CELL CYCLE
2018, VOL. 17, NOS. 21–22, 2447–2459
https://doi.org/10.1080/15384101.2018.1542896

Taylor & Francis
Taylor & Francis Group

RESEARCH PAPER

PHF1 is required for chromosome alignment and asymmetric division during mouse meiotic oocyte maturation

Yi Qu[a], Yang Wang[a], and Jie Qiao [a,b,c,d]

[a]Key Laboratory of Assisted Reproduction, Ministry of Education, Center of Reproductive Medicine, Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing, China; [b]Department of Obstetrics and Gynecology, Beijing Advanced Innovation Center for Genomics, College of Life Sciences, Third Hospital, Peking University, Beijing, China; [c]Biomedical Institute for Pioneering Investigation via Convergence & Key Laboratory of Assisted Reproduction, Ministry of Education, Beijing, China; [d]Peking-Tsinghua Center for Life Sciences, Peking University, Beijing, China

ABSTRACT

In recent years, the etiological study of oocyte maturation failure and other mechanisms of early embryonic development has gradually advanced. However, while some achievements have been made in this field, the intrinsic mechanisms underlying disordered oocyte maturation remain unclear. Polycomb group proteins (PcG) are a family of proteins that are involved in the epigenetic silencing of genes. Many members of this family are reportedly involved in mammalian oocyte maturation and early embryonic development. PHD finger protein 1 (PHF1) is a core member of the polycomblike group of proteins, although its role in oocyte maturation and early embryonic development are unknown. A previous study by our group using single cell transcriptome analysis and high-throughput technology revealed that PHF1 mRNA was elevated in the human oocyte and the early preimplantation embryo. This suggests that PHF1 may play an important role in oocyte maturation and early embryonic development. In the present study, we aimed to reveal the biological function of PHF1 in mouse oocyte maturation and illuminate its regulatory mechanisms. We report here, for the first time, that PHF1 is necessary for the accurate alignment of chromosomes and oocyte euploidy, as well for the regulation of the asymmetric division of oocytes in mouse. The results of the present study may have the potential to provide a new research direction of human oocyte maturation disorder and early embryonic development block. These results may also provide new diagnosis or treatment strategies for clinical patients.

ARTICLE HISTORY
Received 14 June 2018
Revised 17 October 2018
Accepted 19 October 2018

KEYWORDS
PHF1; oocyte maturation; chromosome alignment; asymmetric division; BubR1

Introduction

Due to increasing environmental pollution, progressively delayed reproductive age, a rise in malignant diseases, and other factors, human fertility has begun to face an unprecedented threat in recent years. Some reports suggest that infertility is the third most common disease, following tumors/cancer and cardiovascular/cerebrovascular disease. The prevalence of infertility in developed countries ranges from 8% to 15%, while in developing countries it can be as high as 30% [1]. In China, for example, nearly ten million couples suffer from infertility, and declines in human fertility have become a worldwide concern. Assisted reproductive technology (ART) has emerged in response to this growing societal and medical issue.

ART utilizes hormonal drugs to induce the production of multiple mature or immature eggs in the same cycle. The maturity of these eggs is directly related to the fertilization rate, embryo cleavage rate, and rate of successful pregnancy after implantation [2]. Although ART brings hope to patients with infertility, the widespread development of this technology in clinics is limited and oocyte maturation disorders remain one of the most prominent causes of ART failure [3]. At present, ART has a success rate of approximately only 30%. The successful maturation of oocytes and normal development of early embryos not only promotes the success of ART, but is also key to mammalian reproduction.

In addition to hindering reproduction, oocyte maturation disorders in mammals can lead to abnormal chromosome numbers in mature

CONTACT Jie Qiao jie.qiao@263.net
Supplemental data for this article can be accessed here.

oocytes [4]. Embryos produced after the fertilization of abnormal oocytes tend to exhibit chromosomal aneuploidy. While this will typically result in spontaneous abortion within three months of maternal pregnancy, in those rare cases where spontaneous abortion does not occur, the resulting infant will have a chromosomal disorder [5]. The most common chromosomal disorder caused by aneuploidy is trisomy 21, which causes Down Syndrome [6]. Therefore, exploration of the mechanisms underlying oocyte maturation and early embryonic development is highly relevant to both the development of ART and improving human fertility and embryonic health.

The polycomb protein (PcG) family consists of an important class of transcriptional repressors involved in epigenetics [7]. These proteins play an important biological role in development and differentiation. Mammals express two PcG family complexes – PRC1 and PRC2 [8]. The enzyme EZH2 is a core component of the PRC2 complex. It can directly methylate histone H3 through its catalytic subunit SET domain and has been found to regulate the maturation of mouse oocytes in vitro [9,10]. Additionally, EZH2 may play an important role in early embryonic development. This is supported by findings that, in early embryos lacking maternal EZH2, paternal gene methylation and subsequent embryonic development are affected [11,12].

In addition to EZH2, reports also suggest that the two additional components of the PRC2 complex – EED and SUZ12 – also play an important role in embryonic development in mice. Absence of EED and SUZ12 leads to disturbance in organ development and embryo abortion in transgenic mouse embryos [13–15]. PHF1 is an indispensable factor in the PRC2 complex and has been reported to interact with complex components such as EZH2, EED, and SUZ12. PHF1 can also locate the promoter region of the EZH2 target gene and assist EZH2 in inhibiting the expression of downstream genes such as those in the HOX family [16–18]. In a recent study, the inhibition of PHF1 expression in HeLa cells resulted in decreased H3K27Me3 across the genome as well as accelerated cell proliferation and abnormal cell morphology [18]. In addition, reports suggest that PHF1 can interact with multiple DNA damage-responsive proteins such as RAD50, SMC1, DHX9, and p53 to cause DNA double-strand breaks (DSBs). Studies have also shown that decreased PHF1 protein in HeLa cells can lead to an increase in the sensitivity of cellular DNA to X-ray radiation, increasing the cellular chromosome homologous recombination rate [19].

Collectively, the findings described above suggest some mechanisms for the molecular action of PHF1, though the function of PHF1 in oocyte maturation and early embryonic development remains unclear. To address this gap, a previous study by our group reported single-cell RNA sequencing (RNAseq) profiles of human preimplantation embryos and embryonic stem cells. We found that PHF1 expression is elevated in both stages, suggesting its biological importance to oocyte maturation and early embryonic development [20].

Although etiological studies of oocyte maturation disorders have made some contributions to the scientific understanding of these pathologies in recent years, their specific etiopathological mechanisms remain unclear. In the present study, we examined the function of PHF1 in mouse oocyte maturation to elucidate the molecular mechanisms through which PHF1 regulates downstream genes. The ultimate goal of this work was to provide insights into new diagnosis and treatment strategies for clinically difficult cases of abnormal oocyte maturation.

