 has been shown to perturb neocortical histogenesis and thalamocortical pathway formation (Nakamura et al. 2007; Nakamura et al. 2006). Meiosis is an important process for germ cell development and previous studies have shown prenatal exposure to BPA causes remarkable aberrations in the meiotic prophase, including synaptic defects and increased levels of recombination in the early stage of development, and these aberrations are

translated into an increase in aneuploid eggs and embryos (Hunt et al. 2003; Susiarjo et al. 2007).

Genomic imprinting, a specific epigenetic mechanism in mammals, plays a crucial role in the process of germ cell growth, development and function (Song et al. 2009). DNA methylation is an epigenetic regulator of gene expression and it acts as an important molecular marker for parental-specific expression of genes subjected to genomic imprinting (Reik and Walter 2001). In mice, the sex-specific epigenetic modifications are imposed during gametogenesis whereas it act as markers to distinguish the maternal and paternal alleles (Surani 1998). Insulin like growth factor II receptor (*Igf2r*) and paternally expressed 3 (*Peg3*) are imprinted postnatally from postnatal days (PND) 5 to 25 in the mouse, which is consistent with oocyte and follicle development. Previous studies suggested that there was a close relation between oocyte growth and the establishment of *Igf2r* and *Peg3*'s imprinting (Hiura et al. 2006; Song et al. 2009).

Oocyte growth and maturation are key processes of female gamete development. Many steroids, genes and related proteins are involved in the biological reactions of oogenesis and the maturation process. These factors are easily influenced by exogenous elements (Lutz et al. 2001; Smith and Ecker 1971). Moreover, during the fetal development period, the non-fully developed body, especially the genital system, is susceptible to improper exogenous elements. Whether such influencing factors cause heritable variation to germ cells is currently drawing more attention and many studies have focused on the epigenetic alterations induced by BPA exposure (Bromer et al. 2010; Dolinoy et al. 2007; Ho et al. 2006; Yaoi et al. 2008). We investigated the effects of BPA on DNA methylation on imprinted genes and its potential influence on oocyte development and several associated phenotype alterations. In addition, we preliminarily elucidated the mechanism of BPA action.

Materials and methods

Animals and experimental design

All procedures described in the study were reviewed and approved by the Ethical Committee of Qingdao Agricultural University. 110 CD-1 mice were used in Experiment 1, and 73 mice were used in Experiment 2.

On the basis of previous data from our laboratory, during postnatal days (PND) 7–14, imprinted gene *Igf2r* and *Peg3* were methylated from 52.67 to 81.33% and 33.33 to 76.67% respectively, and nearly fully methylated on PND 21 (Song et al. 2009). To assess the effect of BPA exposure on imprinted gene methylation, in Experiment 1, PND 7 to 14 female mice were given a weight-dependent hypodermi-

cal injection [lower than the "safe" dosage according to the Food and Drug Administration (FDA)] daily and were killed on PND 15. Mice in Experiment 1 were also used to detect DNA methyltransferase expression, as it was approximate the midpoint of *Igf2r* and *Peg3* gene full methylation. For the purpose of imitating the mode of human exposure to BPA (long-term and low dosage), a lower BPA exposure concentration was used in Experiment 2, in which mice were given a weight-dependent injection in a method similar to that of the mice in Experiment 1 from PND 5 to 20 every 5 days (PND5, PND10, PND15 and PND20 respectively). The mice in Experiment 2 were killed on PND 21, and most of the mouse oocytes were prepared for fertilization. For each experiment, three different treatment groups were treated with three different concentrations of BPA (0, 20 and 40 μg/kg dissolved into physiological saline with 0.1% DMSO).

Sample collection and oocyte diameter measuring

The mouse ovaries were collected from Experiment 1 (PND 15) or Experiment 2 (PND 21) mice, and the oocytes were obtained by digested ovary suspension for genome DNA and total RNA extraction. Briefly, the ovaries were firstly rended into as small as possible with eye forceps in the par-enzyme and collagenase mixture (10:1) and then incubated the mixture at 37°C in an incubator for 10 min. At last, 300–500 oocytes per sample were randomly selected using elongated straw under the Olympus stereoscope.

All the oocytes from each group were imaged by an Olympus CKX41 inverted microscope with an Image-Pro® Plus 6.2 system for diameter measuring. The oocyte diameter (after the stage of the primary follicles) was defined as the length of a line that goes through the center of the oocyte sphere from the two sides of the zona pellucid edge. The diameter of oocytes in the primordial follicles was measure without zona pellucida. At least 1,500 oocytes were calculated in each group.

