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Bisphenol A exposure modifies DNA methylation of imprint genes in mouse fetal germ cells

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Abstract Bisphenol A (BPA) is an estrogenic environmental toxin widely used for the production of plastics. Human frequent exposure to this chemical has been proposed to be a potential public health risk. The objective of this study was to assess the effects of BPA on DNA methylation of imprinting genes in fetal mouse germ cell. Pregnant mice were treated with BPA at doses of 0, 40, 80 and 160 μg BPA/kg body weight/day from 0.5 day post coitum. DNA methylation of imprinting genes, *Igf2r*, *Peg3* and *H19*, was decreased with the increase of BPA concentration in fetal mouse germ cells ($p < 0.01$).The relative mRNA levels of *Nobox* were lower in BPA-treated group compared to control (BPA free) in female fetal germ cells, but in male fetal germ cells, a significant higher in *Nobox* expression was observed in BPA-treated group compared to control. Decreased mRNA expression of specific meiotic genes including Stimulated by *Stra8* and *Dazl* were obtained in the female fetal germ cells. In conclusion, BPA exposure can affect the DNA methylation of imprinting genes in fetal mouse germ cells.

Keywords Bisphenol A · Fetal germ cell · Imprinting gene · DNA methylation

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Introduction

Primordial germ cells (PGCs) first appear embryonic at ~6.25 days post coitum (dpc) [1]. Once the founder reaches 40 PGCs, cells migrate to reside in extra-embryonic tissues until gastrulation is complete. PGCs migrate towards genital ridge on 8.5 dpc and arrive there by 10.5 dpc [1]. During the development and differentiation of mouse germ cells, their genome undergoes dramatic epigenetic reprogramming. Imprinting is erased at around 10.5–11.5 dpc in PGCs when they arrive at genital ridges, and imprinting is initially established during gametogenesis [2]. Sexual recognition of germ cell is determined with sex-specific imprinted gene methylation patterns at 10.5–11.5 dpc [3, 4]. Re-establishment of maternal imprints typically does not begin until birth in the phase of germ cell growth. Specific DNA methylation in differentially methylated regions (DMRs) of parental origin allows discrimination between maternal and paternal alleles and leads to mono-allelic expression of imprinted genes [5].

Bisphenol A is a widely used environmental estrogen-like chemical. It is applied in the manufacture of epoxy, polycarbonate and corrosion-resistant polyester-styrene resins of consumer products due to reproductive toxicity. As an endocrine disruptor, BPA has adverse influences in reproductive tract and sexually dimorphic behavior in both males and females [6–9]. Moreover, BPA can lead germ cells to malfunction. Prenatal exposure to low-doses' BPA (20 μg/kg daily) from embryonic day 0.5 has shown to perturb neocortical histogenesis and thalamocortical pathway formation [10, 11]. Our team demonstrated that neonatal exposure to BPA inhibits methylation of imprinted genes during oogenesis via the ER signaling pathway in CD-1 mice [12, 13]. Furthermore, BPA exposure can affect the formation of primordial follicle by inhibiting meiotic

progression of oocytes [12, 13]. To study the effect of BPA exposure during the fetal stages of fetal germ cells, pregnant mice were treated at 0, 40, 80 and 160 μg/kg body weight individually at gestation day 0.5–12.5. Ovaries and testis were isolated from male and female fetuses at 12.5 dpc of gestation, and DNA methylation status of imprinting gene in PGCs were analyzed.

Materials and methods

Animals and BPA dosages

All procedures described in the study were reviewed and approved by the Ethical Committee of Qingdao Agricultural University. CD-1 mice (Vital River, Beijing, China) were housed in temperature-controlled (21–22 °C) and light-controlled (12-h light, 12-h dark cycle) conditions.

Pregnant mice were treated with BPA (Kefeng, Shanghai, China) at doses of 0, 40, 80 and 160 μg BPA/kg body weight everyday in 0.1 % DMSO or 0.1 % DMSO alone as a vehicle control from 0.5 to 12.5 day dpc. Both BPA doses and vehicle control were delivered orally to pregnant females using an Eppendorf pipette with a disposable tip [12, 13].

Fetal mouse germ cells collection

The 12.5 dpc fetal mouse gonads were collected individually without attached mesonephroses and digested with mixture of parenzyme and collagenase at 37 °C incubator for 10 min, then gonads were purified by miniMACS magnetic separation system [14, 15]. Simplified, gonads were isolated from 12.5 dpc mouse embryos and fetal mouse germ cells were immunomagnetically purified by using the monoclonal antibody SSEA-1(MILLIPORE Cat#MAB4301 Temecula, CA, USA) according to the manufacturer's instructions. Techniques for dissecting fetal mouse germ cells containing regions from the embryonic gonads in accordance with embryonic age have been described previously by De Felici and our study [14, 15].

