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ScienceDirect
Theriogenology 74 (2010) 1539–1547
Theriogenology
www.theriojournal.com

Aging changes the chromatin configuration and histone methylation of mouse oocytes at germinal vesicle stage

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Received 3 March 2010; received in revised form 26 May 2010; accepted 21 June 2010

Abstract

Aging decreases the fertility of mammalian females. In old oocytes at metaphase II stage (MII) there are alterations of the chromatin configuration and chromatin modifications such as histone acetylation. Recent data indicate that alterations of histone acetylation at MII initially arise at germinal vesicle stage (GV). Therefore, we hypothesized that the chromatin configuration and histone methylation could also change in old GV oocytes. In agreement with our hypothesis, young GV oocytes had non-surrounded nucleolus (NSN) and surrounded nucleolus (SN) chromatin configurations, while old GV oocytes also had chromatin configurations that could not be classified as NSN or SN. Regarding histone methylation, young GV and MII oocytes showed dimethylation of lysines 4, 9, 36 and 79 in histone 3 (H3K4me2, H3K9me2, H3K36me2, H3K79me2), lysine 20 in histone H4 (H4K20me2) and trimethylation of lysine 9 in histone 3 (H3K9me3) while a significant percentage of old GV and MII oocytes lacked H3K9me3, H3K36me2, H3K79me2 and H4K20me2. The percentage of old oocytes lacking histone methylation was similar at GV and MII suggesting that alterations of histone methylation in old MII oocytes initially arise at GV. Besides, the expression of the histone methylation-related factors Cbx1 and Sirt1 was also found to change in old GV oocytes. In conclusion, our study reports changes of chromatin configuration and histone methylation in old GV oocytes, which could be very useful for further understanding of human infertility caused by aging.

Keywords: Aging; Cbx1; Germinal vesicle; Histone methylation; Oocyte

1. Introduction

Recent changes in lifestyle have led women to postpone childbearing and this has increased the risk of infertility. The principal cause of aging-related infertility in mammals is the poor quality of old oocytes [1]. Mammalian females are born with a limited number of oocytes that are suspended in the prophase I of the meiosis during the life span of the organism. At birth, all the mouse oocytes are at the germinal vesicle stage (GV) and have a diameter lower than 15 μm. At two weeks old, the diameter of fully-grown antral oocytes at GV attains sizes larger than 50 μm (50–80 μm). Oocytes at GV also become larger than 60 μm after PMSG treatment [2,3]. Fully-grown antral oocytes at GV are divided according to the configuration of their chromatin into "non-surrounded nucleolus" (NSN) with decondensed chromatin or "surrounded nucleolus" (SN) with condensed chromatin. Initially, GV oocytes show NSN chromatin configuration, but as they grow, the chromatin of some oocytes changes into the SN configuration

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doi:10.1016/j.theriogenology.2010.06.024

[2,4–6]. After puberty, SN oocytes periodically resume meiosis by the process of germinal vesicle breakdown (GVBD). The GVBD is followed by metaphase I stage, then the first meiotic division and finally the metaphase II stage (MII). MII oocytes isolated from old mice (old MII oocytes) progressively lose the capability to be fertilized and develop normal embryos due to diverse cytological defects [1]. These defects include chromosomal abnormalities such as decondensed chromosomes, aneuploidies, and polyploidies [6–8]. Microarray analysis of old MII oocytes has revealed abnormal expression of genes involved in chromatin modifications [9]. Histone acetylation and DNA methylation are altered by post-ovulatory aging in the oviduct and in culture [10,11]. While all young MII oocytes have the lysine 12 of histone H4 deacetylated, as much as 40% of old MII oocytes have it acetylated [12,13]. In addition, young oocytes have mono-, di-, and tri- methylation of histone H3 at lysine residues 4, 9, and 79. However, the histone methylation in old oocytes has not been studied yet [14–16].

Aging alters the heterochromatin of senescence cells [17]. Changes of the heterochromatin result in the mobilization of HP1 proteins to the pericentromeric regions to suppress abnormal chromosome segregation [18]. HP1 proteins bind to the kinetochores in the regions where methylation in histone H3 at lysine 9, the histone variant H2A2 and RNA components occur [19,20]. Another factor related to histone methylation and aging is Sirt1 [21]. Sirt1 is a NAD+ dependant histone deacetylase, which decreases in prematurely senescent mouse cells and in mice with accelerated aging [22]. Furthermore, Sirt1 regulates the histone methyl-transferase Suv39h1 that induces trimethylation of histone 3 at lysine 9 in heterochromatic regions [23].

In this work, we have investigated changes to the chromatin configuration, histone methylation, and the factors Cbx1 and Sirt1 in GV oocytes from old mice. We found that GV oocytes from old mice show different chromatin configuration, histone methylations, and factors related to histone methylation.

2. Materials and methods

2.1. Animals

Adult CD1 (Charles River) mice were used as oocytes donors. All animals were maintained in accordance with the Animal Experiments Standard. Mice were kept in the Animal Service of CIB (CSIC) in stable conditions of temperature and light (light on at 07:00 hours and off at 19:00 hours) and with food and water *ad libitum*. Females were euthanized using cervical dislocation in accordance with the European Union Agreement about Vertebrate Animal protection (3/18/1986) and with NIH guidelines and the CSIC (Spain) ethics committee for the care and use of laboratory animals. Young mice were two months old while old mice were 11 months old. We chose this age because histone acetylation changes are observed at this age [13].

2.2. Chemicals

All chemicals were obtained from Sigma (St Louis, MO) unless otherwise specified.

2.3. Oocyte collection

To retrieve fully-grown antral oocytes at GV, mice were superovulated by intraperitoneal injection of 7.5 IU pregnant mare's serum gonadotropin (PMSG). The ovaries were removed from the mice 48 h after PMSG treatment and transferred to M2 medium. The ovarian follicles were punctured with a 27G needle to release the oocytes in the cumulus oophorus. The cumulus cells surrounding the oocytes were removed by gentle pippetting through a narrow glass pipette and the oocytes displaying GV were collected. GV oocytes were classified according to their size in smaller or larger than 70 μm using an inverted microscope (Zeiss, Axiovert). To retrieve MII-arrested oocytes, mice were superovulated by intraperitoneal injection of 7.5 IU PMSG followed by 7.5 IU hCG 48 h later. Cumulus enclosed oocyte complexes were collected from the ampulla of oviducts 15 h after hCG injection, placed in M2 medium, and freed of cumulus cells with 1 mg/ml hyaluronidase.

2.4. DAPI staining

GV and MII oocytes were fixed with 4% paraformaldehyde (PFA) overnight and permeabilized with 0.5% Triton X-100 for 15 min. Next, oocytes were incubated with 4-diamidino-2-phenylindole (DAPI) (Roche Diagnostics, Barcelona, Spain) for 5 min and mounted in Mowiol 4-88 antifade medium (Polysciences Inc, Eppelheim, Germany). The fluorescence was captured with a Leica spectral confocal scanning system (LEICA TCS-SP2-AOBS).

2.5. Immunofluorescence

GV and MII oocytes were fixed with 4% PFA overnight and then permeabilized with 0.5% Triton X-100 for 15 min. Oocytes were incubated with antibodies against H3K4me2, H3K9me2, H3K9me3, H3K36me2, H3K79me2, H4K20me2 (1:100, Abcam, Cambridge, UK), Cbx1 (1:100, anti-Hp1beta, Santa Cruz Biotech-