Journal of Animal and Veterinary Advances 11 (10): 1739-1744, 2012
ISSN: 1680-5593

Dynamic Transformation of DNA Methylation and Chromatin Configuration in Porcine Oocyte during Follicular Growth

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Abstract: Oocytes grow and obtain the ability of resume meiosis and maturation during follicular growth. Many regulatory mechanisms play a role in this process. DNA methylation and chromatin configuration are important regulatory mechanisms of gene transformation. The dynamic transformation of DNA methylation and chromatin configuration should be researched during follicular growth. Laser 3D sequence scanning and quantitative analysis fluorescence were used to discover the intensity and position of DNA methylation in porcine oocytes from different diameter follicles. The total fluorescence intensity (9374±2886 vs. 2703±473) and area (40914±7264 vs. 18862±2216) reduced during the follicular growth but the average fluorescence intensity (0.22±0.03 vs. 0.17±0.16) had no significant difference in <1 and >3 mm group although reduced in 1-3 mm group (0.13±0.04). Confocal microscopy was also used to compare the relationship between DNA methylation and chromatin configuration. The position of DNA methylation matched chromatin configuration well in most oocytes but researchers found some different situations that need to be further researched.

Key words: Follicular growth, porcine oocyte, DNA methylation, chromatin configuration, series scanning

INTRODUCTION

When a group of primordial follicles within the mammalian ovary is recruited into the growing phase, oocytes contained in their follicles begin their growth and some of them will be ovulated. The quality of the ovulated oocytes is determined by the complicated transcriptional regulation during oogenesis (Zuccotti *et al*., 2011). Oocyte genomes showed a significant positive correlation between mRNA transcript levels and methylation of the transcribed region (Kobayashi *et al*., 2012). Chromatin configuration also showed a significant positive correlation to the transcription (Tan *et al*., 2009). DNA methylation and chromatin configuration play a vital role during oogenesis but we still do not understand the relationship between them.

DNA methylation occurs in vertebrates, plants and fungi. It is the addition of a methyl group CH_3 at the cytosine of CpG sites of the DNA sequence found in the genomes. Methylation regulates the expression of imprinted and non-imprinted sequences. The majority of the about 90 imprinted genes identified to date in mammals are maternally imprinted, i.e., their sequences are epigenetically modified during oogenesis (Zuccotti *et al*., 2011). CGI methylation in gametes is not entirely related to genomic imprinting but is a strong factor in determining methylation status in preimplantation embryos (Smallwood *et al*., 2011). The methylation status is established during oogenesis and the different status may be responsible for the different abilities of support embryo development in different oocytes.

Chromatin configuration is regarded as a large-scale chromatin structure. Models for the experimental manipulation of large-scale chromatin structure will be important to determine the key cellular pathways and molecules involved in genome-wide chromatin modifications (De La Fuente, 2006). Many people divided the chromatin configuration according to the following method: SN (Surrounded Nucleolus) oocytes with a ring of heterochromatin surrounding the nucleolus and NSN (Not Surrounded Nucleolus) oocytes with more dispersed chromatin not surrounding the nucleolus (Lee *et al*., 2008; Liu *et al*., 2006; Miyara *et al*., 2003; Russo *et al*., 2007). They regard NSN oocytes as having a strong transcriptional activation and the SN oocytes as having poor transcriptional activation (Lodde *et al*., 2008; Wang *et al*., 2009). But Sui *et al*. (2005) did not find the SN like configuration in goat. This reminds us the Quantitive Research Method reported by Liu *et al*. (2012) is more suitable for classification of the chromatin configuration. Laser 3D scan technology used here makes a good supplement to such method.

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The dynamic transformation of DNA methylation in porcine oocyte during follicular growth was still not clear. So, researchers quantitative analysed this transformation with Laser 3D Scan Method and then established the correlation of DNA methylation and chromatin configuration.