DNA isolation and bisulfite sequencing

DNA isolated from 300 to 500 fetal mouse germ cells was extracted using a TIANamp Micro DNA Kit (Tiangen, Beijing, China) according to the manufacturer's instructions. The isolated DNA was treated with sodium bisulfite from a Methylamp™ DNA modification kit (Epigentek) as per the manufacturer's instructions [12, 15]. The bisulfite-treated DNA was amplified by nested (semi-nested) PCR for *Igf2r*, *Peg3* and *H19* genes with primers and PCR conditions were previously described [12, 16, 17] (Table 1). The PCR products were separated by electrophoresis in 1 % agarose gel, and correct bands were excised from the gel and purified with the Wizard SV Gel using PCR Clean-Up System (Promega). Then the purified DNA was cloned into a pDM19-T Vector (TaKaRa, Dalian, China) according to the manufacturer's instructions. The positive clones were obtained by antibiotic selection and the insert was sequenced at invitrogen (Shanghai, China).

RNA extraction and quantitative PCR

Relative mRNA levels were quantified by real-time PCR using cDNA prepared from mouse 300 to 500 fetal mouse germ cells. Total RNA was extracted by an RNAprep pure Micro Kit (Tiangen, Beijing, China), and cDNA was synthesized by a PrimeScript™ RT reagent Kit (TaKaRa, Dalian, China).

Table 1 The primers used for amplification of imprint gene by nested PCR

Gene	PCR	Sequences of primers	Productions (bp)
Igf2r	First round	F: 5′-TAGAGGATTTTAGTATAATTTTAA-3′	
		R: 5′-CACTTTTAAACTTACCTCTCTTAC-3′	
	Second round	F: 5′-GAGGTTAAGGGTGAAAAGTTGTAT-3′	490
		R: 5′-CACTTTTAAACTTACCTCTCTTAC-3′	
Peg3	First round	F: 5′-TTGTTGATGTTAATTTTGTGTTTTGGTG-3′	
		R: 5′-TCAACCTTATCAATTACCCTTAAAAACC-3′	
	Second round	F: 5′-TTTTGTAGAGGATTTTGATAAGGAGGTG-3′	288
		R: 5′-CCCCAAACACCATCTAAACTCTACAAAC-3′	
H19	First round	F: 5′-GAGTATTTAGGAGGTATAAGAATT-3′	
		R: 5′-ATCAAAAACTAACATAAACCCCT-3′	
	Second round	F: 5′-GTAAGGAGATTATGTTTATTTTTGG-3′	421
		R: 5′-CCTCATAAAACCCATAACTAT-3′	

Table 2 Primers used for amplification of specific gene by real-time PCR

Gene	Primer sequence	Amplified fragment length (bp)
Nobox	F: 5′- CTATCCTGACAGTGACAAACGCC- 3′	331
	R: 5′- CACCCTCTCAGCACCCTCATTAT- 3′	
Dazl	F : 5′- TGACGTGGATGTGCAGAAGAT- 3′	244
	R : 5′- AGGAGGATATGCCTGAACATACT- 3′	
Nanog	F: 5′- GCAGAAGTACCTCAGCCTCC- 3′	127
	R : 5′- CCACTGGTTTTTCTGCCAC- 3′	
Stra8	F : 5′- CTCCTCCTCCACTCTGTTGC- 3′	134
	R : 5′- GCGGCAGAGACAATAGGAAG- 3′	
ERα	F : 5′- ACCATTGACAAGAACCGGAG- 3′	170
	R : 5′- CCTGAAGCACCCATTTCATT- 3′	
Vasa	F: 5′-AGGGGATGAAAGAACTATGGTC- 3′	175
	R: 5′-AGCAACAAGAACTGGGCACT- 3′	
Oct4	F: 5′-GGCGTTCTCTTTGGAAAGGT-3′	112
	R: 5′-TCTCATTGTTGTCGGCTTCCT-3′	
β-actin	F : 5′- TCGTGGGCCGCTCTAGGCAC- 3′	255
	R : 5′- TGGCCTTAGGGTTCAGGGGG- 3′	

Experiments in this part were carried out to quantify gene expression with SYBR Premix Ex Taq^{TM} kit (TaKaRa) in an ABI 7300 real-time PCR instrument (Applied Biosystems, Foster City, CA), using the standard curve with *β*-actin as the reference gene. The primer pairs of *β-actin*, Estrogen reaction (*ERα*), *Vasa*, *Oct4*, *Nanog*, retinoic acid gene 8 (*Stra8*), daleted in azoospermia-like (*Dazl*) and newborn ovary homeobox (*Nobox*) gene were designed as shown in Table 2 [12, 13, 15].

Statistical methods

Every independent experiment was duplicated at least three times, and the results were represented as mean ± SEM.