Sodium bisulfite genomic DNA sequencing

DNA was isolated from about 400 oocytes (comes from 10 to 12 ovaries) using a micro DNA isolation kit (Tiangen, Beijing, China) according to the manufacturer's instructions. Briefly, the cell lysate containing nucleic acid released from the oocytes was absorbed by pellosil absorption column with a supplied centrifugal force supplied at 12,000 rpm. After several times of column washing, the DNA was dissolved into eluant. The isolated DNA was treated with sodium bisulfite with a Methylamp™ DNA modification kit (Epigentek, USA) according to the manufacturer's instructions. The bisulfite-treated DNA was amplified by PCR for *Igf2r* (insulin like growth factor 2
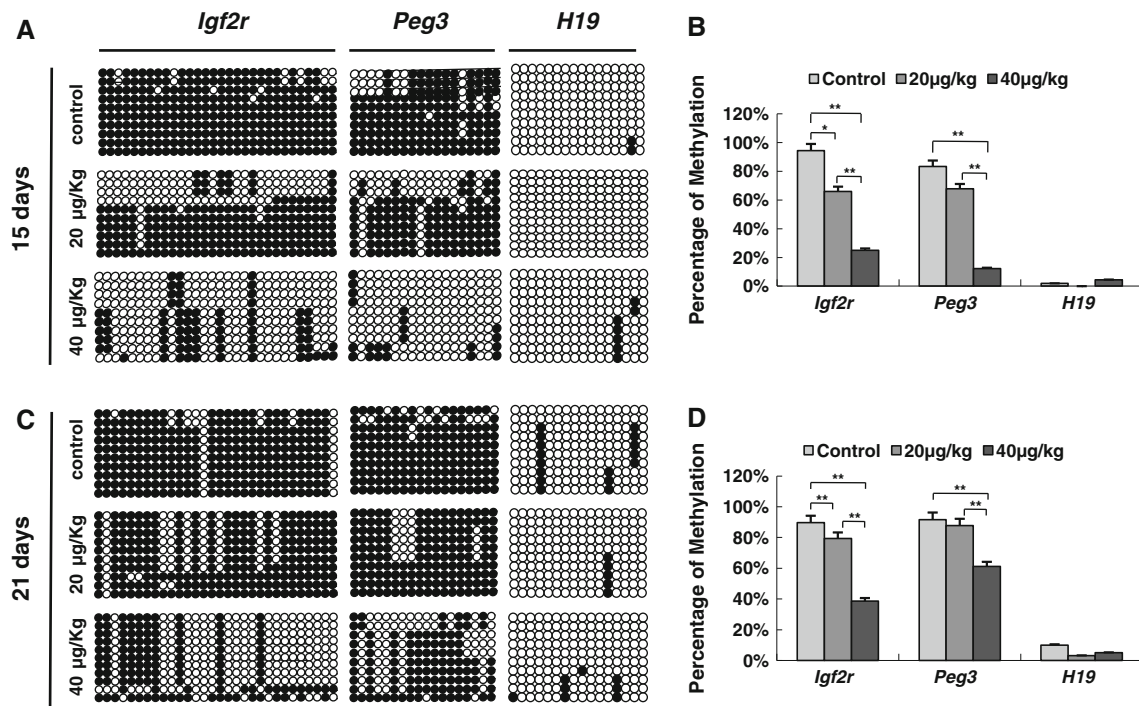
Fig. 1 Methylation analysis of the imprinted genes. **a, c** The methylation status of the imprinted genes *Igf2r*, *peg3* and *H19* in the oocytes in Experiment 1 and 2. *Circles* CpG sites within the regions analyzed; *filled circles* methylated cytosines; *open circles* unmethylated cytosines. **b** The percentage of methylation of imprinted genes in mouse oocytes in Experiment 1. **d** The percentage of methylation of imprinted genes in the mouse oocytes in Experiment 2. The results are presented as mean ± SD. *$p < 0.05$; **$p < 0.01$

receptor), *Peg3* (paternal expressed gene 3) and *H19* genes with primers (Supplemental Table 1) and the PCR conditions previously described (Li et al. 1993, 1999), and the amplification region was shown in Fig. 1a. The PCR products were separated by electrophoresis in 1% agarose gel, and the correct sized bands were isolated from the gel and purified with Wizard SV Gel and a PCR Clean-Up System (Promega, USA). The purified DNA was cloned into a pMD18-T vector (TaKaRa, Dalian, China) according to the manufacturer's instructions. The positive clones were obtained by aminobenzylpenicillin selection and the insert was sequenced at GeneScript (Nanjing, China).