MATERIALS AND METHODS

Collection of oocytes: Porcine ovaries used here were obtained from a slaughterhouse. They were transported to the laboratory at 30°C in normal saline containing 100 IU mL^{-1} penicillin and 0.05 mg mL^{-1} streptomycin within 3 h. Follicles were classified into the following groups: <1, 1-3 and >3 mm. Cumulus-Oocyte Complexes (COCs) within the >1 mm follicles were pumped with 10 mL hypodermic syringe according to the groups separately. COCs within the <1 mm follicles were collected by punctured follicles in Dulbecco's Phosphate Buffered Saline (D-PBS) with needle under a dissecting microscope.

Fluorescent staining: COCs were denuded of cumulus cells by pipetting in D-PBS containing 0.1% (w/v) hyaluronidase (Sigma) with a fine glass tube. The denuded oocytes were fixed for 1 h in 4% paraformaldehyde in D-PBS and permeabilized with 0.2% Triton X-100 in PBS for 30 min at room temperature. The fixed oocytes were incubated in 2M HCl for 30 min and then transfered to 0.1 M Tris/HCl for 10 min. They were blocked in PBS containing 2% BSA for 1 h at room temperature and incubated overnight at 4°C in a 1:100 dilution of primary antibodies Anti-5-methylcytosine (catalogue number A-1014-50, Epigentek Group Inc. USA). After extensive washing in PBS containing 0.1% Tween 20 and 0.01% TritonX-100, the embryos were labeled with secondary FITC conjugated antibody (Goat anti-Mouse IgG whole serum, bs-0296Gs, Bioss Inc. USA) diluted 1:100 for 4 h at room temperature. Nuclear status of embryos was evaluated by staining with 10 μg mL^{-1} propidium iodide (PI, catalogue number P4170, Sigma) for 10 min. Following washes, oocytes were preserved in D-PBS within tissue culture plate at -4°C. Oocytes were washed at least three times between each steps.

Image acquisition and processing: Fluorescence was detected with Leica confocal laser scanning microscopy (TCS-SP5). When the oocytes of each follicular group prepared enough, researchers detected them at once with the same parameters to reduce the influences of conditions. The oocytes were detected within culture dishes to avoid impaction. Sequence scan was used to detect the sections in different z position and the z step was set 1 μm. Each nucleus was scanned different times according to the dispersion degree of fluorescence.

Image-Pro Plus was used to process the images of fluorescence as we used before (Liu *et al*., 2012). In brief, researchers converted the image to gray scale 8 first then snapped picture of image and converted the picture to gray scale 8 again. Calibrated the optical density and counted the region of fluorescence with the automatic dark objects option. The data of one section showed in the statistics. Summate all the sections of one oocyte will be the data of the oocyte.

Statistical analysis: Researchers conducted at least three replicates for each treatment. Data were analyzed by ANOVA and were compared using one-way analysis of variance after being transformed via LSD and $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Dynamic transformation of DNA methylation during oogenesis: Degree of DNA methylation in porcine oocyte was significant reduced when the follicle grew up to 1 mm. It enhanced when the follicle grown up to 3 mm but the degree didn't significantly (Fig. 1a).

Florescent area of DNA methylation in porcine oocyte reduced when the follicle grew up. The florescent area of <1 mm group was significant bigger than the area of >3 mm group (40914±7264 vs. 18862±2216, $p<0.05$). But there were no significant difference between adjacent groups such as <1 vs. 1-3 and 1-3 vs. >3 mm (Fig. 1b).

Florescent degree per unit area (average DOI) gave us more information about the DNA methylation in porcine oocytes. The average DOI was significantly transformed to a weaker status when the follicle grew up to 1 mm. Then, the average DOI was transformed to a stronger status which had no significant difference to the average DOI in the <1 mm group (Fig. 1c).