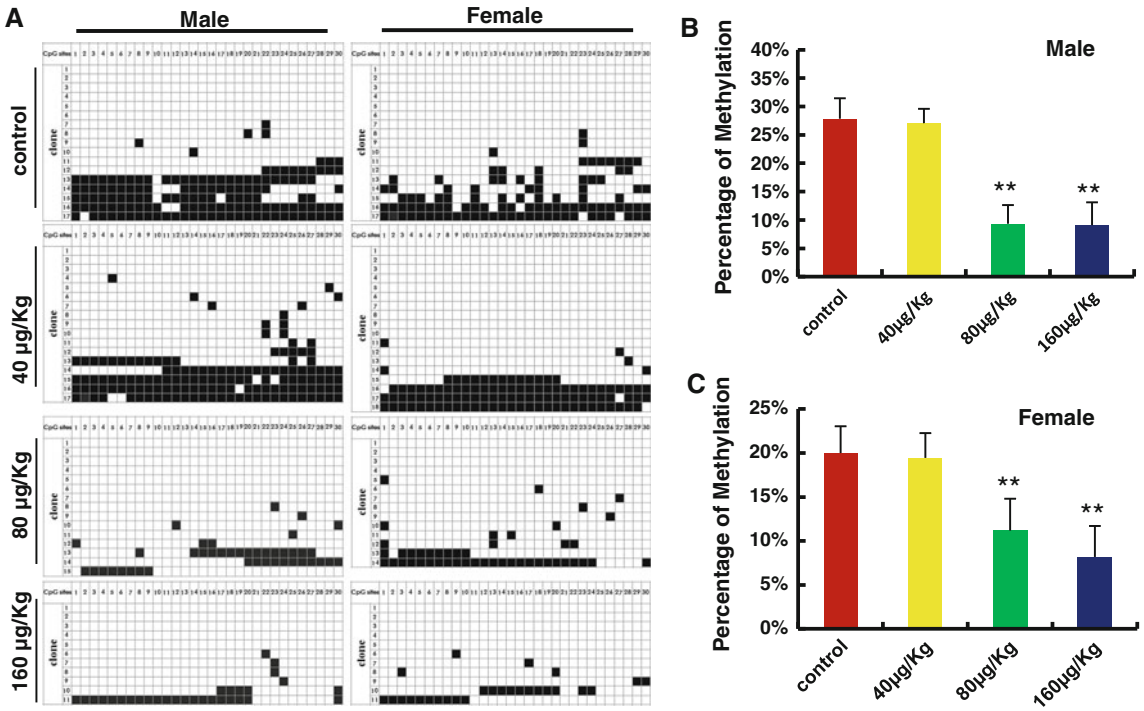

Fig. 1 DNA Methylation analysis for imprinting genes *Igf2r* in fetal germ cells. **a** Methylation status of the DMRs of the imprinted gene *Igf2r* in the fetal germ cells. *Squares* CpG sites within the regions analyzed; *filled squares* methylated cytosines; *open squares* unmethylated cytosines. **b**, **c** Percentage of methylation of imprint gene *Igf2r* in male and female mouse fetal germ cells respectively. The results were presented as mean ± SD. * indicates significance of $p < 0.05$; ** indicates significance of $p < 0.01$

