Theriogenology 83 (2015) 1154–1161

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

The Tet1 and histone methylation expression pattern in dairy goat testis

Liming Zheng, Haijing Zhu, Furong Tang, Hailong Mu, Na Li, Jiang Wu, Jinlian Hua*

College of Veterinary Medicine, Shaanxi Centre of Stem Cells Engineering & Technology, Northwest A&F University, Yangling, Shaanxi, China

ARTICLE INFO

Article history:
Received 6 August 2014
Received in revised form 11 December 2014
Accepted 13 December 2014

Keywords:
Tet (ten-eleven translocation) protein 1
DNA (de)methylation
5-Hydroxymethylcytosine
Histone H3 lysine 9 and 27
Spermatogonia stem cell
Dairy goat

ABSTRACT

DNA methylation and histone methylation are critical for mammalian development. Ten-eleven translocation (Tet1), a key regulator of DNA methylation, has been identified as a key enzyme for the activation of DNA demethylation; histone H3 lysine 9 (H3K9) and 27 (H3K27) methylation repress gene expression. Significant progress on the biological functions of Tet proteins has been made in mice and humans. However, their expression pattern and function in the male germ cells in the dairy goat testis are still unclear. The present study described the expression pattern of Tet1, H3K9, and H3K27 in the dairy goat testis and cultured goat spermatogonia stem cells (gSSCs). The results showed that Tet1 was weakly expressed in the dairy goat's testis compared to other organ tissues. Tet1, 5-hydroxymethylcytosine, H3K9, and H3K27 expressions were positive and dynamically changing during spermatogenesis; however, they showed weak expression in neonate stage *in vivo*. Tet1 and 5-hydroxymethylcytosine showed low expression in gSSCs *in vitro* in differentiated cultures. These will provide new perspectives for DNA methylation/demethylation and better regulation of epigenetic modifications in gSSCs.

1. Introduction

DNA methylation is identified as a major regulator in epigenetic modifications involved in gene regulation, genomic imprinting, X-chromosome inactivation, and carcinogenesis [1]. Although DNA methylation at the fifth position of cytosine (5 mC) has been extensively studied, the viewpoint that 5 mC is the substrate of ten-eleven translocation (Tet) protein in DNA demethylation was little known until recently. 5-Hydroxymethylcytosine (5hmC) is the direct derivative of 5 mC, and the 5-formylcytosine and 5-carboxylcytosine are the other subsequent derivatives [2].

Tet1 is highly expressed in embryonic stem cells (ESCs). It plays an important role in active DNA demethylation, and many studies have elucidated the functions of Tet1 in mouse ESCs [3], neuronal cells [4], human ESCs [5], and brain tissues [6]. Previous studies showed that overexpression of Tet1 and Tet2 *in vivo* conversed global 5 mC to 5hmC, which could accelerate DNA demethylation and transcriptional regulation [3].

In the developing primordial germ cells, Tet1 is required for proper erasure of genomic imprints [7]. However, Tet1 and Tet2 are not involved in the initial genome-wide DNA demethylation and shaping primordial germ cell epigenome in a locus-specific way [3].

Mammalian spermatogenesis is a balance between the self-renewal of spermatogonia stem cells (SSCs) and their differentiation into spermatocyte and spermatid, with the former governs the continuity of spermatogenesis and formation efficiency of spermatid. A recent study showed

* Corresponding author. Tel.: 029-87080068; fax: +86 29 87080068.
E-mail address: jinlianhua@nwsuaf.edu.cn (J. Hua).

0093-691X/$ – see front matter © 2015 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2014.12.020

that DNA methylation patterns during mammalian spermatogenesis are changing [8]. Histone modifications, especially histone methylation, also play important roles in spermatogenesis. During spermatogenesis, histone displacement is essential for the formation of histone variants, which is associated with genes expression and function in the testis by a reverse reciprocate manner [9]. Compared with DNA methylation, lysine methylation functions in both chromatin structure repression and activation in a context-specific manner, such as lysine 9 and 27 on histone H3 for gene repression and lysine 4, 36, and 79 on histone H3 for gene activation when they are methylated [10,11]. The different methylation patterns of K3K4me3 and H3K27me3 consisted Tet1 showed a special role in DNA demethylation mediated by Tet1 [12]. There are currently little information on the expression pattern of Tet1 and histone methylation during the spermatogenesis in mammals [8].