DNA methylation transformed their occurred position during the follicular growth. Researchers scaned the oocyte section by section and each section had 1 μm interval. The number of scanned section reflected the distribution of the florescence. The DNA methylation occurred in a broad range in <1 mm group. The occurred position transforms more narrow limitation during the follicle grown up. The DNA methylation of oocyte occurred in the narrowest area when the follicle diameter grown to 3 mm ($p<0.05$, Fig. 1d).

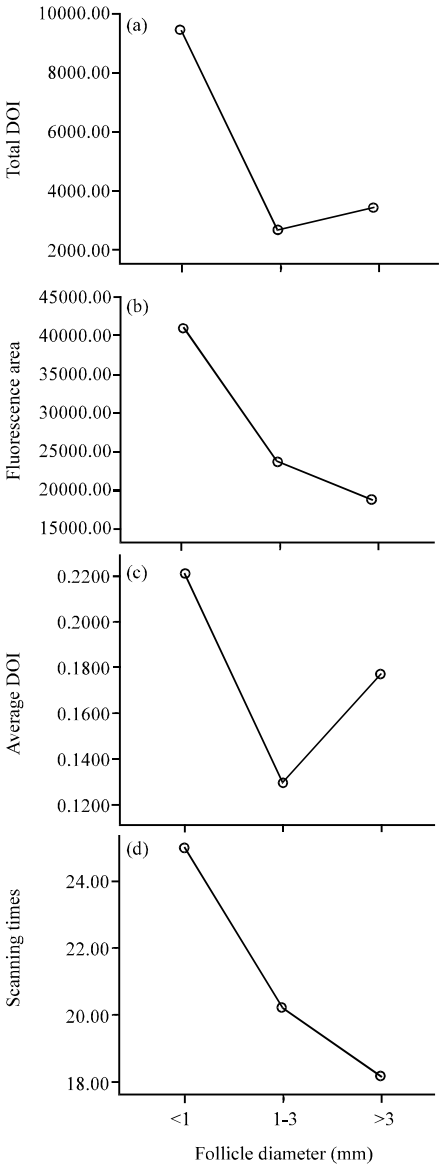

Fig. 1: Line charts show the dynamic transformation of DNA methylation in porcine oocytes during follicular growth; a) shows the total DOI of oocytes from different diameter follicles. The total DOI is calculated by summates the DOIs in all sections of one oocyte; b) shows the fluorescence area of oocytes from different diameter follicles. Fluorescence area means the sum of fluorescence area in all sections of one oocyte; c) shows the average DOI of oocytes from different diameter follicles. The average DOI means the DOI per area and d) shows the scanning times of oocytes from different diameter follicles. Scanning times is the number of sections we had scanned. It means the separation of DNA methylation location

DNA methylation of different chromatin configuration: According to the classification of chromatin configuration discussed the relationship between DNA methylation and chromatin configuration. The position of DNA methylation was similar to chromatin configuration (Fig. 2 and 3). In GV0 configuration, the diffuse DNA methylation position distributed over the nuclear area (Fig. 2). In GV1 configuration, the position of DNA methylation distributed around the nucleoli (Fig. 3).

Laser 3D scan showed more details of DNA methylation position: The position of DNA methylation in porcine oocyte has regular distribution as shown in Fig. 1d. It also had relationship with chromatin configuration as Fig. 2 and 3 shows. But a specific was fond on the base of vast compared pictures (Fig. 4). The position of DNA methylation overlaps the chromatin in most porcine oocytes (Fig. 4b). But in some oocytes, the position of DNA methylation was entirely different to the chromatin (Fig. 4a).

Series scanning was used to detect the DNA methylation in porcine oocytes. This method could gather the fluorescence in all sections of oocyte. It meliorated the normal detection method which scanned only one section. Researchers measured each section separately to avoid super saturation when merged all the sections although much more measurement had to do for this method. Sequence scanning was used to avoid mutual interference of different excitation wavelength. These methods were good supplements to the quantitative analysis method of chromatin configuration (Liu *et al*., 2012).