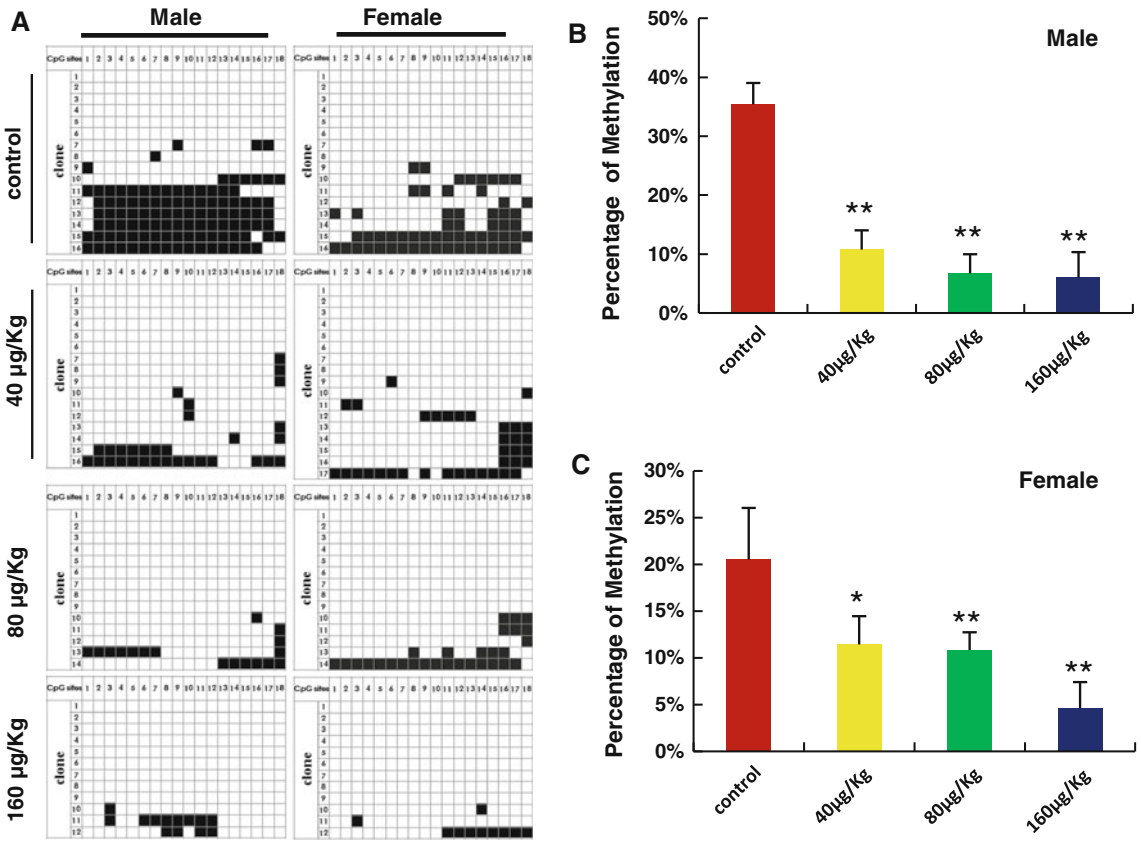

Fig. 2 DNA Methylation analysis for imprinting genes *Peg3* in fetal germ cells. **a** Methylation status of the DMRs of the imprinted gene *Peg3* in the fetal germ cells. *Squares* CpG sites within the regions analyzed; *filled squares* methylated cytosines; *open squares* unmethylated cytosines. **b**, **c** Percentage of methylation of imprint gene *Igf2r* in male and female mouse fetal germ cells respectively. The results were presented as mean ± SD. * indicates significance of $p < 0.05$; ** indicates significance of $p < 0.01$

The differences between treatments and controls were analyzed by ANOVA, and differences were calculated by Turkey's test. The significance of results was determined at $p < 0.05$. All numerical comparisons were carried out with the statistical analysis system.

Results

BPA exposure affects the DNA methylation of imprinting gene in fetal mouse PGC

The fetal germ cells within 12.5 dpc mouse gonads were collected using the miniMACS technology. Alkaline phosphatase staining of the germ cells presents in the eluted fraction from disaggregation of germ cells-containing tissues. The recovery rates of purified positive germ cells were more than 90 %.

To evaluate the effects of BPA on reprogramming of imprinting genes during the gestation while development of fetal mouse germ cells, the DNA methylation status of *Igf2r*, *Peg3* and *H19* were analyzed. The results revealed, in male fatal mice, the increased BPA level remarkably decreased the methylation pattern of two maternal imprinting genes and one paternal imprinting gene.

The percentages of methylated CpG sites in *Igf2r* DMRs, in male fatal mice, were 27.65, 26.86, 9.33 and 9.1 % in 0 (control), 40, 80 and 160 μg/kg group, respectively ($p < 0.01$) (Fig. 1a, b). In female fatal mice, the DNA methylation of *Igf2r* gene were 19.6, 19.44, 11.2 and 8.2 % in 0 (control), 40, 80 and 160 μg/kg group, respectively ($p < 0.01$) (Fig. 1a, c).

Regarding *Peg3* DMRs, the percentages of methylated CpG sites, in male fatal mice, were 36.8, 10.76, 6.75 and 6.0 % in 0 (control), 40, 80 and 160 μg/kg group, respectively ($p < 0.01$) (Fig. 2a, b). In female fatal mice, the DNA methylation of *Peg3* gene were 20.49, 11.76, 12.05 and 5.2 % in 0 (control), 40, 80 and 160 μg/kg group, respectively ($p < 0.01$) (Fig. 2a, c).

The percentages of methylated CpG sites in *H19* DMRs, in male fatal mice, were 30.86, 30.86, 5.82 and 2.27 % in 0 (control), 40, 80 and 160 μg/kg group, respectively ($p < 0.01$)