Results

Subcellular localization and expression of PHF1 during mouse oocyte meiotic maturation

To explore the function of PHF1 in mouse oocyte meiotic maturation, we first examined the subcellular localization of PHF1. Mouse oocytes were collected at the GV (Germinal vesicle), GVBD (Germinal vesicle breakdown), Pro-Met I (Prometaphase I), MI (Metaphase I), AI-TI (Anaphase I-telophase I), and MII (Metaphase II) stages of meiosis. Using immunofluorescent staining with a PHF1 antibody and additional positive control, we observed that PHF1 was mainly distributed in the nucleus at GV stage (Figure 1(a)). Shortly after GVBD, PHF1 began to migrate to the periphery of chromosomes. Until formation of the

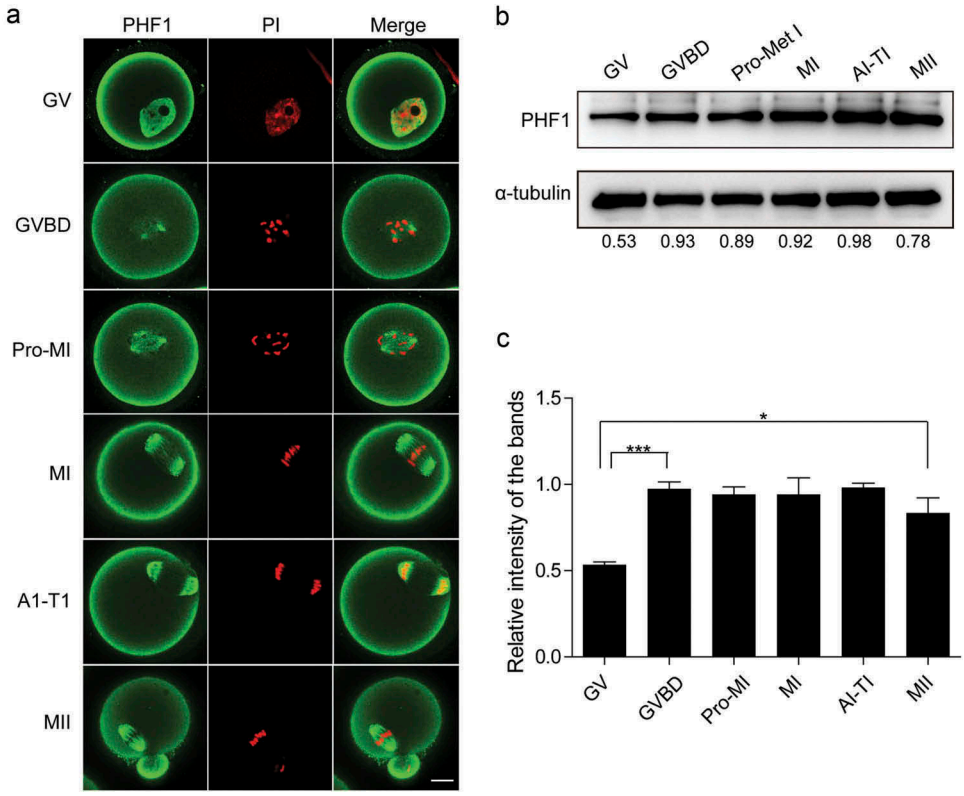

Figure 1. Expression and subcellular localization of PHF1 during oocyte maturation. (a) Subcellular localization of PHF1 (green) at GV, GVBD, pre-MI, MI, AI-TI and MII stages by confocal microscope scanning. DNA of chromosomes was stained with PI (Propidium iodide, red). Scale bar = 20 μm. (b) Oocytes of mouse at different stages of meiotic maturation were collected for Western blot. Proteins from a total of 100 oocytes were loaded for each sample. PHF1 level were detected by anti-PHF1 antibody and α-tubulin was used as a loading control. PHF1 amount at each stage from a representative experiment was quantified and displayed as indicated. (c) PHF1 amount at each stage from 4 independent Western blot analysis experiments was quantified. Data were normalized to the abundance of internal control α-tubulin. Data are shown as mean ± SEM from three independent experiments, $p < 0.001$ or 0.05.

MI spindle, PHF1 seemed to localize to the spindle and cortical regions at during the MI, AI-TI, and MII stages (Figure 1(a)). To avoid false positives, we purchased another PHF1 antibody and repeated the above immunofluorescence experiments. The results PHF1 was localized to the nucleus at during the GV stage and moved to the spindle at during the MI and MII stages (Supplementary Figure 1). The use of two antibodies that delivered the same results suggested that our subcellular PHF1 localization experiments were reliable. Since the new antibody did not show the remarkable result that the original one did, the following results are demonstrated by using the original antibody. Furthermore, to examine the expression of PHF1 in mouse oocytes, 100 oocytes were collected at different stages of meiotic maturation. Western blotting revealed that the PHF1 protein was present throughout all of meiosis and expressed at similar levels from GV to MII (Figure 1(b)). Quantification data from the 4 set samples showed that PHF1 protein gradually increased from the GV to AI-TI stages with a peak at AI-TI and there was a moderate decline at the MII stage (Figure 1(c) and the other three analysis results are presented in Supplementary Figure 2). The sustained expression of PHF1 during maturation suggested that PHF1 may play a role in subsequent fertilization and embryonic development processes.

Localization of PHF1 in mouse oocytes treated with spindle-perturbing agents

To further define the localization of PHF1, we double-stained oocytes with α-tubulin and PHF1 antibodies. This labeling demonstrated PHF1 almost overlapped significantly with α-tubulin at

during the MI and MII stages (Figure 2(a)). Furthermore, given that PHF1 was primarily localized to the spindle after Pro-Met I, we investigated the correlation between PHF1 and microtubule dynamics. First, we treated the oocytes with Taxol, a microtubule-stabilizing agent, at the MI stage. From this we found that microtubule fibers in these oocytes were