DNA methylation of porcine oocyte was heterogeneous in the same grown period. Different configuration of DNA methylation was discovered in the experiments although, the oocytes were collected from the same follicular diameter. The results provided more reasons for explaining why the same period oocytes had different abilities of resumed meiosis, maturation and supported development (Koike *et al*., 2010; Somfai *et al*., 2011). It also gave us some clue to explain the heterogeneous chromatin configuration in homologous oocytes (Tan *et al*., 2009).

The positional transformation of DNA methylation during the follicular growth was discovered in Fig. 1d, 2 and 3. DNA methylation occurred through the whole nucleus when the follicular diameter <1 mm. When the follicular diameter >3 mm, the DNA methylation occurred only around the nucleoli. There were two reasons maybe answered for this transformation. First the heterochromatin moved from a spread configuration to a surrounding configuration during porcine follicular

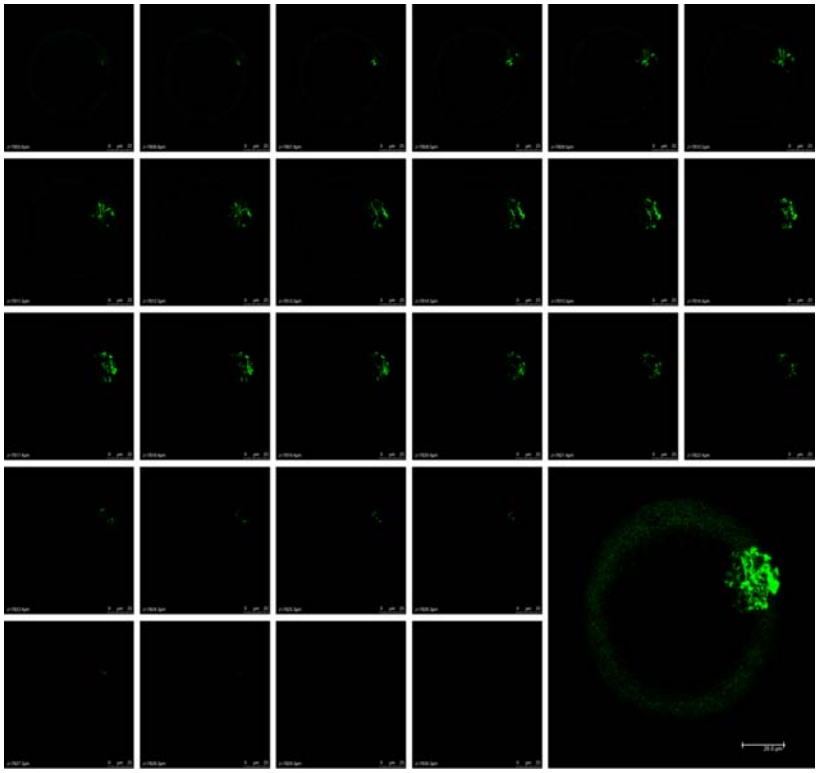

Fig. 2: Photographs show the DNA methylation of a porcine oocyte from <1 mm follicle. Smaller photographs show the DNA methylation in each focal plane continuously with 1 μm step in z axis. The bigger photograph in the right bottom shows the overlying of all the smaller photographs with the maximum projection function in Leica SP5