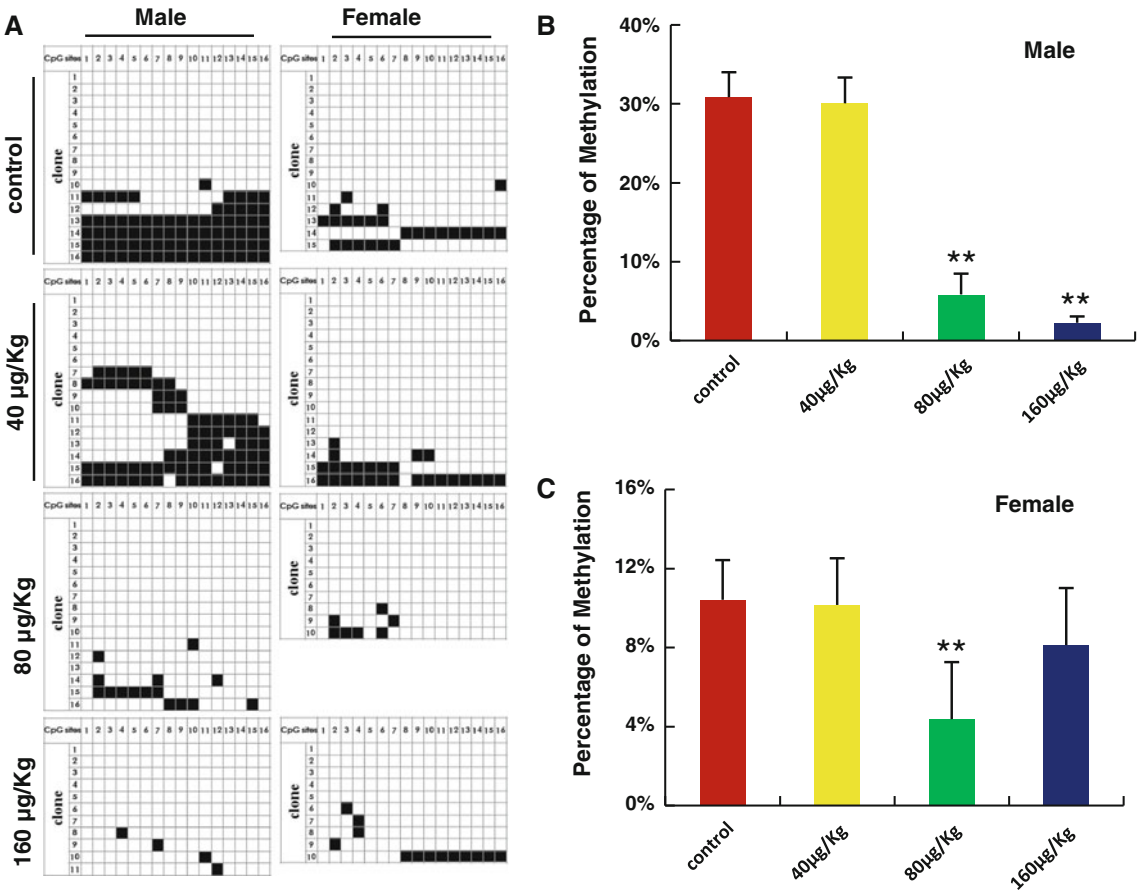

Fig. 3 DNA Methylation analysis for imprinting genes *H19* in fetal germ cells. **a** Methylation status of the DMRs of the imprinted gene *H19* in the fetal germ cells. *Squares* CpG sites within the regions analyzed; *filled squares* methylated cytosines; *open squares* unmethylated cytosines. **b, c** Percentage of methylation of imprint gene *Igf2r* in male and female mouse fetal germ cells respectively. The results were presented as mean ± SD. * indicates significance of $p < 0.05$; ** indicates significance of $p < 0.01$

(Fig. 3a, b). In female fatal mice, the DNA methylation of *H19* gene were 9.6, 10.15, 4.38 and 8.13 % in control (0), 40, 80 and 160 μg/kg group, respectively (Fig. 3a, c).

The dosage effect of BPA, the higher the dose showed the higher influence whether in male or in female mouse fetal germ cells.

BPA affects the expression of germ cell specific genes in fetal mouse germ cells

The mRNA expression of germ cell specific genes was examined, including *Vasa*, *Oct4*, *Nanog* and *Nobox* genes at 12.5 dpc of fetal mouse fetal mouse germ cells. In male fetal germ cells, the relative mRNA levels of *Vasa* were lower in BPA-treated group compared to control ($p < 0.01$), and a significant higher expression in *Nobox* was observed between the treated and control groups ($p < 0.01$) (Fig. 4a). In female fetal mouse germ cells, the mRNA expressions of *Nobox* in experimental group were significantly lower than that of the control group ($p < 0.01$) (Fig. 4b).

During the development of female germ cells, the mRNA expression of meiosis-specific genes, such as *Dazl* and *Stra8*, is able to be detected in 12.5 dpc. To evaluate the effect of BPA exposure on the meiosis of female germ cells, the mRNA expression of meiosis-specific genes were checked using the real time PCR. The results demonstrated that, after BPA exposure, the expressions of *Stra8* (0.297) and *Dazl* (0.300) in 160 μg/kg group were significantly lower than that of the control group ($p < 0.01$).

BPA promotes the expression of estrogen receptor (ER) genes

ER is regarded as a ligand-dependent transcription factor, since it recruits coactivator complexes with histone acetyltransferase or methyltransferase activities to valid downstream target genes [18]. Here, we tried to elucidate whether BPA, an environmental xenoestrogen, exerted its biological function via ER signaling pathways. The results demonstrated that BPA exposure significantly up-regulated *ERα* at the mRNA levels, in the male fetal germ cells,