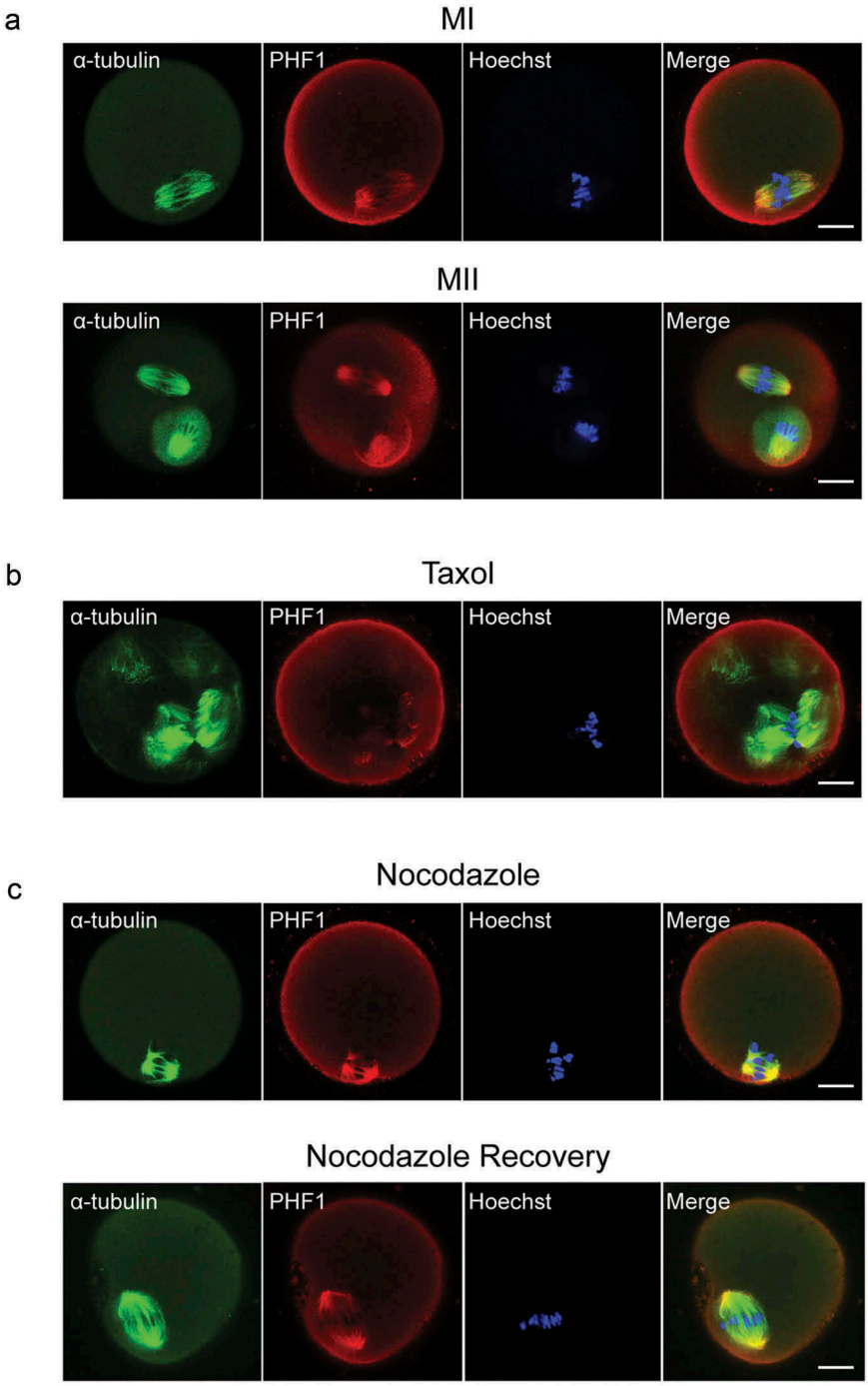

Figure 2. Localization of PHF1 in mouse oocytes treated with taxol and nocodazole. (a) Oocytes cultured for 7 h and 13 h, corresponding to MI and MII stages, respectively. These oocytes were fixed and co-stained with PHF1 (red) and α-tubulin (green). DNA (blue) was visualized with Hoechst 33342 staining. Scale bar = 20 μm. (b) Oocytes at MI stage were incubated in M2 medium with 10 μM taxol for 45 min and then double stained with PHF (red) and α-tubulin (green). The sample was counterstained with Hoechst 33342 to visualize DNA. Scale bar = 20 μm. (c) The MI oocyte first treated with M2 medium containing 10 mg/ml nocodazole, and then washed thoroughly with fresh M2 medium. After recovering for 30 min the oocytes were fixed and stained for PHF1 (red), α-tubulin (green) and DNA (blue). Scale bar = 20 μm.

excessively polymerized, exhibited significantly enlarged spindles, and contained numerous cytoplasmic asters. Additionally, these abnormal spindle fibers and cytoplasmic asters were positive for PHF1 (Figure 2(b)).

Next, we briefly exposed MI stage oocytes in M2 medium containing Nocodazole (20 μg/ml), a microtubule-depolymerizing drug. The microtubules in these oocytes appeared partially depolymerized, though an intact spindle morphology was retained. We further observed that most PHF1 was altered, an endophenotype that correlated with the partial disassembly of spindle microtubules (Figure 2(c), upper). After washing these oocytes with fresh M2 medium and culturing them for an addition 30 min, we found that PHF1 remained associated with microtubules even following spindle re-assembly (Figure 2(c), lower). These findings clearly demonstrated that PHF1 was consistently localized to and dependent on spindle microtubules.

PHF1 depletion affected polar body extrusion (PBE) and chromosome alignment

To further dissect the role of PHF1 during mouse oocyte meiotic maturation, control siRNA or PHF1-siRNA were microinjected into GV stage oocytes. When compared with controls, PHF1 protein was significantly reduced after PHF1-siRNA treatment according to assessment by western blot (Figure 3(a)). Furthermore, after depletion of PHF1, we found that the PB1 extrusion rate in oocytes was also decreased at 10, 12, and 13h after treatment (Figure 3(b); Control: 10 h: 19.7%, 12 h: 58.1%, 13 h: 69.6%, n = 135; PHF1-siRNA: 10 h: 2.9%, 12 h: 44.9%, 13 h: 54.4%, n = 136). As PHF1 was found to be localized to the spindle after

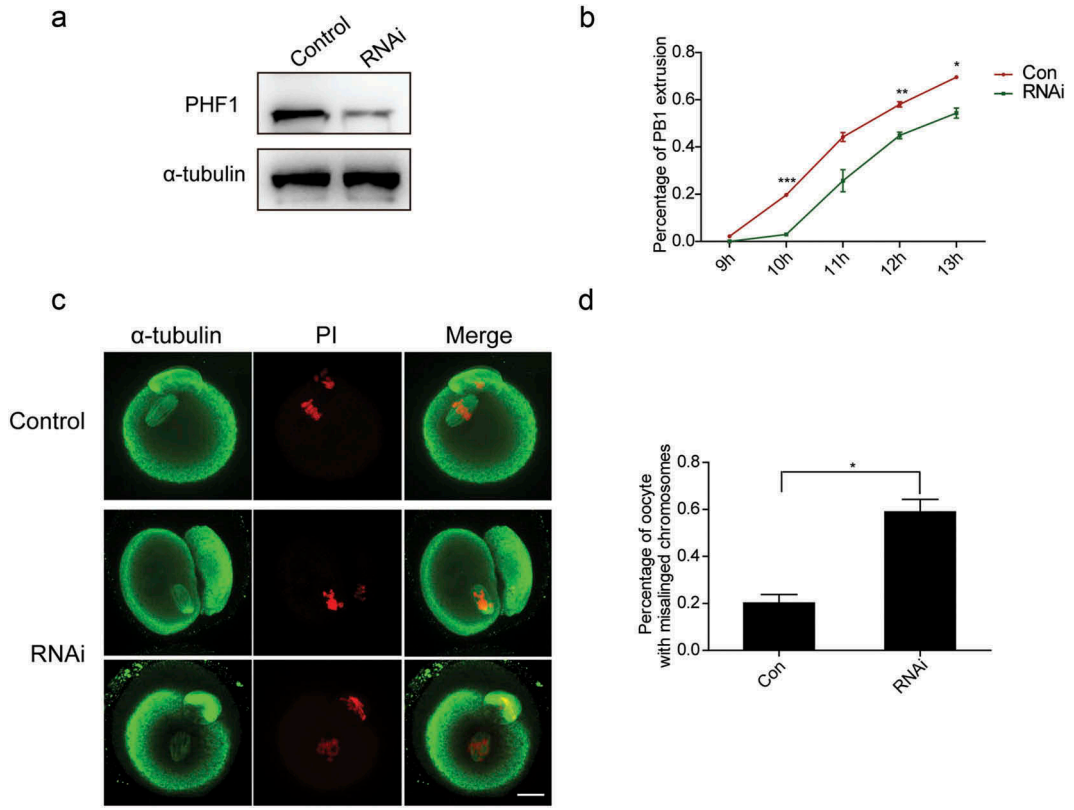

Figure 3. Lack of PHF1 affects the first polar body extrusion and leads to chromosome misalignment. (a) Depletion of PHF1 was determined by Western blot analysis using PHF1 antibody after endogenous PHF1 was depleted by siRNA. And α-tubulin was used as a loading control. (b) PB1extrusion rates were decreased after siRNA treatment. Data were presented as mean ± SEM from three independent experiments. Different superscripts indicate statistical difference at 10 h, 12 h and 13 h ($p < 0.05$). (c) Oocytes microinjected with the PHF-siRNA displayed chromosome misalignment. Part of them demonstrated "2-cell-like" oocytes (Panel 2). (d) Percentage of oocytes with chromosome misalignment in the PHF1-siRNA microinjected group and Control. Data were presented as mean ± SEM from three independent experiments, $p < 0.05$.

