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Histone lysine methylation exhibits a distinct distribution during spermatogenesis in pigs

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ABSTRACT

Spermatogenesis is a continual process throughout the adult life of a male, which is governed by unique transcriptional regulation and massive alterations of chromatin. Histone modification was one of the underlying epigenetic mechanisms during spermatogenesis. It has been shown that methylation of histone lysine exhibits a distinct distribution in mice during spermatogenesis and some histone lysine methylation is essential for male fertility. However, the dynamic change of methylated histone in porcine testis tissue was largely unknown. Here, we studied the dynamic modulation of three types of methylation (monomethylation, dimethylation, and trimethylation) of H3K4, H3K27, and H4K20 during spermatogenesis in pigs. The results showed that H3K4me2/3, H3K27me3, and H4K20me1/2/3 were extensively localized in adult pig testis. Interestingly, we found that undifferentiated spermatogonia contained strongly H4K20me2 and H4K20me3, but little H4K20me1, whereas the differentiated spermatogonia possessed H4K20me1 and H4K20me2 and little H4K20me3. The findings of this study help for the understanding of epigenetic modifications during spermatogenesis in pigs and provide information for further studies.

1. Introduction

As a basic component of the nucleosome, histone proteins of H2A, H2B, H3, and H4 form an octamer structure which is wrapped by DNA [1,2]. It has been found that there are several types of modification in the N-terminal tail of these four histones such as phosphorylation, acetylation, methylation, and ubiquitination [3,4]. These modifications were related to cellular processes, including transcriptional regulation, heterochromatin formation, DNA recombination, DNA damage response, and X-chromosome inactivation [5–8].

Among these modifications, histone methylation is extensively associated with both activation and repression of gene expression in euchromatic and heterochromatic regions [9]. There are three distinct methylation states, which are mono- (me1), di- (me2), and tri- (me3) methylation that occur at lysine (K) of histone H3 or H4 (such as K4, K9, and K27 of histone H3 and K20 of histone H4), and the function of methylation status of each lysine is independent. For instance, H3K4 and H3K36 are related to transcriptional activation, whereas H3K9, H3K27, and H4K20 are related to transcriptional repression [10].

Spermatogenesis is a continual process throughout the adult life of the male, consisting of cell proliferation and differentiation and transformation of spermatozoa from round spermatids [11]. The study of epigenetic regulation, especially histone modifications and chromatin remodeling during spermatogenesis, has attracted much attention in the field of reproductive biology [12]. It has been reported that several histone methyltransferases, such as Suv39h1, are essential for spermatogenesis [13]. To date, the methylation profile of H3K4, H3K27, and H4K20 has been

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reported in mice [12,14], but such information is remained unknown in pigs.

In this study, we examined the histone methylation profile (H3K4me1/2/3, H3K27me1/2/3, and H4K20me1/2/3) in testicular tissue of pigs by immunohistochemistry. In addition, the distribution of H4K20me1/2/3 was also examined in undifferentiated spermatogonia and differentiated spermatogonia *via* co-immunofluorescence. Our work disclosed the dynamic pattern of histone methylation at H3K4, H3K27, and H4K20 during porcine spermatogenesis, which could be a foundation for future research.

2. Materials and methods

2.1. Materials and reagents

All reagents including PBS (calcium and magnesium free), EDTA, and trizma base were obtained from Hyclone (Logan, UT, USA) or Sigma (St. Louis, MO, USA). All solutions were prepared using ultrapurified water supplied by a Milli-Q system (Millipore, Billerica, MA, USA).

2.2. Animals

All experimental procedures involving animals were approved by the Northwest A&F University's Institutional Animal Care and Use Committee. Testis samples were collected with surgery from 10-day-old Landrace hybrid piglets and Landrace hybrid adult boars and transported to the laboratory in Dulbecco's PBS within 2 hours.