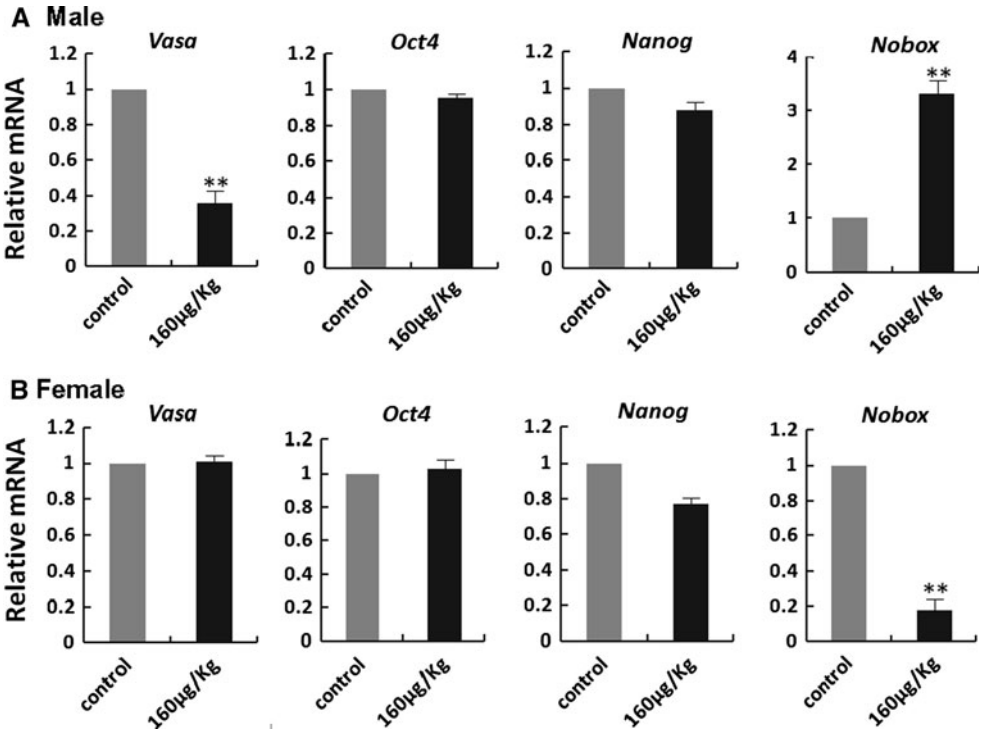

Fig. 4 The mRNA expression level of specific gene of male (**a**) and female (**b**) PGCs under the exposure of BPA. The results were presented as mean ± SD. * indicates significance of $p < 0.05$; ** indicates significance of $p < 0.01$

~2.35-fold over control levels ($p < 0.01$) (Fig. 5b). Furthermore, the same dosage of BPA exposure increased *ERα* expression ~1.63-fold over control levels, in female fetal germ cells ($p < 0.01$) (Fig. 5b).

Discussion

DNA methylation plays a key role in regulating eukaryotic gene expression and acts as an important molecular marker underlying the parental-specific expression of genes in growth, differentiation, development and other processes [19–21]. Imprinting is erased in PGCs in mice, and then re-established during gametogenesis [2, 22]. Many imprinted genes, including the maternally methylated *Igf2r* and *Peg3* and the paternally methylated *H19*, are erased when PGCs arrive at genital ridges; similarly, methylation in imprinted genes is erased when bulk demethylation occurs between 11.5 and 12.5 dpc [23–25]. Patterns of DNA methylation frequently change in response to cell differentiation, disease and environmental influences. The data indicated that BPA exposure dramatically affect the DNA methylation processing of *Igf2r*, *Peg3* and *H19* regardless of male or female (Figs. 1, 2, 3).

Newborn ovary homeobox (NOBOX) preferentially expressed in oocytes and encode a homeobox transcriptional regulator, is an oocyte-specific homeobox gene essential for folliculogenesis and ovarian development in mice [26–30]. Abnormal expression and deficiency of

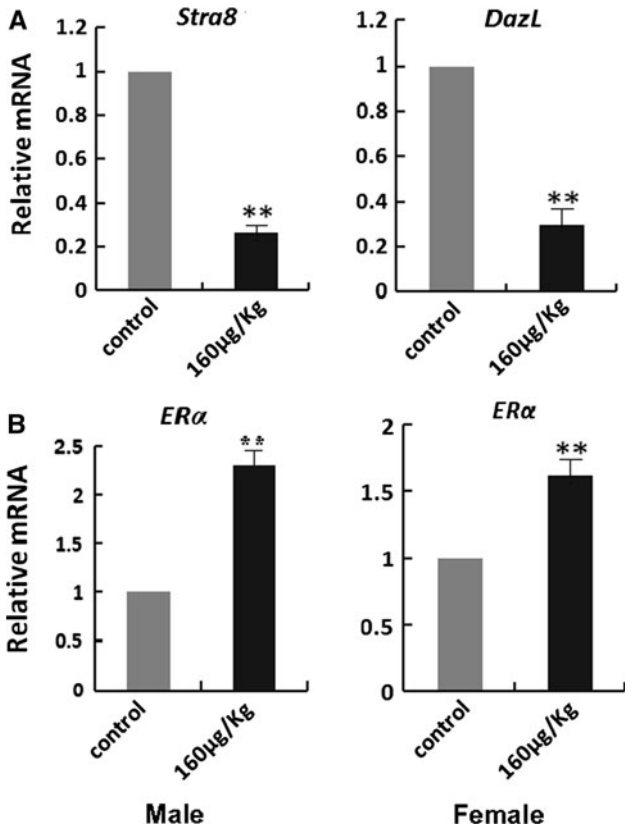

Fig. 5 The mRNA expression levels of meiosis-specific gene (**a**) and estrogen receptors-α (ERα) (**b**) in mouse fetal germ cells. The results were presented as mean ± SD. * indicates significance of $p < 0.05$; ** indicates significance of $p < 0.01$