the Pro-Met I stage, we depleted PHF1 in MII stage oocytes by siRNA and stained with α-tubulin antibody and PI. By immunofluorescent staining, we further identified that in the PHF1-siRNA group, oocytes also exhibited chromosomal defects (Figure 3(c)). The ratio of misaligned chromosomes in the PHF1-siRNA microinjected group was much higher than that in the control group (Figure 3(d); Control: 20.2%± 3.7%, n = 80; PHF1-siRNA: 58.9%± 5.4%, n = 71; $p < 0.05$). In addition, we found that the absence of PHF1 does not seem to affect spindle morphology. In both control and PHF1-siRNA-treated oocytes, spindles were bipolar, not aberrantly nonpolar or multipolar, because γ-tubulin can affect the spindle structure. To further explain this observation, after depletion of PHF1, we used immunofluorescence to assess the cellular localization of γ-tubulin. As expected, PHF1 depletion had no influence on γ-tubulin localization (Supplementary Figure 3).

PHF1 depletion caused failure of cortical reorganization, disrupted K-MT attachment, and led to aneuploidy

We sought to further investigate the causes of the above-described oocyte abnormalities after PHF1 depletion or knock-down. As we found that depletion of PHF1 caused MI stage arrest as well as a two-cell-like MII oocyte phenotype (Figure 3(c), second parallel row), we first examined actin cap formation, a marker of oocyte polarity. We labeled F-actin with phalloidin to detect actin cap formation. After being cultured for 8.5 h, an actin cap appeared prominently in control oocytes while PHF1-siRNA oocytes exhibited centrally localized metaphase I chromosomes but lacked a cortical actin cap (Figure 4(a)). Later, we quantified the protein expression of F-actin after knocking down PHF1 in the oocyte. However, when we compared it with the control group, the level of F-actin in the knockdown group decreased, but the change was not statistically significant (Supplementary Figure 4). Therefore, we speculate that PHF1 can only affect the distribution of F-actin but not its expression in oocytes. As instability in the K-TM attachments can lead to chromosomal misalignment, CREST can be used to study the structural dynamics of constitutive centromere proteins in the kinetochores in mammalian oocytes. The kinetochores associated with the centromeres of chromosomes are responsible for maintaining the connection with the microtubules in the mitotic and meiotic spindles. Therefore, we further used CREST antibody, α-tubulin antibody, and the cold treatment assay to detect whether PHF1 was also associated with K-MT attachments. After 10 min incubation in 4°C M2 medium, the control group spindle microtubules remained connected to the kinetochore while in PHF1 knockdown oocytes, some kinetochores were not attached to the spindle (Figure 4(b)). Given that PHF1 depletion disrupted chromosomal alignment and K-MT attachments, we used a chromosome spreading assay to examine whether the chromosomes were undergoing appropriate segregation. The results of this assay indicated that, in the PHF1-siRNA group, the proportion of aneuploidy oocytes was significantly elevated (Figure 4(c,d), Control: 20.8%± 1.2%, n = 23; PHF1-siRNA: 41.6%± 2.3%, n = 19, $p < 0.05$).

Increased levels of PHF1 led to impaired PB1 extrusion and chromosome misalignment

Collectively, the above results indicate that an absence of PHF1 caused meiotic abnormalities in oocytes. Given this, we wondered whether increased PHF1 might also affect oocyte maturation. To examine this question, we microinjected myc-PHF1 mRNA into mouse oocytes at the GV stage and, after 6 h of inhibition by milrinone, the oocytes were collected for analysis by western blot using an Myc antibody for detection (Figure 5(a)). In another experiment, oocytes were microinjected with mRNA-PHF1 and transferred into milrinone-free M2 medium. The percentage of MII oocytes at 9, 10, 11, 12, and 13 h was then assessed. At 11 and 13 h, the ratio of failure to extrude a PB was higher in the mRNA-PHF1 group than in the control group (Figure 5(b); control: 11 h: 40.3%, 13 h: 72.4%, n = 95; mRNA-PHF1: 11 h: 34.1%, 13 h: 56.7%, n = 129). We also found that, after overexpression of PHF1, the arrangement of chromosomes in MII oocytes was also disordered (Figure 5(c,d); Control: 19.6%± 3.1%, n = 56, mRNA-PHF1: 46.5%± 4.4%, n = 64). Furthermore, two-cell-like abnormalities