RNA extraction and real-time PCR

Total RNA was extracted from about 400 oocytes (from 10 to 12 ovaries) using an RNAprep pure Micro Kit (Tiangen, Beijing, China) according to the manufacturer's instructions. Oocytes were cracked and total RNA was absorbed by special silicone jelly material in the adsorption column. After several times operation of proteins and DNA elution, the RNA was resuspended into 11 μl nuclease-free water. For isolating the total RNA from the mouse ovaries, the ovaries were cracked by use of an RNAiso reagent (Takara, Dalian, China). After the chloroform extraction and isopropanol precipitation, the total ovarian RNA was dissolved in 22 μl RNase-free water. The oocyte and ovarian cDNA was

synthesized using a PrimeScript™ RT Reagent Kit (TaKaRa, Dalian, China) according to the manufacturer's instructions. Reaction conditions: 25 pmol Oligo dT Primer, 50 pmol random primers, and 300 ng RNA, 5×PrimerScript™ Buffer up to a 20 μl final volume. Experiments in this part were carried out to quantify gene expression with SYBR Premix Ex Taq™ kit (TaKaRa, Dalian, China) in an ABI 7300 real-time PCR instrument (Applied Biosystems, Foster City, CA), and using the standard curve method with *β-actin* as the reference gene. The primers used to amplify *ERα, ERβ, Dnmt1, Dnmt3a, Dnmt3b, Dnmt3l* and *β-actin* have been previously described (Kipp et al. 2007; La Salle et al. 2004; Tanikawa et al. 1998) (Supplemental Table 2). Amplification was performed in 20 μl reaction volume according to the manufacturer's instructions, containing 10 μl SYBR Premix Ex Taq™, 0.4 μl ROX Reference Dye II, 0.8 μl (0.4 μM) forward primer and reverse primer, 2 μl cDNA and 6 μl ddH₂O.

Western blot

The proteins were released from the ovaries (from 6 to 8 different treated mice) with an RIPA lysis solution (Beyotime, Jiangsu, China). Western blotting analysis was performed according to standard methods (Zhang et al. 2010). Briefly, after 10% SDS-PAGE electrophoresis, proteins were transferred onto a polyvinylidene fluoride membrane,

blocked with TBST containing 10% bovine serum albumin (BSA) and incubated with specific primary antibodies: anti-actin (Abcam ab8226, Hong Kong) at the concentration of 1.0 µg/ml and anti-ERα (Abcam ab2746) at the concentration of 0.5 µg/ml. After exposure to secondary antibodies (Beyotime), the blots were developed by enhanced chemiluminescence (Beyotime). The band intensity was quantified using actin as an internal quantitative control and IPWIN software was used for intensity measuring.

Ovarian culture in vitro

To investigate the relationship between estrogen receptor expression and imprinted gene DNA hypomethylation, an in vitro culture system was employed (an in vitro study compared to Experiment 1). Briefly, ovaries were separated mechanically from PND 7 mice and cultured in basic medium [α-MEM + DMEM/F12 (1:1) (Hyclone), 1% penicillin–streptomycin, 1% sodium pyruvate, 0.3% BSA (Roche)] for 8 days. All of the ovaries were cultured at 37°C, 5% CO_2 and saturated humidity condition. The medium was half-changed every 2 days. At the end of the culture, ovaries were sorted out of the medium and digested with trypsin and collagenase mixture for 10 min. The control group contained basic medium, the BPA group contained basic medium with 4.34 µM BPA (1.25 µg/ml), and the BPA + ICI182780 (ER inhibitor, Santa Cruz, USA) group contained basic medium with 4.34 µM BPA and 0.434 µM (0.26 µg/ml) ICI182780.

Maturation of oocytes in vitro

PND 21 mouse ovaries from the 0 (control), 20 and 40 µg/kg groups were collected. Antral follicle oocytes were released by puncturing the ovaries and washed three times in semi-maturation medium (maturation medium without FSH and EGF) before culturing in maturation medium [α-MEM, 10% fetal calf serum (FCS), 1% penicillin–streptomycin, 1% sodium pyruvate (Hyclone), 100 mIU/ml FSH, 1 ng/ml EGF (Sigma)]. The maturation medium droplets in mineral oil were pre-incubated at 37°C, 5% CO_2 and saturated humidity conditions for 12 h before use. The oocytes were cultured in the maturation medium for 16–18 h before the oocytes and their oocyte maturation status was scored. Oocyte maturation status was scored as germinal vesicle (GV) when the germinal vesicle was present, as germinal vesicle breakdown (GVBD) when the germinal vesicle had broken down, and as metaphase II (MII) when the first polar body had been extruded. The percentage of GVBD is the percentage of oocytes that progressed to GVBD in the total number of mature GV stage oocytes, while the percentage of MII is that of progressed polar body extrusion oocytes in the total number of GVBD oocytes.

Spindle assembling analysis

Antral follicle oocytes were released by puncturing PND 21 mouse ovaries and then sorted out using an elongated straw and matured for 16–18 h in vitro. The GVBD and MII stage oocytes were incubated in 4% paraformaldehyde for 30 min at room temperature (RT). The zona pellucida (ZP) was then removed with acidic M2 (Millipor MR-015-D, USA, adjust to pH = 2.5 before use) and incubated with α-tublin antibody (Sigma F2168, USA) in cassette for 2 h at RT and stained with propidium iodide (PI) for 15 min. Finally, slices were covered by coverslip supplied with DABCO antifade (Li et al. 2009). The oocytes were examined with a confocal laser-scanning microscope (Zeiss LSM 510 META, Germany).