2.3. Histology

The testis tissues were cut into small pieces. The tissues were fixed in Bouin's solution overnight and embedded in paraffin. Consecutive serial cross sections were prepared at an interval of 100 μm. After immunohistochemistry, five of the cross sections were analyzed and cells with positive signals were counted.

2.4. Immunohistochemical analysis

Slides were deparaffinized in xylene and hydrated in an ethanol series from 100% (v:v) to 70% (v:v). The sections were washed three times, each for 5 minutes in ultrapurified water, followed by exposure to 0.5% Triton-X 100 in ultrapurified water for 10 minutes at room temperature. The slides were boiled in a solution of 0.01-M Tris-EDTA (pH = 9) for 15 minutes for antigen retrieval. The sections were then incubated with 3% H_2O_2 for 10 minutes at room temperature and washed three times with Tris-buffered saline (TBS). All sections were treated with 10% goat serum for blocking nonspecific reactions at room temperature for 2 hours and incubated with the primary antibodies overnight at 4 °C. Next day, the sections were rinsed in TBS and incubated with goat anti-rabbit IgG (Bio-Rad) for 15 minutes at 37 °C. The sections were rinsed in TBS and exposed to the horseradish peroxidase at 37 °C for 15 minutes. The color was developed with 3,3′-diaminobenzidin at room temperature in dark for 5 minutes and counterstained with Mayer's hematoxylin solution. The negative control group was obtained with the same protocol as described previously, but the primary antibody was omitted. Digital images were captured with the Nikon Eclipse 80i fluorescence microscope camera (Tokyo, Japan).

2.5. Immunofluorescence labeling

Immunofluorescence staining was done basically using the same procedure as of immunohistochemistry with minor modification. Briefly, the slides were boiled in a solution of 0.01-M Tris-EDTA (pH = 9) for 15 minutes for antigen retrieval. All sections were treated with 10% donkey serum for blocking nonspecific reactions at room temperature for 2 hours and incubated with the primary antibodies overnight at 4 °C. Next day, the sections were rinsed in TBS and incubated with Alexa Fluor 488 donkey anti-Goat and 594 donkey anti-Rabbit (Invitrogen) antibodies for immunofluorescence labeling. Digital images were captured with the Nikon Eclipse 80i fluorescence microscope camera (Tokyo, Japan).

2.6. Antibodies

Rabbit polyclonal immunoglobulin G antibodies against monomethyl-, dimethyl-, and trimethyl-H3K4, H3K27, and H4K20 were generated and kindly provided by Dr Charlie Degui Chen (Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, China). All antibodies were used at a dilution of 1:100. Goat polyclonal anti-KIT and anti-promyelocytic leukemia zinc finger (PLZF; Santa Cruz Biotechnology) were diluted at 1:25. To detect the primary antibodies, Alexa Fluor 488 donkey anti-Goat and 594 donkey anti-Rabbit (Invitrogen) were diluted at 1:400.

3. Results

3.1. The methylated H3K4 shows extensive distribution in spermatogonia

In adult porcine testis tissue, all three types of methylated H3K4 were detected in spermatogonia. H3K4me1 existed strongly in type In- (intermediate) and B-spermatogonia and pachytene spermatocytes but not in elongating spermatids. H3K4me2 was located in spermatogonia and spermatids but absent or weak in different types of spermatocytes. H3K4me3 was detected strongly in spermatogonia and round spermatids but not in elongated spermatids (Table 1, Fig. 1, and Fig. S1). In the seminiferous tubules of piglets, all the three forms of H3K4 methylations were present in Sertoli cells. H3K4me1 was absent in germ cells (also known as gonocytes), whereas H3K4me2 and H3K4me3 were detected weakly in gonocytes (Fig. 1, Table 1). These observations suggest that the establishment of H3K4me1 in germ cells is mainly at the stage of spermatogonia initiation.

3.2. The methylation of H3K27 was increased during spermatogenesis

We analyzed the expression pattern of H3K27 in both piglet and adult testis cross sections. In piglets, the