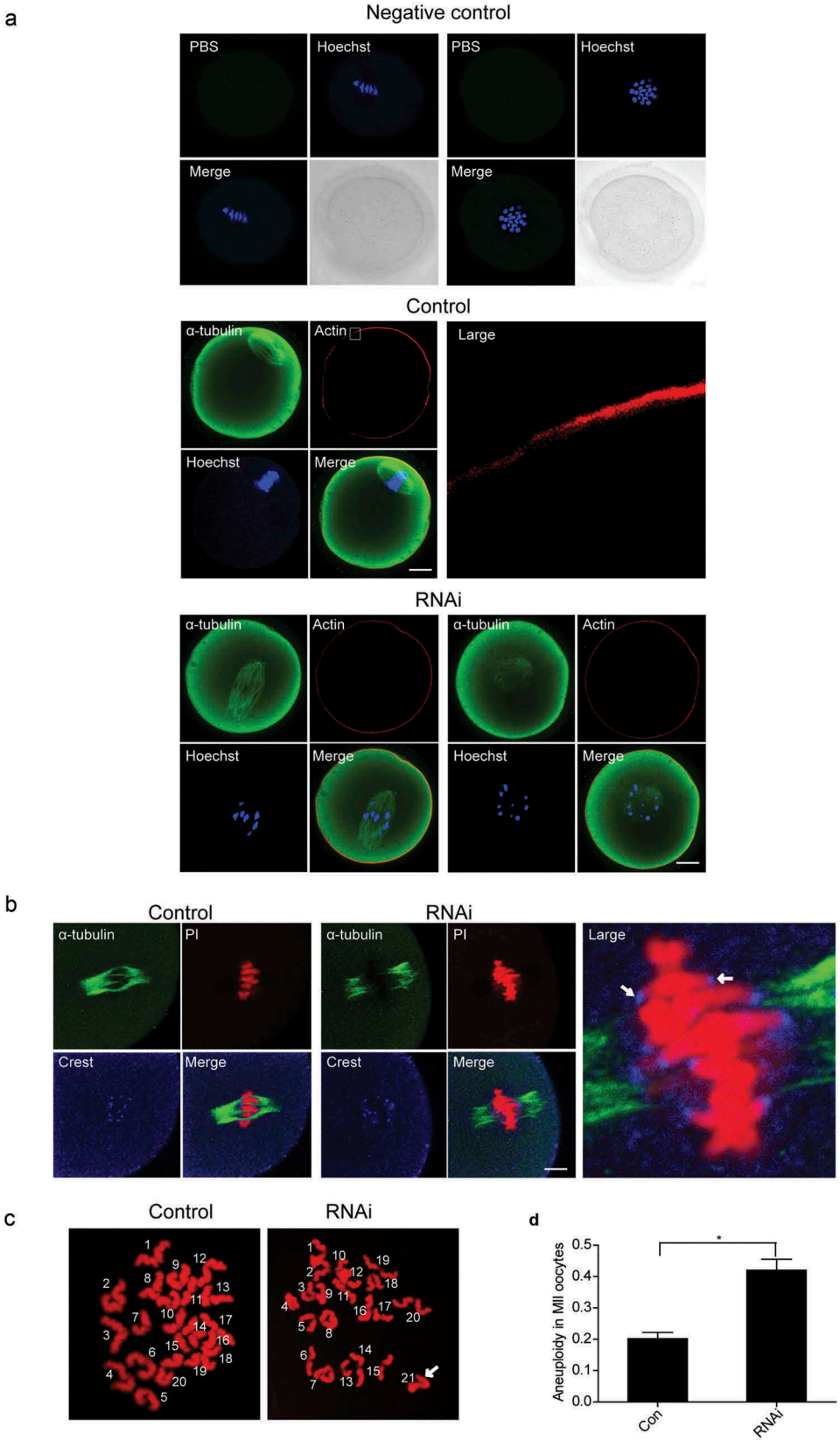

Figure 4. Effects of PHF1 depletion on actin cap formation, K-MT attachment and oocyte karyotype. (a) At the MI stage, no actin cap formed in PHF-siRNA group while an actin cap formed in the control group, and antibody injection oocytes. These oocytes were co-stain with a-tubulin (green) and Phalloidin (red). DNA of chromosomes was stained with Hochest33341. Scale bar = 20 μm. (b) The MI oocytes were cultured at 4°C for 10 min after microinjected with Control/PHF-siRNA. Images were acquired with a laser confocal microscope. Green: α-tubulin; Blue: Crest; Red: DNA. Scale bar = 5 μm. (c) Chromosome spreading was performed of MII oocytes after Control and PHF1-siRNA injection. Representative images of euploid and aneuploid oocytes were shown. (d) Quantification of aneuploidy in the control and PHF1 knockdown oocytes. The data were presented as mean ± SEM, $p < 0.05$.

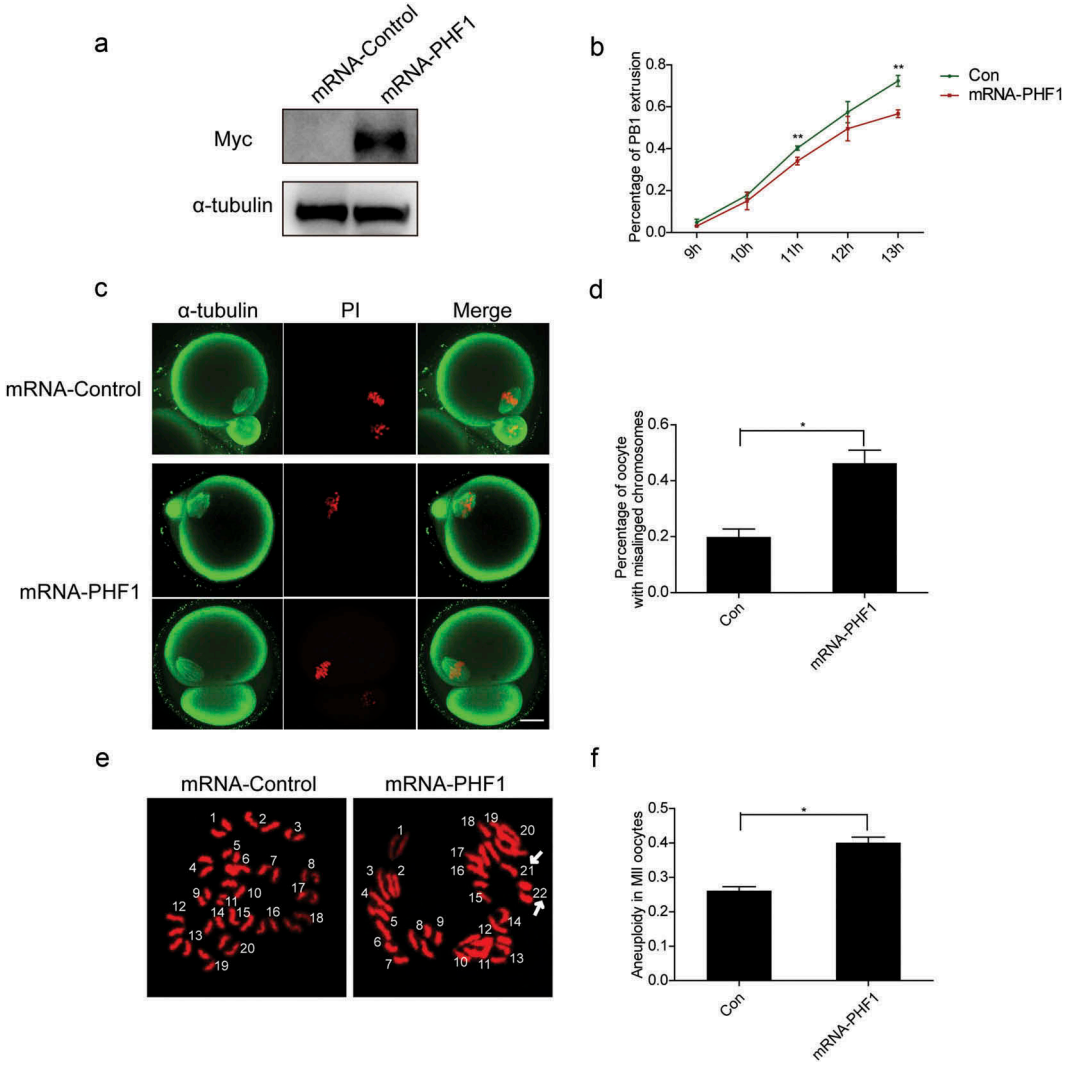

Figure 5. Raised level of PHF1 in oocytes disturbs the first polar body extrusion and chromosome alignment. (a) Western blot analysis was applied to confirm the microinjection of Myc-EZH2 into oocyte with a Myc antibody. The α-tubulin antibody was used as a loading control. (b) The rate of PB1extrusion between the control and mRNA-PHF1 groups at 11 h and 13 h were determined separately. Data were presented as mean ± SEM from three independent experiments, $p < 0.01$. (c) Increased level of PHF1 causes chromosome misalignment. Green: α-tubulin; Red: DNA. Scale bar = 20 μm. (d) Percentage of oocytes with chromosome misalignment at MII stage was quantified. Data were expressed as mean ± SEM from three independent experiments, $p < 0.05$. (e, f) Raised level of PHF1 in oocytes leads to aneuploidy. Chromosome spreading was performed in oocytes microinjected with the control or Myc-PHF1 mRNA and aneuploidy was seen (e). Percentage of aneuploidy occurrence was quantified (f). Data were expressed as mean ± SEM from three independent experiments, $p < 0.05$.

were also apparent in MII stage oocytes after the overexpression of PHF1 (Figure 5(c), third parallel row). Subsequent karyotype analyses showed some MII oocytes with aneuploidy due to increased level of PHF1 (Figure 5(e)), which were quantified as shown in Figure 5(f) (Control: 26.3%± 1.1%, n = 20; PHF1-mRNA: 40.5%± 1.9%, n = 25, $p < 0.05$).