Nobox in mice and humans result in the premature ovarian failure syndrome characterized by postnatal oocyte loss and the replacement of follicles by fibrous tissue [31–33]. Bouilly et al. [34] has showed that Nobox absence leads to primary ovarian insufficiency (POI) to female infertility in humans. This study indicated that BPA exposure remarkably increase the expression of *Nobox* gene in male, but the opposite effect in female (Fig. 4). Furthermore, BPA exposure significantly decreased the expression of meiosis-specific gene in female germ cells. However, there was no a significant change in the proportion of male and female mice offspring after BPA treatment (data not shown).

As an estrogenic environmental toxin, BPA exerts its biological activities via estrogen receptors [35]. There are two classical nuclear estrogen receptors, ERα and ERβ. This may be due to influences of BPA through the classical nuclear estrogen receptors, ERα and ERβ. It showed that BPA acts as a SERM (selective estrogen receptor modulator), and it possess functions similar to estradiol. BPA can interact with the ligand binding domain of ERs [36] and display differential binding affinity for ERα and ERβ in targeted cells [37, 38]. Since BPA is an estrogen-like compound, the relationship between ER and BPA was analyzed in our study. In our previous study, the mRNA expression levels of ER in the BPA-treated and control groups at 17.5 dpc were observed [13], by the way it revealed that BPA significantly increased ERα expression ($p < 0.05$) and show no effect on ERβ expression (data not shown). *ER* over-expression and DNA methylation modification of imprint genes regulate the relative gene expression. However, it is unknown whether the expression of *ER* is regulated by the DNA methylation. In our previous study, BPA-induced imprinting gene hypomethylation was eliminated by an *ER* inhibitor ICI182780, suggesting that ER was the mediator in the process of BPA induced hypomethylation [12]. In a conclusion, BPA influences DNA methylation of the imprinting genes via regulating ER expression.

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References

1. McLaren A (2003) Primordial germ cells in the mouse. Dev Biol 262:1–15
2. Lee J, Inoue K, Ono R, Ogonuki N, Kohda T, Kaneko IT, Ogura A, Ishino F (2002) Erasing genomic imprinting memory in mouse clone embryos produced from day 11.5 primordial germ cells. Development 129:1807–1817
3. Reik W (2007) Stability and flexibility of epigenetic gene regulation in mammalian development. Nature 447:425–432
4. Saffman EE, Lasko P (1999) Germline development in vertebrates and invertebrates. Cell Mol Life Sci 55(8–9):1141–1163
5. Bird AP (1986) CpG-rich islands and the function of DNA methylation. Nature 321:209–213
6. Chapin RE, Adams J, Boekelheide K, Gray J, Hayward SW, Lees PS, McIntyre BS, Portier KM, Schnorr TM, Selevan SG, Vandenbergh JG, Woskie SR (2008) NTP-CERHR expert panel report on the reproductive and developmental toxicity of bisphenol A. Birth Defects Res 83:157–395
7. Ho SM, Tang WY, de Belmonte Frausto J, Prins GS (2006) Developmental exposure to estradiol and bisphenol A increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4. Cancer Res 66: 5624–5632
8. Markey CM, Luque EH, Munoz De Toro M, Sonnenschein C, Soto AM (2001) In utero exposure to bisphenol A alters the development and tissue organization of the mouse mammary gland. Biol Reprod 65:1215–1223
9. Rubin BS, Lenkowski JR, Schaeberle CM, Vandenberg LN, Ronsheim PM, Soto AM (2006) Evidence of altered brain sexual differentiation in mice exposed perinatally to low, environmentally relevant levels of bisphenol A. Endocrinology 147: 3681–3691
10. Nakamura K, Itoh K, Sugimoto T, Fushiki S (2007) Prenatal exposure to bisphenol A affects adult murine neocortical structure. Neurosci Lett 420:100–105
11. Nakamura K, Itoh K, Yaoi T, Fujiwara Y, Sugimoto T, Fushiki S (2006) Murine neocortical histogenesis is perturbed by prenatal exposure to low doses of bisphenol A. J Neurosci Res 84: 1197–1205
12. Chao HH, Zhang XF, Chen B, Pan B, Zhang LJ, Li L, Sun XF, Shi QH, Shen W (2012) Bisphenol A exposure modifies methylation of imprinted genes in mouse oocytes via the estrogen receptor signaling pathway. Histochem Cell Biol 137:249–259
13. Zhang HQ, Zhang XF, Zhang LJ, Chao HH, Pan B, Feng YM, Li L, Sun XF, Shen W (2012) Fetal exposure to bisphenol A affects the primordial follicle formation by inhibiting the meiotic progression of oocytes. Mol Biol Rep 39:5651–5657
14. Pesce M, De Felici M (1995) Purification of mouse primordial germ cells by MiniMACS magnetic separation system. Dev Biol 170:722–725
15. Pan B, Chao HH, Chen B, Zhang LJ, Li L, Sun XF, Shen W (2011) DNA methylation of germ cell-specific basic helix-loop-helix (HLH) of transcription factors, Sohlh2 and Figα, during germ cell development. Mol Hum Reprod 17:550–561
16. Li E, Beard C, Jaenisch R (1993) Role for DNA methylation in genomic imprinting. Nature 366:362–365
17. Li L, Keverne EB, Aparicio SA, Ishino F, Barton SC, Surani MA (1999) Regulation of maternal behavior and offspring growth by paternally expressed Peg3. Science 284:330–333
18. Hall JM, McDonnell DP (2005) Coregulators in nuclear estrogen receptor action: from concept to therapeutic targeting. Mol Interv 5:343–357
19. Reik W, Walter J (2001) Genomic imprinting: parental influence on the genome. Nat Rev Genet 2:21–32
20. McGrath J, Solter D (1984) Completion of mouse embryogenesis requires both the maternal and paternal genomes. Cell 37: 179–183
21. Surani MA, Barton SC, Norris ML (1984) Development of reconstituted mouse eggs suggests imprinting of the genome during gametogenesis. Nature 30:548–550