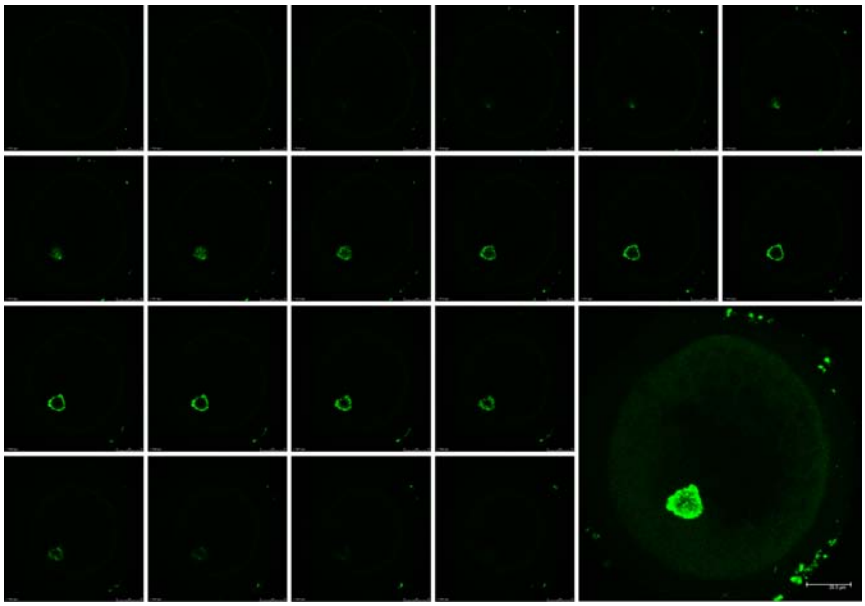

Fig. 3: Photographs show the DNA methylation of a porcine oocyte from >3 mm follicle. Smaller photographs show the DNA methylation in each focal plane continuously with 1 μm step in z axis. The bigger photograph in the right bottom shows the overlying of all the smaller photographs with the maximum projection function in Leica SP5

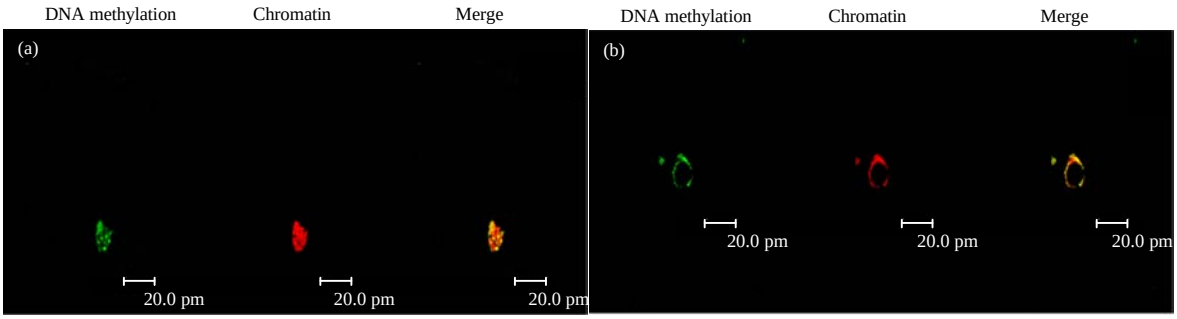

Fig. 4: Photographs show the relative position of DNA methylation and chromatin. a) DNA methylation occurred the same position as chromatin in some oocytes. But in some oocytes, b) the DNA methylation occurred position not fit the chromatin position well shows

growth (Sun *et al*., 2004). The DNA methylation mainly occurred in the heterochromatin so researchers got such transformation of DNA methylation. The other reason was the alteration of gene which needed to be methylated. Different gene needed to transcribe in different period during follicular growth. The results give such alteration a support: DNA methylation weakened both in total fluorescence intensity and average fluorescence intensity in the 1-3 mm group.

CONCLUSION

The intensity, area and position of DNA methylation was transformed during porcine follicular growth. Configuration of DNA methylation in porcine oocyte is similar to the chromatin configuration.

ACKNOWLEDGEMENTS

This research was supported by grants from the National Natural Science Foundation of China (No.: 30871415 and 31071310) and the Key Grant of the Natural Science Research Program of Anhui Higher Education Institutions of China (No.: KJ2009A030, KJ2011B121 and KJ2012B132).

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