Despite the fact that both knockdown and overexpression of PHF1 both resulted in multiple and aneuploidy, abnormal changes to oocytes, these abnormalities were more pronounced in PHF1 knockdown oocytes. This may be due to the relatively high initial expression of PHF1 in oocytes, meaning that a lack of PHF1 is more likely to affect oocyte maturation.

PHF1 may affect the expression of BubR1 in mouse oocytes

Evidence suggests that SAC proteins maintain correct chromosome alignment during meiosis, potentially via EZH2, another component of the

PRC2 complex that interacts with PHF1 and may serve to stabilize BubR1 expression during meiosis. Given this, so we wondered whether PHF1 could also affect BubR1expression. To this end, we found that endogenous BubR1 protein levels were decreased after knockdown of endogenous PHF1 by siRNA in mouse oocytes (Figure 6(a), left panel). When PHF1 expression was enhanced by microinjection of PHF1 mRNA into oocytes, endogenous BubR1 also increased (Figure 6(a), right panel). However, we found no obvious co-localization of PHF1 and BubR1 (Figure 6(b)).

Discussion

Oocyte maturation is a very important part of female reproduction, with many involved molecules and proteins. In the present study, we demonstrated the biological function of PHF1 in mouse oocyte meiosis. Specifically, we identified that PHF1 was required for the accurate alignment of chromosome and oocyte euploidy. Additionally, we found that PHF1 may also affect the asymmetric division of oocytes. To our knowledge, this represents the first evidence that PHF1 plays an important regulatory role in oocyte maturation.

PHF1 (PCL1), MTF2 (PCL2), and PHF19 (PCL3) are three polycomblike (PCL) genes in the human genome [21]. Mammalian PHF1 is a 456 amino acid protein which contains an N-terminal domain, a Tudor domain, and two PHD domains. PCL, the Drosophila ortholog of PHF1, is considered to be a component of PRC2 in the 1 MDa complex [22]. Critically, components of the PRC2 complex including EZH2, SUZ12, and EED have been reported to be involved in mammalian meiosis and subsequent embryonic development [10–15]. In mammalian mitosis, PHF1 is mainly localized to the nucleus. The function of these two PHD domains is thought to be assisting the PRC2 complex in inhibiting downstream gene expression and thus affecting the biological

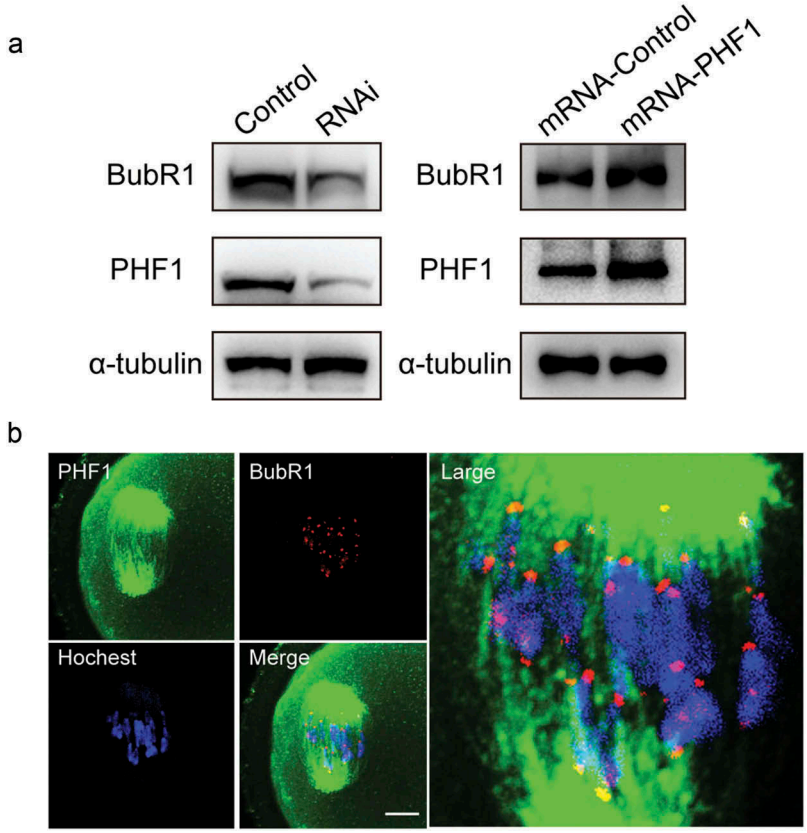

Figure 6. PHF1 regulate the expression of BubR1 in oocytes. (a) Western blot analysis showed that BubR1 protein level was decreased by siRNA depletion of endogenous PHF1 in oocytes (Left panel), and raised in oocytes with microinjection of PHF1 mRNA (Right panel). (b) Immunofluorescence images demonstrate no apparent co-localization of PHF1 and BubR1 in oocytes. Oocytes were stained with an EZH2 antibody (green), BubR1 was stained with a goat antibody (red) and chromosome were stained by Hoechst 33342. Scale bar = 5 μm.

functions of EZH2 [23]. In the present study, PHF1 was localized to the nucleus only at the GV stage and transferred to the spindle, cytoplasm, and cortex at the MI and MII stages in mouse oocytes. These results suggest that after GVBD, the role of PHF1 in meiosis may not be the same as in mitosis. It has been reported that EHZ2 is required for the maturation of the mouse oocyte, which does not rely on methylase activity. Furthermore, the mechanism by which EZH2 affects chromosome arrangement depends on its formation of a trimolecular complex with BubR1 and PCAF [10]. According to the localization of PHF1 in oocyte meiosis, we speculate that in meiosis, the role of PHF1 may be similar to that of EZH2, both of which may proceed regardless of H3K27 methylation.

Previous work has demonstrated that the SAC protein BubR1 is necessary for proper chromosome alignment and the stabilization of kinetochore-microtubule interactions [24,25]. As we mentioned above, EZH2 was reported to participate in the maturation of the mouse oocyte by stabilizing BubR1 expression during meiosis [10]. However, in the present study, while changes in PHF1 expression led to chromosomal misalignment and disordered K-TM attachment in mouse oocytes, PHF1 was not found to interact with BubR1 in mouse oocytes. As depicted in Figure 6, the level of BubR1 in oocytes depends on the presence of PHF1, but there is no co-localization of these two proteins in the subcellular fraction. Therefore, we speculate that PHF1 may be able to affect the expression of BubR1 at the mRNA level or by another, heretofore unidentified pathway. This requires future exploration.