Statistical methods

For each set of results, independent experiments were repeated at least three times, with data representing the mean ± SD of all repeats within an individual experiment. Data were analyzed by *t* test or one-way analysis of variance (ANOVA) followed by the Tukey test for multiple comparisons to determine statistical differences between groups (denoted by a star or different letters) using GraphPad Prism analysis software. Results were considered significant at $p < 0.05$.

Results

BPA affects the establishment of imprinted gene methylation

To evaluate the effects of BPA on the reprogramming of imprinted genes during the postnatal development of oocytes, the DNA methylation status of *Igf2r*, *Peg3* and *H19* was analyzed (Supplemental Fig. 1; Fig. 1). The results showed that the increased concentration of BPA remarkably decreased the methylation pattern of two maternal imprinted genes in both Experiment 1 and 2. In Experiment 1, mice were given weight-dependent hypodermic injections daily from PND 7 to 14 and were killed on PND 15. The percentages of methylated CpG sites in *Igf2r* DMRs were 94.33, 66 and 25% in the 0, 20 and 40 µg/kg groups, respectively. The percentages of methylated CpG sites in *Peg3* DMRs were 83.33, 67.78 and 12.22% in 0, 20 and 40 µg/kg groups, respectively (Fig. 1a, b). In Experiment 2, mice were given a weight-dependent injection in a method similar to that of Experiment 1 from PND 5 to 20 every 5 days (PND5, PND10, PND15 and PND20 respectively) and were killed on PND 21. The percentages of methylated CpG sites in *Igf2r* DMRs were 89.67, 79.33 and

Fig. 2 The mRNA expression level of DNA methylation transferases under exposure to BPA in Experiment 1. The results are presented as mean ± SD. *$p < 0.05$; **$p < 0.01$
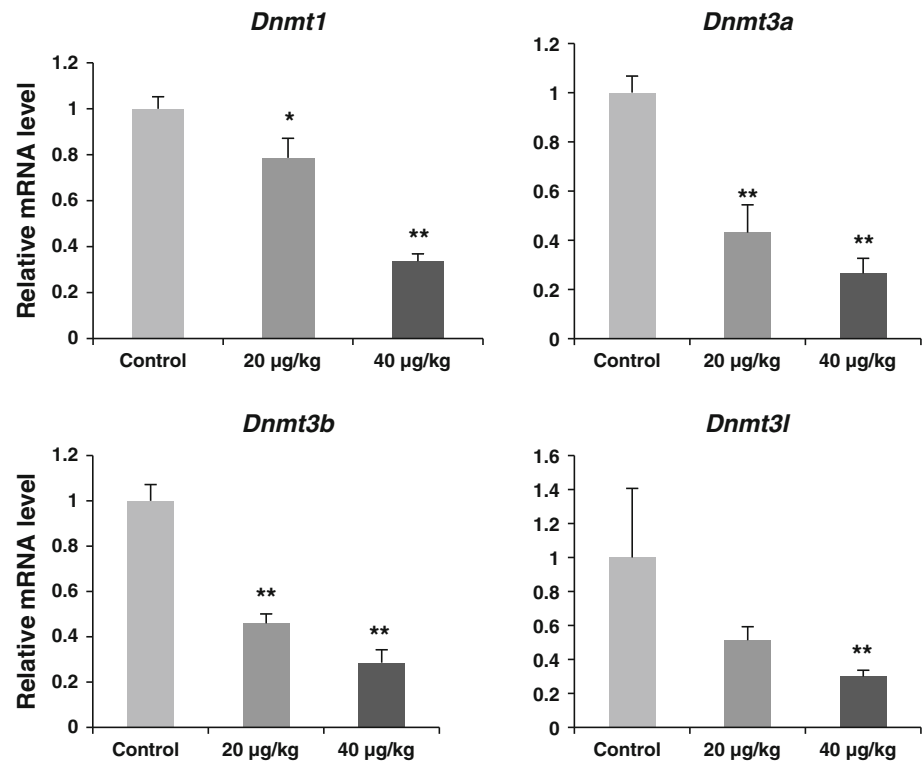
39.67% in the 0, 20 and 40 μg/kg groups, respectively. The percentages of methylated CpG sites in *Peg3* DMRs were 91.67, 87.78 and 66.11% in the 0, 20 and 40 μg/kg groups, respectively ($p < 0.01$) (Fig. 1c, d). Under the same BPA concentration exposure (20 μg/kg), the DNA methylation status in the earlier developmental stage of oocytes in Experiment 1 was lower than that of the later ones in Experiment 2, as the establishment of imprinted gene methylation was development dependent. *H19*, a paternal imprinted gene, was also analyzed to assure the purity of the germ cell genomic DNA. Our results demonstrated that the percentages of methylated CpG sites in *H19* DMRs were 1.88, 0 and 4.38% in the 0, 20 and 40 μg/kg groups in Experiment 1, respectively, and 10, 3.13 and 5% in the 0, 20 and 40 μg/kg groups in Experiment 2, respectively. These results showed that BPA affected the methylation establishment of these two maternal imprinted genes.

