Biochemical and Biophysical Research Communications xxx (2017) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Peroxiredoxins are required for spindle assembly, chromosome organization, and polarization in mouse oocytes

Hyuk-Joon Jeon [a], Yong Seok Park [a], Dong-Hyung Cho [b], Jae-Sung Kim [c], Eunji Kim [d], Ho Zoon Chae [d], Sang-Young Chun [d, **], Jeong Su Oh [a, *]

[a] Department of Genetic Engineering, College of Biotechnology and Bioengineering, Sungkyunkwan University, Suwon, South Korea
[b] Department of East-West Medical Science, Graduate School of East-West Medical Science, Kyung Hee University, Yongin, South Korea
[c] Division of Radiation Cancer Research, Korea Institute of Radiological and Medical Sciences, Seoul, South Korea
[d] School of Biological Sciences and Technology, Faculty of Life Science, Chonnam National University, Gwangju, South Korea

ARTICLE INFO

Article history:
Received 8 May 2017
Accepted 22 May 2017
Available online xxx

Chemical compounds studied in this article:
Conoidin A (PubChem CID: 511509)

Keywords:
Oocyte
Peroxiredoxin
Meiosis

ABSTRACT

Peroxiredoxins (Prxs) are highly conserved antioxidant enzymes and are implicated in multiple biological processes; however, their function in oocyte meiosis has not been studied. Here we show that inhibition of Prx I and II results in spindle defects, chromosome disorganization, and impaired polarization in mouse oocytes. Prx I was specifically localized at the spindle, whereas Prx II was enriched at the oocyte cortex and chromosomes. Inhibition of Prx activity with conoidin A disturbed assembly of the microtubule organizing center (MTOC) through Aurora A regulation, leading to defects in spindle formation. Moreover, conoidin A impaired actin filament and cortical granule (CG) distribution, disrupting actin cap and CG formation, respectively. Conoidin A also increased DNA damage without significantly increasing reactive oxygen species (ROS) levels, suggesting that the effects of conoidin A on meiotic maturation are not likely associated with ROS scavenging pathways. Therefore, our data suggest that Prxs are required for spindle assembly, chromosome organization, and polarization during meiotic maturation.

1. Introduction

Mammalian oocytes are arrested within the ovarian follicles at the prophase of the first meiosis, which is morphologically identified by the presence of a large, vesicular nucleus called the germinal vesicle (GV). Following the luteinizing hormone surge, oocytes resume meiosis, as indicated by GV breakdown (GVBD). Microtubules then assemble at the microtubule organizing centers (MTOCs) and form a bipolar spindle, which migrates to the oocyte cortex, capturing the chromosomes [1–3]. Actin filaments and cortical granules (CGs) redistribute to form an actin cap and CG-free domain (CGFD), respectively, polarizing the oocyte [3–5]. Accurately controlling these processes is essential for orderly meiosis during oocyte maturation. Any error in spindle assembly, chromosome organization, or polarization can cause serious defects including chromosome missegregation, aneuploidy, and symmetric division, which contribute to early abortion and female infertility [6,7]. Although numerous molecules responsible for meiotic defects have been proposed, the pathways and underlying mechanisms that modulate the meiotic apparatus remain largely unknown.

Production of reactive oxygen species (ROS) is unavoidable in aerobic organisms. Although limited amounts of ROS mediate some cellular signaling, elevated ROS levels and the subsequent oxidative stress are detrimental to most cells, including mammalian oocytes and embryos [8–13]. To fine-tune ROS levels and protect themselves from oxidative stress, cells have evolved several antioxidant systems. These include glutathione and classical enzymatic antioxidants, such as catalase, superoxide dismutase, and glutathione peroxidase [14]. In addition, peroxiredoxins (Prxs) have been recently discovered as novel antioxidant enzymes. Prxs comprise six related isoforms (Prx I-VI) in mammals and are widely distributed in many organisms, maintaining a high degree of conservation [15–17]. The six mammalian isoforms of Prx can be further divided into three subtypes: typical 2-Cys Prxs (Prx I-IV), atypical 2-Cys Prxs (Prx V), and 1-Cys Prx (Prx VI). Prxs reduce hydrogen peroxide and other peroxide substrates by oxidizing a conserved