In addition to affecting the maturation of mouse oocytes via BubR1, PHF1 may also regulate meiosis in oocytes via additional molecular mechanisms. In breast cancer, for example, PHF1 directly interacts with p53 proteins to act as a tumor suppressor gene [26]. Additionally, p53 is involved in homologous recombination and checkpoint function in meiotic male germ cells [27]. At the same time, intriguingly, Sherley's group demonstrated that, p53 plays a direct and central role in regulating the switch between asymmetric and symmetric cell divisions of stem cells [28]. Later, Insinga et al. proved that after DNA damage in normal stem cells, the inhibition of p53 activity could shift the cell divisions from asymmetric to symmetric self-renewal [29]. In our study, we also found that PHF1 is localized in the cortical region of the oocyte and involved in the formation of actin cap as well as the asymmetric division of oocyte. Therefore, we surmised that PHF1 may affect the maturation of oocytes by regulating or interacting with p53 during meiosis in oocytes.

Given that PHF1 directly contributed to HOX gene (the HOX family is widely expressed in mouse oocytes) silencing by promoting the recruitment of the PRC2 complex [16,18,30], PHF1 may also regulate the HOX family during oocytes meiosis. While compelling, this hypothesis requires further experimentation to verify.

In summary, in the present study we explored a new role for PHF1 in female mouse reproduction and demonstrated that PHF1 is required for oocyte meiotic maturation. We further revealed that PHF1 regulated the arrangement of chromosomes and maintained oocyte euploidy via regulating oocyte expression of BubR1. We also found that PHF1 influenced extrusion of the first polar body by preserving oocyte polarity. Collectively, our results demonstrate that PHF1 is a crucial regulator of mouse oocyte maturation and shed light on mechanisms which might be targeted by future clinical therapeutics.

Methods and materials

Antibodies

Rabbit monoclonal anti-PHF1 antibody (ab184951 and ab80042), rabbit monoclonal anti-α-tubulin antibody (ab52866), goat polyclonal anti-BubR1 antibody (ab28193) and rabbit monoclonal anti-γ-tubulin antibody (ab179503) were purchased from Abcam; and mouse monoclonal anti-α-Tubulin-FITC antibody (F2168) was purchased from Sigma; rabbit polyclonal anti-Myc (562) was purchased from MBL; and CREST, human anti-centromere antibody (HCT-0100) was brought from Immunovision. Alexa Fluor 488 donkey anti-rabbit IgG (H + L) (A-21206), Alexa Fluor 568 donkey anti-goat IgG (H + L) (A-11057) and Alexa Fluor 555 donkey anti-rabbit IgG (H + L)

(A-31572) were purchased from Invitrogen; and CY5-conjugated goat anti-human IgG was purchased from Jackson ImmunoResearch.

Mouse Oocyte collection and culture

Animal care and handling were conducted in accordance with the Institutional Animal Welfare and Ethics Committee policies of Peking University. 6–8 week female ICR mouse were sacrificed by cervical dislocation, and the ovaries were isolated from abdominal cavity. Large antral follicles were punctured under a stereoscopic microscope to release GV oocytes. The GV stage oocytes were cultured in M2 medium under mineral oil at 37°C in an atmosphere of 5% CO2 in air. At different times of culture, oocytes were collected at GVBD, Pro-Met I, MI, AI-TI or MII stages, respectively.

PHF1 mRNA synthesis

Myc-PHF1-PCS2+ plasmids were purchased from Vigene Biosciences. Then the plasmids were linearized and purified by gel extraction kit (QIAGEN, 28704) and SP6 mMessage mMachine (Ambion, AM1340) were used to obtain capped mRNA.

Mouse oocyte microinjection and drug treatment

For oocyte microinjection, 50 μM PHF1-siRNA (5′-GGAGAAGGAGAGGGCGCAUTT-3′) were injected into the GV oocytes cytoplasm to deplete PHF1 and the same concentration control siRNA was used as control. Milrinone, a selective inhibitor of oocyte-specific cyclic nucleotide phosphodiesterase, can block the GV oocytes that remain in this stage and prevent them progressing into the GVBD stage. Therefore, after injection, both the control siRNA group and PHF-siRNA group oocytes were cultured for 24 h in M2 medium containing 2.5 μM milrinone to maximize the function of siRNA and were then transferred to fresh medium. The following overexpression of PHF1, about 5 to 10 pl Myc–PHF1 mRNA in RNase-free PBS solution (2.5 mg/ml) was injected into cytoplasm of GV oocytes. After arresting at the GV stage in M2 medium for 3 h, the oocytes were released in fresh M2 medium for subsequent experiments.

For drug treatment, 5 mM Taxol (Selleck Chemicals, S1150) in DMSO stock solution was diluted to 10 μM in M2 medium and MI stage oocytes were incubated in it for 45 min. At the same time, the other MI stage oocytes were incubated in 10 mg/ml Nocodazole (Sigma, M1404), which were diluted in DMSO stock (Sigma, D2650) and further diluted in M2 medium to a final concentration of 20 μg/ml. After Nocodazole treatment, the oocytes were washed thoroughly and cultured in fresh M2 medium for 30 min to recovery.

Immunofluorescent analysis

Oocytes were fixed with 4% paraformaldehyde in PBS (PH 7.4) for at least 1 hour at room temperature, and permeabilization with 0.5% Triton X-100 at room temperature for 30 min, followed by blocking in 1% BSA-supplemented PBS for 1 h and then incubated overnight at 4°C with rabbit monoclonal anti-PHF1 antibody (1:50), rabbit monoclonal anti-α-tubulin antibody (1:50), mouse monoclonal anti-α-Tubulin-FITC antibody (1:50), human anti-centromere antibody (1:40) or Phalloidin-TRITC (1:100). And then, after 3 times washes in PBS for 5 min each, the oocytes were labeled with Alexa Fluor 488 donkey anti-rabbit IgG, Alexa Fluor 568 donkey anti-goat IgG, Alexa Fluor 555 donkey anti-rabbit IgG and CY5-conjugated goat anti-human IgG (1:200) for 2 h at room temperature, and then washed three times with PBS. The oocytes were further stained with propidium iodide (PI; red, 10 μg/ml) for 10 min or Hoechst 33342 (blue, 5 μg/ml) for 20 min and finally the oocytes were viewed a confocal laser-scanning microscope (Carl Zeiss LSM780).

Western Blot analysis

Oocytes were collected in Laemmli sample buffer and boiled for 5 min at 100°C. The protein were separated by 10% SDS-PAGE and transferred to polyvinylidene fluoride membranes. Following transfer, the membranes were blocked with 5% (w/ml) fat-free dry milk/TBST at room temperature for 1 h and incubated with rabbit monoclonal anti-PHF1 antibody (1:1000), rabbit monoclonal anti-α-tubulin antibody (1:1000) or goat polyclonal anti-BubR1 antibody (1:1000) overnight at 4°C. Followed by three 10 min wash in TBST, the

membranes were incubated with its corresponding secondary antibody for 1 hour at room temperature. Finally, the membranes were washed in TBST for three times and immobilized antibodies were detected by the enhanced chemiLuminescence detection system (Bio-Rad).