DNA methylation transferases (*Dnmts*) is a family of enzymes indispensable during the process of gene methylation modification (Robertson 2001). As the establishment and maintenance of DNA methylation pattern are ongoing during oogenesis, we examined the expression dynamics of *Dnmt1*, *Dnmt3a*, *Dnmt3b* and *Dnmt3L* during oogenesis under exposure to BPA using quantitative RT-PCR. As shown in Fig. 2, after BPA exposure in Experiment 1, the expressions of four types of *Dnmts* were all suppressed with the increase of the BPA treatment concentration: *Dnmt1* (0.7864 ± 0.0853), *Dnmt3a* (0.4313 ± 0.1127), *Dnmt3b* (0.459 ± 0.0414) and *Dnmt3l* (0.5129 ± 0.0792)

in the 20 μg/kg group and *Dnmt1* (0.3368 ± 0.0316), *Dnmt3a* (0.2655 ± 0.0612), *Dnmt3b* (0.2852 ± 0.057) and *Dnmt3l* (0.2987 ± 0.037) in the 40 μg/kg group.

BPA promotes the expression of estrogen receptors

The estrogen receptor (ER) is regarded as a ligand-dependent transcription factor and recruits co-activator complexes with histone acetyltransferase or methyltransferase activities to activate downstream target genes (Hall and McDonnell 2005). Here, we tried to elucidate whether BPA, an environmental xenoestrogen, exerts its biological function via ER signaling pathways. In Experiment 1, there was a significant difference in *ERα* expression between the treatment group (40 μg/kg) and control group ($p < 0.05$) (Fig. 3a). BPA exposure significantly up-regulated the mRNA expression of *ERα* approximately 3.85-fold in comparison to the control. The same dosage of BPA exposure increased *ERα* expression 1.92-fold versus the control group in Experiment 2 ($p < 0.05$). However, there was no statistical difference between the control and treatment group in the mRNA expression of the *ERβ* gene. Western blot was used to detect the protein expression of ERα. As shown in Fig. 3b, our results revealed a positive correlation between BPA exposure concentration and ERα protein expression. With the exposure to BPA, the ERα protein was 1.6 ± 0.23 and 1.8 ± 0.19 times overexpressed in the treatment groups of 20 and 40 μg/kg than in the treatment group of 0 μg/kg (control) in Experiment 1, and 1.5 ± 0.11 and
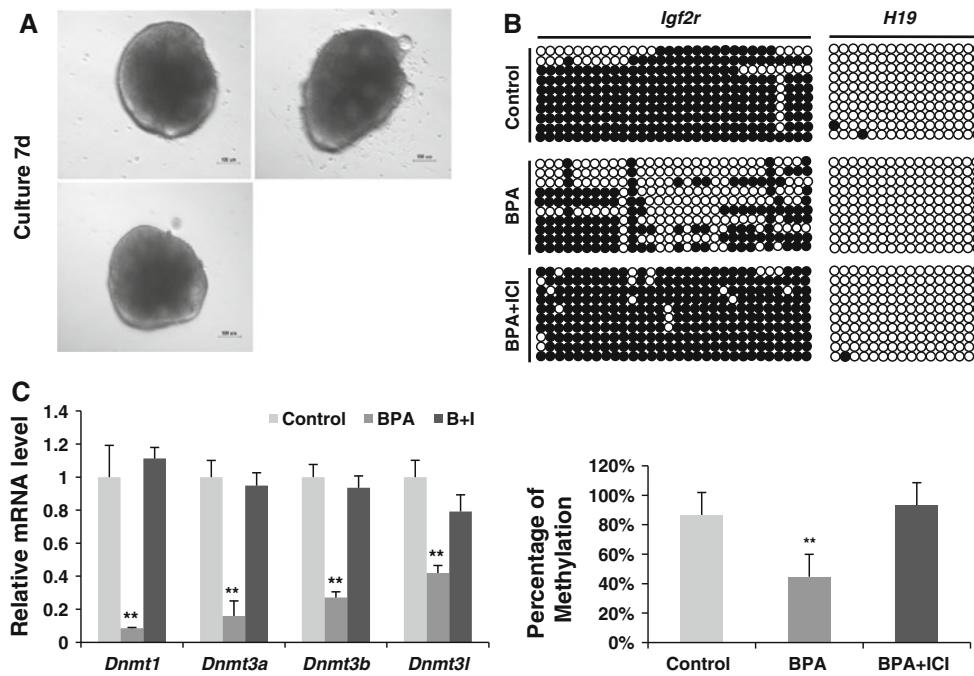
Fig. 4 BPA induces hypomethylation via affecting estrogen receptor and *Dnmts* expression. **a** Mouse ovaries were cultured for 8 days in vitro with or without ER inhibitor ICI182780. **b** The methylation status of *Igf2r* and *H19* gene in three different treatment groups (control, BPA and BPA + ICI group). *Circles* CpG sites within the regions analyzed; *filled circles* methylated cytosines; *open circles* unmethylated cytosines. **c** The *Dnmts* expression in cultured oocytes with or without ER inhibitor ICI182780. The results were presented as mean ± SD. *$p < 0.05$; **$p < 0.01$