22. Hajkova P, Erhardt S, Lane N, Haaf T, El-Maarri O, Reik W, Walter J, Surani MA (2002) Epigenetic reprogramming in mouse primordial germ cells. Mech Dev 117:15–23
23. Li JY, Lees–Murdock DJ, Xu GL (2004) Timing of establishment of paternal methylation imprints in the mouse. Genomics 84: 952–960
24. Yamazaki Y, Low EW, Marikawa Y (2005) Adult mice cloned from migrating primordial germ cells. Proc Natl Acad Sci USA 102:11361–11366
25. Yamazaki Y, Mann MR, Lee SS, Marh J, McCarrey JR, Yanagimachi R, Bartolomei MS (2003) Reprogramming of primordial germ cells begins before migration into the genital ridge, making these cells inadequate donors for reproductive cloning. Proc Natl Acad Sci USA 100:12207–12212
26. Choi Y, Qin Y, Berger MF, Ballow DJ, Bulyk ML, Rajkovic A (2007) A Microarray analyses of newborn mouse ovaries lacking Nobox. Biol Reprod 77:312–319
27. Suzumori N, Yan C, Matzuk MM, Rajkovic A (2002) Nobox is a homeobox encoding gene preferentially expressed in primordial and growing oocytes. Mech Dev 111:137–141
28. Huntriss J, Hinkins M, Picton HM (2006) cDNA cloning and expression of the human NOBOX gene in oocytes and ovarian follicles. Mol Hum Reprod 12:283–289
29. Qin Y, Choi Y, Zhao H, Simpson JL, Chen ZJ, Rajkovic A (2007) NOBOX homeobox mutation causes premature ovarian failure. Am J Hum Genet 81:576–581
30. Qin Y, Shi Y, Zhao Y, Carson SA, Simpson JL, Chen ZJ (2009) Mutation analysis of NOBOX homeodomain in Chinese women with premature ovarian failure. Fertil Steril 91:1507–1509
31. Simpson JL (2008) Genetic and phenotypic heterogeneity in ovarian failure: overview of selected candidate genes. Ann NY Acad Sci 1135:146–154
32. Rajkovic A, Pangas SA, Ballow D, Suzumori N, Matzuk MM (2004) NOBOX deficiency disrupts early folliculogenesis and oocyte-specific gene expression. Science 305:1157–1159
33. Lechowska A, Bilinski S, Choi Y, Shin Y, Kloc M, Rajkovic A (2011) Premature ovarian failure in nobox-deficient mice is caused by defects in somatic cell invasion and germ cell cyst breakdown. J Assist Reprod Genet 28:583–589
34. Bouilly J, Bachelot A, Broutin I, Touraine P, Binart N (2011) Novel NOBOX loss of function mutations account for 6.2 % of cases in a large primary ovarian insufficiency cohort. Hum Mutat 32:1108–1113
35. Krishnan AV, Stathis P, Permuth SF, Tokes L, Feldman D (1993) Bisphenol-A: an estrogenic substance is released from polycarbonate flasks during autoclaving. Endocrinology 132:2279–2286
36. Gould JC, Leonard LS, Maness SC, Wagner BL, Conner K, Zacharewski T, Safe S, McDonnell DP, Gaido KW (1998) Bisphenol A interacts with the estrogen receptor alpha in a distinct manner from estradiol. Mol Cell Endocrinol 142:203–214
37. Routledge EJ, White R, Parker MG, Sumpter JP (2000) Differential effects of xenoestrogens on coactivator recruitment by estrogen receptor (ER) alpha and ERbeta. J Biol Chem 275: 35986–35993
38. Kuiper GG, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S, Gustafsson JA (1997) Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology 138:863–870