Chromosome spreading assay and cold treatment

For chromosome spreading assay, MII oocytes were left in 1% sodium citrate for 15 min at room temperature and placed to the glass slide one by one. About 100 μL methanol: glacial acetic acid (3:1) was dropped onto the glass to fix the oocyte. After the chromosomes were stained with PI (10 mg/ml) for 10 min, the specimen was examined by the confocal laser-scanning microscope (Carl Zeiss LSM780).

For cold treatment, the GV oocytes were blocked in M2 medium containing 2.5 μM milrinone for 24 hour after injecting the PHF1-siRNA or control-siRNA. Followed by washing and culture in fresh M2 medium for 8 hour, the oocytes were transfer to pre-cooled M2 medium for 10 min at 4°C. And then, the oocytes were used for immunofluorescent experiments with human anti-centromere antibody and rabbit monoclonal anti-α-tubulin antibody.

Statistical analysis

The data were performed at least for three times independently and all percentage data were shown as mean ± SEM. Differences between groups were evaluated by one-way ANOVA or t test and $p < 0.05$ was considered statistically significant.

Acknowledgments

We are grateful to Professor Hongquan Zhang (Peking University Health Science Center, Beijing, China) for providing technical assistance.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This study was supported by grants from the National Natural Science Foundation of China (81521002, 81730038 and 31801244).

ORCID

Jie Qiao http://orcid.org/0000-0003-2126-1376

References

[1] World Health Organization. Infections, pregnancies, and infertility: perspectives on prevention. Fertil Steril. 1987;47:964–968.
[2] Beall S, Brenner C, Segars J. Oocyte maturation failure: a syndrome of bad eggs. Fertil Steril. 2010;94:2507–2513.
[3] Engmann L, Romak J, Nulsen J, et al. In vitro viability and secretory capacity of human luteinized granulosa cells after gonadotropin-releasing hormone agonist trigger of oocyte maturation. Fertil Steril. 2011;96:198–202.
[4] Decordier I, Cundari E, Kirsch-Volders M. Mitotic checkpoints and the maintenance of the chromosome karyotype. Mutat Res. 2008;651:3–13.
[5] Menasha J, Levy B, Hirschhorn K, et al. Incidence and spectrum of chromosome abnormalities in spontaneous abortions: new insights from a 12-year study. Genet Med Off J Am Coll Med Genet. 2005;7:251–263.
[6] Schultz RM, Letourneau GE, Wassarman PM. Program of early development in the mammal: changes in patterns and absolute rates of tubulin and total protein synthesis during oogenesis and early embryogenesis in the mouse. Dev Biol. 1979;68:341–359.
[7] Simon JA, Kingston RE. Mechanisms of polycomb gene silencing: knowns and unknowns. Nature Rev Mol Cell Biol. 2009;10:697–708.
[8] Muller J, Hart CM, Francis NJ, et al. Histone methyltransferase activity of a Drosophila Polycomb group repressor complex. Cell. 2002;111:197–208.
[9] Cao R, Wang L, Wang H, et al. Role of histone H3 lysine 27 methylation in Polycomb-group silencing. Science. 2002;298:1039–1043.
[10] Qu Y, Lu D, Jiang H, et al. EZH2 is required for mouse oocyte meiotic maturation by interacting with and stabilizing spindle assembly checkpoint protein BubRI. Nucleic Acids Res. 2016;44:7659–7672.
[11] Erhardt S, Su IH, Schneider R, et al. Consequences of the depletion of zygotic and embryonic enhancer of zeste 2 during preimplantation mouse development. Development. 2003;130:4235–4248.
[12] Huang XJ, Wang X, Ma X, et al. EZH2 is essential for development of mouse preimplantation embryos. Reprod Fertil Dev. 2014;26:1166–1175.
[13] Pasini D, Bracken AP, Jensen MR, et al. Suz12 is essential for mouse development and for EZH2 histone methyltransferase activity. Embo J. 2004;23:4061–4071.

[14] Yu W, Zhang F, Wang S, et al. Depletion of polycomb repressive complex 2 core component EED impairs fetal hematopoiesis. Cell Death Dis. 2017;8:e2744.
[15] Faust C, Lawson KA, Schork NJ, et al. The Polycomb-group gene eed is required for normal morphogenetic movements during gastrulation in the mouse embryo. Development. 1998;125:4495–4506.
[16] Cao R, Wang H, He J, et al. Role of hPHF1 in H3K27 methylation and Hox gene silencing. Mol Cell Biol. 2008;28:1862–1872.
[17] Musselman CA, Gibson MD, Hartwick EW, et al. Binding of PHF1 Tudor to H3K36me3 enhances nucleosome accessibility. Nat Commun. 2013;4:2969.
[18] Sarma K, Margueron R, Ivanov A, et al. Ezh2 requires PHF1 to efficiently catalyze H3 lysine 27 trimethylation in vivo. Mol Cell Biol. 2008;28:2718–2731.
[19] Hong Z, Jiang J, Lan L, et al. A polycomb group protein, PHF1, is involved in the response to DNA double-strand breaks in human cell. Nucleic Acids Res. 2008;36:2939–2947.
[20] Yan L, Yang M, Guo H, et al. Single-cell RNA-Seq profiling of human preimplantation embryos and embryonic stem cells. Nat Struct Mol Biol. 2013;20:1131–1139.
[21] Li H, Liefke R, Jiang J, et al. Polycomb-like proteins link the PRC2 complex to CpG islands. Nature. 2017;549:287–291.
[22] Tie F, Prasad-Sinha J, Birve A, et al. A 1-megadalton ESC/E(Z) complex from Drosophila that contains polycomblike and RPD3. Mol Cell Biol. 2003;23:3352–3362.
[23] Gibson MD, Gatchalian J, Slater A, et al. PHF1 Tudor and N-terminal domains synergistically target partially unwrapped nucleosomes to increase DNA accessibility. Nucleic Acids Res. 2017;45:3767–3776.
[24] Wei L, Liang XW, Zhang QH, et al. BubR1 is a spindle assembly checkpoint protein regulating meiotic cell cycle progression of mouse oocyte. Cell Cycle. 2010;9:1112–1121.
[25] Touati SA, Buffin E, Cladiere D, et al. Mouse oocytes depend on BubR1 for proper chromosome segregation but not for prophase I arrest. Nat Commun. 2015;6:6946.
[26] Yang Y, Wang C, Zhang P, et al. Polycomb group protein PHF1 regulates p53-dependent cell growth arrest and apoptosis. J Biol Chem. 2013;288:529–539.
[27] Habu T, Wakabayashi N, Yoshida K, et al. p53 Protein interacts specifically with the meiosis-specific mammalian RecA-like protein DMC1 in meiosis. Carcinogenesis. 2004;25:889–893.
[28] Merok JR, Lansita JA, Tunstead JR, et al. Cosegregation of chromosomes containing immortal DNA strands in cells that cycle with asymmetric stem cell kinetics. Cancer Res. 2002;62:6791–6795.
[29] Insinga A, Cicalese A, Faretta M, et al. DNA damage in stem cells activates p21, inhibits p53, and induces symmetric self-renewing divisions. Proc Natl Acad Sci. 2013;110:3931–3936.
[30] Paul D, Bridoux L, Rezsohazy R, et al. HOX genes are expressed in bovine and mouse oocytes and early embryos. Mol Reprod Dev. 2011;78:436–449.