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G9a-mediated histone methylation regulates cadmium-induced male fertility damage in pubertal mice

Min Li[a,1], Chuan Liu[a,1], Lingling Yang[a], Lei Zhang[a], Chunhai Chen[a], Mindi He[a], Yonghui Lu[a], Wei Feng[b], Huifeng Pi[a], Yanwen Zhang[a], Min Zhong[a], Zhengping Yu[a,2,*], Zhou Zhou[a,2,*]

[a] Department of Occupational Health, Third Military Medical University, Chongqing 400038, People's Republic of China
[b] Department of Occupational and Environmental Health, School of Public Health and Health Management, Chongqing Medical University, Chongqing 400016, People's Republic of China

HIGHLIGHTS

- Pubertal Cd exposure induced male fertility damage and testicular cell apoptosis.
- Pubertal Cd exposure induced elevation of G9a-mediated histone methylation.
- BIX-01294 blocked Cd-induced male fertility damage and testicular cell apoptosis.

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ABSTRACT

Increasing evidence suggests that cadmium (Cd) is associated with male fertility damage. However, the effects of histone modification on Cd-induced male fertility damage remain obscure. This study aims to evaluate the roles of histone methylation mediated by euchromatin histone methyltransferase (EHMT2/G9a) in regulating Cd-induced male fertility damage. We exposed 4-week-old male C57BL/6J mice to Cd by intraperitoneal injection at 2 mg/kg for 1, 3 and 5 days. Our data showed that Cd exposure decreased the numbers of impregnated females and litter sizes, which was concomitant with sperm count reduction, histological changes in the cauda epididymal ducts and seminiferous epithelium, and testicular cell apoptosis as evaluated by terminal dUTP nick-end labeling (TUNEL) assay and immunoblotting with increased levels of cleaved caspase 3, PARP and Bax and a decreased level of Bcl-2. Cd-induced male fertility damage was accompanied by enhanced G9a activity followed by increased histone H3 lysine 9 monomethylation (H3K9me1) and dimethylation (H3K9me2) in testes. Furthermore, inhibition of G9a by BIX-01294 normalized H3K9me1 and H3K9me2 to basal levels and prevented Cd-induced male fertility damage and testicular cell apoptosis. Our present study revealed that G9a-mediated histone methylation plays a critical role in Cd-induced male fertility damage and testicular cell apoptosis.

1. Introduction

Cadmium (Cd), a highly toxic heavy metal, is a major environmental and occupational contaminant found in soils, water and air (Satarug et al., 2003). The general public is exposed to Cd via industrial contamination, drinking water, food and tobacco smoke (Perlman et al., 2012). A vast number of studies show that Cd exposure is correlated with the increasing incidence of male fertility damage (Benoff et al., 2000). Epidemiological investigation suggests that Cd exposure adversely affects sex hormone concentration, sperm parameters and male fertility (Benoff et al., 2000). Besides, seminal fluid Cd level is associated with male fertility and poor semen quality in humans (Al-Azemi et al., 2010; Pant et al., 2003). Accordingly, a recent study shows

* Corresponding authors.
E-mail addresses: yuzping_tmmu@126.com (Z. Yu), lunazhou00@163.com (Z. Zhou).
[1] These authors contributed equally to this work.
[2] These authors share last authorship.

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that Cd exposure via drinking water reduces sperm count and seminal plasma in humans. Moreover, a high correlation is observed between altered semen parameters and lower level of accessory sex gland markers such as fructose, acid phosphatase, and neutral α-glucosidase (Sengupta et al., 2013). Even a low level of Cd accumulation in semen may reduce sperm quality and result in male infertility (Wu et al., 2008).

In rodent animals, exposure to Cd can adversely affect the male reproductive system via degenerative alterations in the seminal vesicles, epididymis and testis, including severe testicular degeneration, seminiferous tubule damage and hemorrhagic necrosis (Burukoglu and Baycu, 2008). There is a positive correlation between Cd exposure and asthenozoospermia in animal models (Benoff et al., 2008). Moreover, Cd induces testicular cellular toxicity by causing oxidative stress and endoplasmic reticulum (ER) stress, resulting in abnormal DNA repair mechanisms and cell apoptosis (Ji et al., 2012; Valko et al., 2006). More specifically, Cd exposure induces the generation of testicular reactive oxygen species (ROS) and elevated lipid peroxidation, which can impair intracellular defenses and cause oxidative damage, and then altered spermatogenesis (Acharya et al., 2008). In addition, it has been reported that epigenetics is implicated in male fertility (Liu et al., 2015), although the role of epigenetic mechanism in Cd-induced male fertility damage is unknown.