* Corresponding author.
** Corresponding author.
E-mail addresses: sychun@chonnam.ac.kr (S.-Y. Chun), ohjs@skku.edu (J.S. Oh).

http://dx.doi.org/10.1016/j.bbrc.2017.05.127
0006-291X/

cysteine residue, which can form disulfide bond with the corresponding cysteine of an adjacent Prx subunit [18]. Therefore, Prxs undergo redox-dependent conformational changes, which are linked to Prx function. Although the primary function of Prxs appears to be ROS scavenging, they have been also implicated in various cellular processes, such as cell proliferation, differentiation, cell death, and intracellular signaling [19–21]. Prxs have also been associated with cancer, cardiovascular dysfunction, and neurodegeneration [22,23].

Despite the significant potential to regulate a number of cellular processes, surprisingly little is known about Prx function during oocyte maturation. In the present study, we examined the expression, localization, and function of Prx I and II during meiotic maturation. Our data revealed that Prx I and II activity are required for spindle assembly, chromosome organization and oocyte polarization during meiotic maturation.

2. Materials and methods

2.1. Oocyte collection and culture

All procedures regarding mouse care and use were conducted in accordance with the guidelines and approved by the Institutional Animal Care and Use Committees of Sungkyunkwan University (approval ID:SKKU 12–37). Ovaries were isolated from 3-6-week-old CD-1 female mice (Koatech, Pyeongtaek, Korea) 46–48 h after intraperitoneal injection of 5 IU of pregnant mare's serum gonadotropin (PMSG; Sigma, St. Louis, MO, USA). Oocytes at the GV stage were released from the ovaries by puncturing with a fine needle and were recovered in M2 medium (Sigma) supplemented with 100 μM 3-isobutyl-1-methylxanthine (IBMX; Sigma) to prevent meiotic resumption. For in vitro maturation, oocytes were washed and cultured in IBMX-free M16 medium (Millipore, Madison, WI, USA) at 37 °C in a 5% CO_2 atmosphere.

For conoidin A treatment, oocytes were cultured with 0, 2, 4, or 8 μM conoidin A (2,3-bis(bromomethyl)-quinoxaline 1,4-dioxide; Millipore) dissolved in dimethyl sulfoxide (DMSO). Control oocytes were treated with an equal volume of DMSO. For cycloheximde treatment, oocytes were cultured with 20 μg/ml cycloheximde (Sigma) for 24 h.

2.2. RT-PCR

Total RNA was extracted from 20 oocytes with an RNeasy Plus Micro kit (Qiagen, Hilden, Germany). First-strand cDNA was synthesized from the RNA synthesis using the Sensiscript RT kit (Qiagen). PCR was performed with the following primers: Prx I, 5′-GATAACAATAAACGATCTTCCCGTTGG -3′ and 5′- GAAA-TACTCTTTGCTCTTATTGACATCAG -3′; Prx II, 5′-TCCTTCGCCA-GATCACAG-3′ and 5′ CTTGATGGTGTCACTGCC-3’; GAPDH, 5′-ACCACAGTCCATGCCATCAC-3′ and 5′-TCCACCACCCTGTTGCTGTA-3’. The PCR products were visualized with agarose gel electrophoresis.