of oocytes in Experiment 2 mice by using an in vitro maturation system. Figure 6a, b illustrates that a high concentration of BPA exposure inhibits the oocyte GVBD. Of 587 oocytes, 398 (67.80%) progressed into GVBD in the BPA exposure group (40 μg/kg), which was significantly lower than the control (536/666, 80.48%) and the 20 μg/kg group (612/748, 81.82%). However, there was no difference in the MII stage.

To investigate the developmental quality of oocytes under BPA exposure, the spindle assembling of oocytes in Experiment 2 mice were analyzed to detect whether oocyte maturation failure induced by BPA exposure was due to abnormality of spindle assembling (Fig. 6c). The ratio of oocytes with abnormal spindle assembling in MI or MII was calculated. As indicated in Fig. 6d, the ratio in MI were 6.5% (8/123), 22.14% (31/140) and 21.23% (38/179) in the 0, 20 and 40 μg/kg groups, respectively, whereas the abnormal ratio in MII were 15.46% (15/97), 18.18% (16/88) and 23.68% (27/114) in the 0, 20 and 40 μg/kg groups, respectively. Together, our results demonstrated that BPA exposure significantly raised the abnormal ratio of spindle assembling in meiosis I ($p < 0.05$), and that there was no statistical difference between the control and treatment groups in meiosis II (Fig. 6d).

Discussion

In recent years, studies have revealed that BPA could function as an endocrine disruptor, meiotic resumption inhibitor and cell cycle alteration factor (Can et al. 2005; Eichenlaub-Ritter et al. 2008; George et al. 2008; Hunt et al. 2003; Maffini et al. 2006; Pfeiffer et al. 1997; Uzumcu and Zachow 2007) and that it was involved in different biological activities during the development of mammal histogenesis. The aim of this study was to assess the extent of BPA-induced alteration of the imprinted gene methylation during oogenesis and folliculogenesis, and oocyte meiotic maturation. Our results demonstrated that BPA exposure resulted in hypomethylation of imprinted gene *Igf2r* and *Peg3* during oocyte growth and enhanced estrogen receptor (ER) expression. In addition, BPA promoted primordial follicular development and suppressed the meiotic maturation of oocytes because of abnormal spindle assembling in meiosis I.