Histone modification is a primary component of epigenetic programing, which plays a dominant role in both normal and disease processes (Anway et al., 2005; Chen et al., 2015). One of the most important histone methyltransferases (HMTases), G9a, has an essential role in performing the monomethylation and dimethylation of H3K9 (marked by H3K9me1 and H3K9me2, respectively) (Shinkai and Tachibana, 2011), which has a critical role in heterochromatin formation and transcriptional silencing. Moreover, germ-lineage-specific deletion of the G9a gene induces an observable loss of H3K9me1/2 during meiotic prophase, accompanied with developmental defects at the pachytene stage, and a marked loss of germ cells, thereby leading to male fertility damage (Tachibana et al., 2007). Therefore, the balance of histone methylation mediated by G9a is of paramount importance to the male reproductive system.

Several lines of evidences show that the interference of H3K9me2, which is implicated in the regulation of gene expression, may alter the susceptibility or tolerance of cells to stress, and induce apoptosis via a caspase-dependent pathway (Yu et al., 2013). Furthermore, G9a negatively affects proliferation and cell viability by upregulating the p21 (Oh et al., 2014), which can increase levels of Bax, decrease levels of Bcl-2, and then induce p53-null H1299 cell apoptosis (Chresta et al., 1996). In addition, in the developing brain, G9a-mediated H3K9me2 critically regulates ethanol-induced neurodegeneration (Subbanna et al., 2013). Nevertheless, whether Cd-associated male fertility damage involves alterations of G9a-mediated H3K9 methylation is still unknown.

Based on these considerations, we hypothesized that G9a-mediated histone methylation may play important roles in Cd-induced male fertility damage. So we conducted the present study, in which Cd was administered to pubertal mice via intraperitoneal injection at 2 mg/kg of body weight for 1, 3, and 5 days. After Cd exposure, testicular cell apoptosis was assessed using a terminal dUTP nick-end labeling (TUNEL) assay and western blotting with cleaved caspase 3, PARP, Bax and Bcl-2. Additionally, histone methylation was evaluated using immunofluorescence and western blotting for H3K9me1 and H3K9me2.

2. Materials and methods

2.1. Animals and treatments

Male (3 weeks old) and female (8 weeks old) C57BL/6J mice were purchased from the Laboratory Animal Center of the Third Military Medical University (Chongqing, China) and were acclimatized in our Laboratory for one week before the experiment. All animals were kept under standard conditions (23 ± 1 °C, 55 ± 5% humidity, 12-h light/dark cycle), and were given access to a standard laboratory diet and water ad libitum. All of the animals were treated humanely and with regard for the alleviation of suffering. This study was approved by the Third Military Medical University Institutional Animal Care and Use Committee.

The mice were randomly divided into either a control group or an experimental group. Mice in the control group were administered normal saline (the solvent of cadmium), and mice in the other groups were injected intraperitoneally (i.p.) with 2 mg/kg of body weight of cadmium (Sigma-Aldrich, St. Louis, MO, USA) for 1, 3 and 5 days, once a day (Ji et al., 2012; Li et al., 2016). For the BIX-01294 pretreatment, BIX-01294 (Cayman, Michigan, USA) was dissolved in sterile deionized water and then diluted with sterile saline. The BIX-01294 solution was injected by i.p. 30 min before Cd treatment.

2.2. Epididymal sperm count

The epididymides were dissected immediately from mice testis after euthanasia, and then the cauda epididymides were transferred to 400 μl of pre-warmed Ham's F-12 (GIBCO, Grand Island, NY, USA) and pierced with needle to allow spermatozoa to swim out for 5 min at 37 °C in 5% CO_2. Sperm concentration was evaluated using computer-assisted sperm analysis (CASA) (Hamilton Thorne, USA) based on WHO guidelines.

2.3. Male fertility assay

Male mice were kept until 8 weeks after the last treatment, and then individually housed and partnered with three virgin female C57BL/6J mice of 8 weeks old for 5 consecutive days. Female were examined every morning for vaginal plugs during cohabitation to determine if mating had occurred. The plug-positive females were separated and the pregnancies were allowed to progress (Fan et al., 2015). Approximately twenty-one days after the last day of cohabitation, the number of pregnant females and the litter size of each male mouse were counted.

2.4. Testis and epididymides histology

After performing a perfusion with saline and paraformaldehyde, testis were pierced with a needle and post-fixed in Bouin's Solution overnight. Then, the tissue were dehydrated in gradient alcohol, followed by embedding in paraffin and sectioned at 5 μm. The slices were then deparaffinized with xylene and rehydrated with ethanol, prepared for routine hematoxylin and eosin staining. Images were captured under the microscope at 200 × magnification (Leica DM6000B, Wetzlar, Germany). Testicular sections used to assess both qualitative evaluation and quantitative structural modifications. A total of 30 cross-sections of seminiferous tubules were randomly analyzed for each animal and each tubule was scored according to the level of damage, such that no damage, mild damage, moderate damage and extensive damage were scored as 0, 1, 2 and 3, respectively (Padmanabhan et al., 2009). The final seminiferous tubule damage score was acquired by the sum of the score of the chosen tubules.