2.3. Immunoblotting

Oocytes were lysed in SDS sample buffer at 95 °C for 5 min. The lysates were subjected to SDS-PAGE and transferred to an Immobilon-P membrane (Millipore). After blocking in TBST (0.1% Tween-20, 3% BSA), the membranes were incubated with primary antibodies overnight at 4 °C. The membranes were washed three times in TBST and incubated with secondary antibodies for 1 h. The blots were developed with an ECL Western Blotting Detection kit (GE Healthcare Life Science, Marlborough, MA, USA). The primary antibodies included anti-Prx I and II antibodies (1:1000, a kind gift from Prof. H. Z. Chae, Chonnam National University, Gwangju, Korea) and anti-β-actin (Cell Signaling, Beverly, MA, USA, 1:5000). HRP-labeled mouse and rabbit antibodies (Jackson ImmunoResearch, Westgrove, PA, USA) were used as the secondary antibodies.

2.4. Immunostaining

Oocytes were fixed in 4% paraformaldehyde for 10 min and then permeabilized in phosphate buffered saline (PBS) with 0.1% Triton X-100 and 0.01% Tween-20 for 30 min. After blocking in PBS with 3% bovine serum albumin (BSA), immunostaining was performed with anti-Prx I and II (1:500, a kind gift from Prof. H. Z. Chae, Chonnam National University, Gwangju, Korea), anti-Aurora A (Cell Signaling, 1:250), anti-PCNT (BD Biosciences, San Jose, CA, 1:100), anti-γ-H2A.X (Abcam, 1:500), and anti-α-tubulin (Sigma, 1:500) antibodies, followed by Alexa Fluor-conjugated 488 and 594 secondary antibodies (Jackson ImmunoResearch). F-actin and CGs were labeled with phalloidin-FITC (Sigma) and lectin-FITC (Sigma), respectively. DNA was counterstained with DAPI. Images were obtained on an LSM 700 laser scanning confocal microscope (Zeiss, Berlin, Germany) with a C-Apochromat 63 × /1.2 water immersion objective. Data were analyzed using ZEN 2012 Black (Zeiss) and ImageJ software (National Institutes of Health, MD, USA).

2.5. Measurement of intracellular ROS levels

To determine intracellular ROS levels, oocytes were incubated with 20 μM dihydrorhodamine 123 (DHR-123; Sigma) for 30 min. After washing, oocytes were observed under a Nikon Eclipse Ti inverted microscope with a CCD cooled camera (DS-Qi1Mc, Nikon; Tokyo, Japan). The images were quantified using ImageJ software (National Institutes of Health).

2.6. Statistical analysis

Statistical analysis was performed with GraphPad Prism (GraphPad Software; San Diego, CA). Data are representative of at least three independent experiments, unless otherwise specified. Each experimental group included at least 20 oocytes. Significant differences between groups were analyzed with Student's t-test. P-values < 0.05 were considered statistically significant.

3. Results

3.1. Expression and localization of Prx I and II during meiotic maturation

We first examined Prx expression during meiotic maturation. RT-PCR analysis showed that transcripts of both Prx I and II were constantly expressed during oocyte meiosis (Fig. 1A). Immunoblot analysis also showed that Prx I and II were expressed in oocytes with a gradual increase during meiotic maturation (Fig. 1B). Because Prxs undergo major redox-dependent conformational changes, which are linked to their enzymatic function, we wanted to determine the oligomeric state of Prxs during meiotic maturation. Therefore, we performed immunoblot analysis under non-reducing conditions. Both Prx I and II were predominantly present as dimers during meiotic maturation. However, multimeric forms of Prx I and II were weakly detectable at the GV stage and increased at the MI and MII stages (Fig. 1C). This result suggests that Prx I and II undergo conformational changes during meiotic maturation, implying changes in their activity during oocyte meiosis.

We next determined the localization of Prxs at different stages of oocyte meiosis. Prx I was uniformly distributed in the cytoplasm

Please cite this article in press as: H.-J. Jeon, et al., Peroxiredoxins are required for spindle assembly, chromosome organization, and polarization in mouse oocytes, Biochemical and Biophysical Research Communications (2017), http://dx.doi.org/10.1016/j.bbrc.2017.05.127