With the establishment of maternal imprinted genes, such as *Igf2R*, *Peg3* and *Snrpn*, the oocyte develops until fertilizing ability is accessed. Previous research demonstrated that *Igf2r* and *Peg3* were nearly fully methylated at PND 21 (95.33% for *Igf2r* and 88.98% for *Peg3*) (Song et al. 2009). Our data indicated that BPA exposure markedly reduced these indexes (Fig. 1), suggesting a confirmed
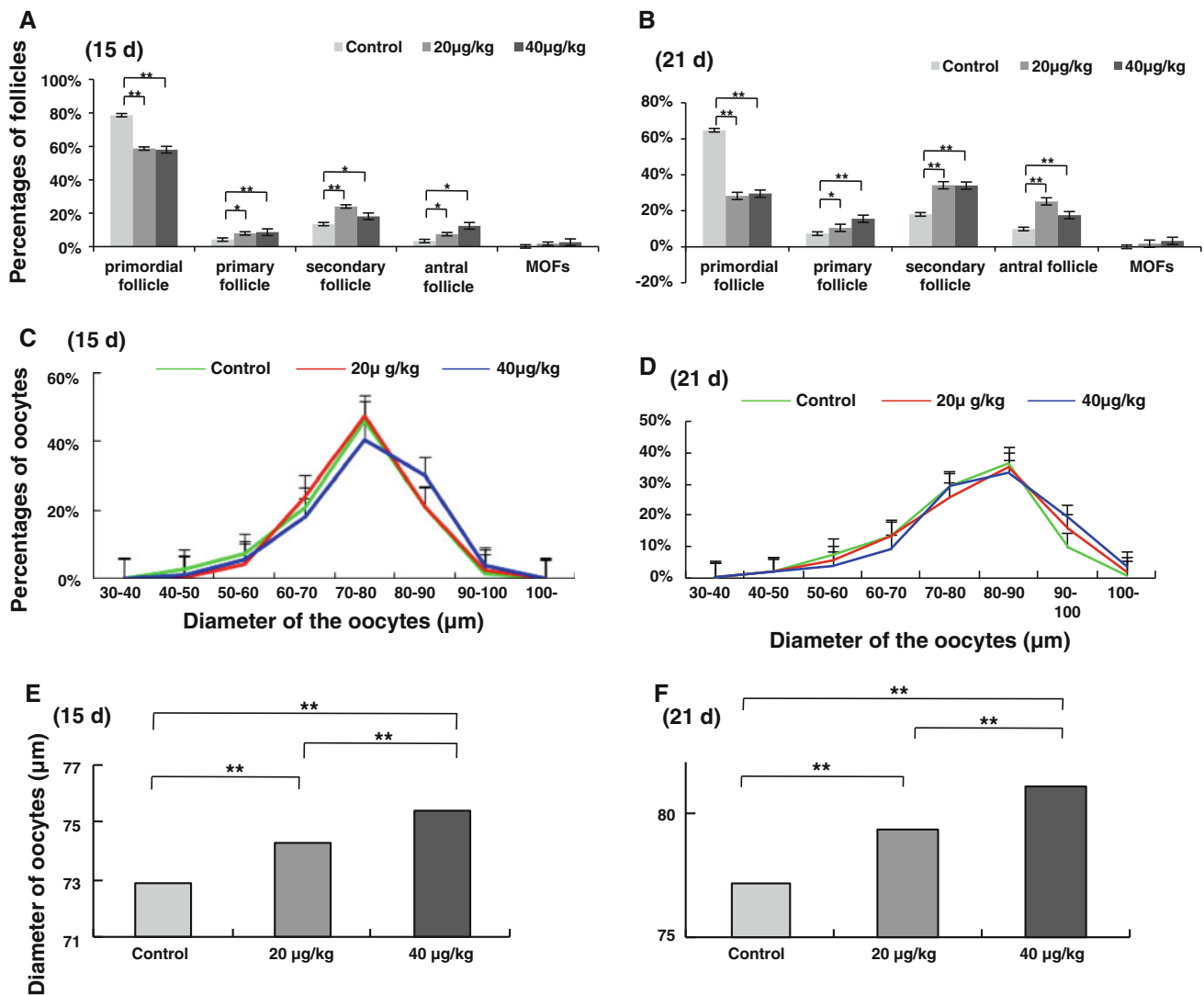
Fig. 5 The effects of BPA on the follicular development and oocyte growth. **a**, **b** The ratio of follicle and multiple oocytes follicle (MOF) in different developmental stages. **c**, **d** The number of oocytes in differ-ent diameter sections counting. **e**, **f** The diameter of oocytes grown under the exposure to BPA. The results are presented as mean ± SD. *$p < 0.05$; **$p < 0.01$

effect of BPA on the DNA methylation processing of these maternal imprinted genes. Consistently, Dolinoy et al. (2007) also demonstrated that BPA exposure induced the methylation modification alteration of an intracisternal A particle retrotransposon upstream of the *Agouti* gene and CDK5 activator-binding protein (CabpIAP), which pro-vides a clue that BPA may affect oocyte maturation via direct or indirect epigenetic modification of related genes. In addition, we detected the expression of DNA methyla-tion transferase, which has an intimate relationship with gene methylation modification and found a suppressive effect of BPA on the expression of *Dnmts* (Fig. 2). In view of the different roles played by thw four types of employed *Dnmts* (*Dnmt1, Dnmt3a, Dnmt3b* and *Dnmt3l*) in the pro-cess of gene methylation, they induced gene methylation maintenance (*Dnmt1*), de-novo (*Dnmt3a* and *Dnmt3b*) and

the establishment of maternal methylation imprints in the female germ line (*Dnmt3l*) (Bourc'his et al. 2001; Hata et al. 2002; Lees-Murdock et al. 2005; Okano et al. 1999; Shovlin et al. 2007). We tested the mRNA expression of these four types of *Dnmts* and found that their expression was inhibited under exposure to BPA, indicating that BPA has vast effects on the process of gene methylation in oocytes, but the study by Bromer et al. (2010) showed that the hypomethylation of *Hoxa10* induced by BPA was not due to persistent repression of DNA methyltransferases in utero; therefore, BPA-induced *Dnmts* low expression may be tissue-specific. In addition, researchers found that other endocrine disrupting chemicals such as methoxychlor (an estrogenic compound) and vinclozolin (an antiandrogenic compound) also altered DNA methylation patterns in the germ line and induced some reproductive defects in the