In our study, we investigated whether Tet1 existed during dairy goat spermatogenesis and analyzed Tet1 expression and 5hmC pattern *in vivo* and *in vitro*. Furthermore, the methylation patterns of lysine 4, 9, and 27 on histone H3 were analyzed. This study reports that Tet1 and histone methylation expression pattern in dairy goats and evidenced that they are critical for the spermatogenesis *in vivo* and the self-renewal of goat SSCs (gSSCs) *in vitro*.

2. Materials and methods

2.1. Collection of dairy goat testes

Guanzhong dairy goat testes were obtained from an abattoir in Yaoan, which were collected from goats at 0 day post postnatal (dpp), 3, 6, 9, 10, 12, and 18 months postnatal and treated as previously described [13]. Goat testes were transported to the laboratory within 4 hours of collection and subsequently prepared for cell isolation and culture. Goats were sacrificed, and all procedures were approved by Shaanxi Centre of Stem Cell Engineering & Technology, Northwest A&F University.

2.2. Cell culture

After collection, goat testicular tissues were prepared as described previously [13]. Briefly, the testicular tissues were washed 5 to 8 times in PBS containing penicillin (100 U/mL) and streptomycin (100 mg/mL). Seminiferous tubules from each testis were cut into small pieces by forceps and scissors. Seminiferous epithelial cells were dissociated by modified enzymatic digestion, followed by a two-step successive differential plating method to plate cells. The cells were cultured in Petri dishes treated by polylysine [14].

2.3. Immunohistochemistry and immunocytochemistry

Immunocytochemistry with Tet1 antibodies on fixed seminiferous tubules of goat at different stages (0 dpp, 6 and 18 months) was used to detect their localization and expression on protein levels as previously described [13]. The testicular tissues were dissected and fixed in 4% paraformaldehyde (PFA) for 24 hours and embedded in paraffin wax, before being sectioned, deparaffinized, and rehydrated following standard methods [15]. The slides were soaked in boiling citrate buffer for 15 to 25 minutes to get natural cooling and then washed in cold PBS for three times, each for 5 minutes. The cell samples were directly fixed in 4% PFA for 15 minutes without antigen retrieval treatment. The testicular tissues and cells were then incubated in 3% H_2O_2 for 10 minutes and then rinsed in PBS. Blocking was performed with 4% goat serum at room temperature for 30 minutes. The samples were then exposed to the primary antibody (anti-Tet1, 1:200; Bioworld, Nanjing, China) overnight at 4 °C. Appropriate horseradish peroxidase–conjugated secondary antibodies and chromogen solution, 3,3′-diaminobenzidine, were used according to the manufacturer's manual (Beijing Zhongshan Golden Bridge Biochemical Factory, Beijing, China). Testes from different age stages (three samples from each stage) were fixed, sectioned, and stained for Tet1 expression. Average percentages of Tet1-positive seminiferous tubules cross section (number of Tet1-positive cells per seminiferous tubule/total number of cells in each seminiferous tubule cross section) were plotted.

2.4. Immunofluorescence

Immunofluorescence of histone methylation antibodies on fixed seminiferous tubules of goat at different stages (3, 6, and 18 months) was performed to detect their localization and dynamic expression on protein level, and the testicular tissues were treated as previously described [13]. The testicular tissues were dissected and fixed in 4% PFA for 24 hours and embedded in paraffin wax, and then sections were cut, deparaffinized, and rehydrated following standard methods [15]. The slides were soaked in boiling citrate buffer for 15 to 25 minutes to get natural cooling, then to be washed in cold PBS for three times, each for 5 minutes. After three washes in PBS, the testicular tissues were blocked in 4% goat serum at room temperature for 30 minutes and then exposed to the primary antibody against H3K9me2, H3K9me3, H3K27me3, and H3K4me2 (1:200; Sino Biological Inc., Beijing, China) overnight at 4 °C. Then, to be washed in PBS for three times, each for 5 minutes and incubated in secondary fluorescein isothiocyanate (FITC)-conjugated antibodies (1:500; Chemicon) at room temperature for 30 minutes followed by three washes in PBS. Nuclei of cells were stained using 4′,6-diamidino-2-phenylindole. Images were captured using a fluorescence microscope (Leica, Hicksville, NY, USA) [16].

2.5. Immunofluorescence of primary cells cultured in vitro

To ensure the primary cells cultured *in vitro* were maintained SSCs rather than differentiated cells, they were cultured to form colonies [17], several specific markers of pluripotent stem cells, gSSCs markers, and histone methylation markers analyzed by immunofluorescence. The cell samples were directly fixed in 4% PFA for 15 minutes and 0.5% Triton X-100 for 10 minutes, blocked in 4% goat serum at room temperature for 30 minutes, and then