Fig. 6 The alteration of oocyte maturation (**a**, **b**) and spindle assembling (**c**, **d**) under BPA exposure. **a** The maturation status of the cultured oocytes from BPA-treated mice. The oocytes were termed as GV stage oocyte (*red arrow*) when the germinal vesicles were present, as GVBD stage oocyte when the germinal vesicle was broken down (*yellow arrow*), and as MII stage when the first polocyte was present (*blue arrow*). **b** The percentage of oocytes progressed to GVBD or reached the MII stage, respectively. The spindle assembling status of oocytes is shown in (**c**) and (**d**). The results are presented as mean ± SD. *$p < 0.05$; **$p < 0.01$
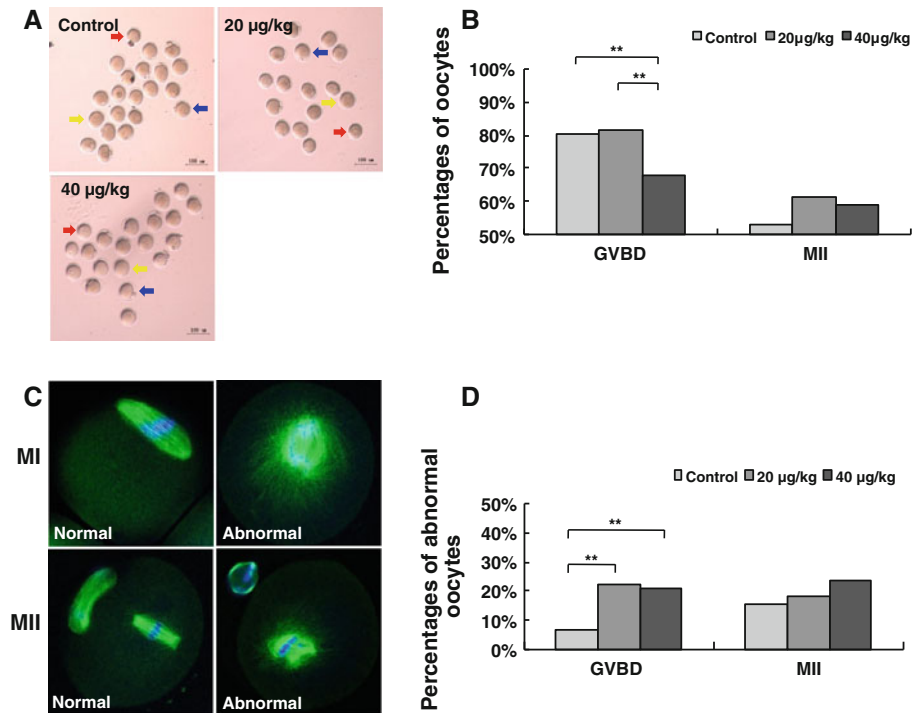
subsequent generations (Anway et al. 2005; Stouder and Paoloni-Giacobino 2010).

As BPA is an estrogen-like chemical, the estrogen receptor must be the most probable target of BPA. Krishnan et al. (1993) substantiated that BPA exerted its biological activities via estrogen receptors. Several previous studies also showed that BPA had weak estrogenic activity (Roy et al. 1997). In addition, Bouskine et al. reported that after binding to a membrane receptor G-protein-coupled receptor (GPCR), BPA transfers the extracellular signal by the PKA and PKG pathway to induce a remarkable proliferation of JKT-1 cells (Bouskine et al. 2009). In the present study, we found an increased expression of estrogen receptors in mouse ovaries treated with BPA. The mammalian development after birth is accompanied by a continuous increase in estrogen, and the reproductive system is intensively influenced by estrogen. Our data suggest that BPA promotes oocyte growth and follicular development, which results in the acceleration of the transformation of the primordial to primary follicle. As it mimics estrogen, we speculate that BPA may play a role in the process of mammalian reproductive activities to some extent. *ER* overexpression and DNA methylation modification of maternal imprinted genes regulate the related gene expression, but little is known about whether the *ER* exerts its action via regulating DNA methylation. In this study, BPA-induced imprinted gene hypomethylation was eliminated by *ER* inhibitor ICI182780 (Fig. 4), suggesting that ER was the mediator in the process of BPA-induced hypomethylation. In conclusion, BPA induced imprinted genes hypomethylation via the ER signaling pathway during the imprint establishment.

The meiotic process of oocytes is arrested in the diplotene stage of the meiosis I prophase around the time of birth until proper stimulation is given. Therefore, meiotic resumption is a key point of female gamete development (Roy et al. 1997). Spindle assembling is one of the momentous subcellular structure activities during oocyte meiotic resumption. Previous studies suggest that BPA induces cell cycle delay and spindle microtubular organization failure during oocyte meiosis (Can et al. 2005). In the present study, we also observed a remarkable increase in spindle assembling abnormality in meiosis I. The conversion of germinal vesicle breakdown (GVBD) to the first polar body extrusion is a successive course in the process of oocyte meiotic resumption; thus, GVBD is termed the key process of oocyte maturation. Here, our results show that BPA inhibits GVBD, presenting adverse effects on oocyte postnatal development, especially oocyte maturation.

Conflict of interest These authors fully declare any financial or other potential conflict of interest.

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