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Effects of scriptaid on the histone acetylation of buffalo oocytes and their ability to support the development of somatic cell nuclear transfer embryos

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ABSTRACT

The present study was undertaken to investigate the effect of scriptaid treatment on histone H3 on lysine 18 (H3K18) acetylation and relative expression levels of genes related to histone acetylation (*HAT1*, *CBP*, and *p300*) in buffalo oocytes during IVM. Meanwhile, the embryonic developmental ability of buffalo oocytes after SCNT was also examined. The H3K18 acetylation in oocytes increased from the germinal vesicle (GV) stage to the GV breakdown (GVBD) stage and arrived at a high acetylation level at the GVBD stage. Then, the H3K18 deacetylated completely at the metaphase I (MI) and acetylated again at the MII stage. However, addition of 500-nM scriptaid to the maturation medium resulted in a significant increase in the H3K18 acetylation at the MI stage. The expression profiles of genes related to histone acetylation (*HAT1*, *CBP*, and *p300*) in the meiosis stages of oocytes matured in the medium supplemented with 500-nM scriptaid were significantly higher than those of the oocytes matured in the medium without scriptaid ($P < 0.05$) with the exception of *p300* at the GVBD stage. More SCNT embryos reconstructed with oocytes matured in the medium supplemented with 500-nM scriptaid developed to blastocysts (23.1%) in comparison with oocytes matured in the medium without scriptaid (13.8%, $P < 0.05$). These results indicate that scriptaid can increase the histone acetylation of buffalo oocytes during meiotic maturation and improve their ability to support the development of SCNT embryos.

1. Introduction

Oocyte maturation is one of the most critical steps for the development of modern biotechnology, including IVF [1], SCNT [2], and intracytoplasmic sperm injection [3,4]. Oocytes are arrested at dictyate of the first meiotic prophase for long periods and resume meiosis in response to a hormonal stimulus. During this process, oocytes undergo nuclear progression from dictyate of the first meiotic prophase to metaphase II (MII), which is characterized by dissolution of the nuclear membrane, emission of the first polar body, and arrest of meiosis on MII till to fertilization or parthenogenetic activation. Buffalo oocytes recovered from 2- to 6-mm follicles are also arrested at dictyate of the first meiotic prophase and matured to MII after IVM of 22 hours [5].

Epigenetic modification can affect the transcriptions [6], which includes methylation, histone acetylation, phosphorylation, and polyubiquitination [7]. The histone acetylation was first detected to be associated with the level of transcriptions in 1964 [8]. The acetylation of core histones is

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considered to play a key role in various biological functions (oocyte maturation and preimplantation embryonic development). The core histones (H2A, H2B, H3, and H4) are wrapped by 147 base pairs of DNA and constitute the octamer of nucleosome which is the fundamental unit of chromatin [9]. Acetylation of histones H3 and H4 is more extensive than that of histones H2A and H2B. The core acetylation sites of histones are highly conserved, which are located at lysines in histones H3 (K9, K14, K18, and K27) and H4 (K5, K8, K12, and K16) [10,11]. Among these acetylation sites of histones, acetylation of histone H3 at position lysine 18 was proved to be associated with developmental potential of SCNT embryos [12,13].

Histone acetylation is a process of dynamic modification, which is catalyzed by histone acetyltransferases (HATs) and histone deacetylases (HDACs). Histone acetyltransferases are divided into three families: Gcn5/PCAF, p300/CBP, and MYST. The high expression of *HAT1* and *Gcn5* mRNA was detected in bovine oocytes at the germinal vesicle (GV) and MII stages [14]. The HDACs are also classified into three categories: class I (HDACs 1, 2, 3, and 8), class II (HDACs 4, 5, 6, 7, 9, and 10), and class III (SIRTs 1, 2, 3, 4, 5, 6, and 7) [11]. HDAC1 is localized in the nucleus of fully grown mouse oocytes [15] and associated with chromosomes congressed on metaphase plate at the MI and MII stage during meiotic maturation [16]. Wang et al. [17] reported that HDAC1 localized in the chromatin-depleted space at the GV stage but translocated to the periphery of condensed chromosomes with the meiotic resumption.

Histone acetylation was revealed to play a key role in various chromatin-based activities in meiosis [18]. DNA replication within chromosomal domain was associated with acetylation and deacetylation of histone H4 [19]. Increased acetylation level of histones can shorten the timing of DNA replication [20,21], which can be easily achieved by the HDAC inhibitor such as scriptaid [22], trichostatin A [23], and sodium butyrate [24]. During maturation process, the onset of GV breakdown (GVBD) in porcine oocytes was markedly delayed by the HDAC inhibitor [25]. De La Fuente et al. [26] revealed that mouse oocytes were arrested at MI stage when the HDAC inhibitor was added to the maturation medium. The lysine residue acetylation of histone in oocytes might vary in diverse species [25]. Thus, the effect of histone acetylation and deacetylation on oocyte maturation remains to be elucidated.

Therefore, the present study was undertaken to examine the effect of scriptaid on H3K18 acetylation and relative mRNA expression levels of genes related to histone acetylation (*HAT1, CBP*, and *p300*) in buffalo oocytes during the IVM, and then, the ability of oocytes to support the development of SCNT embryos was also investigated.

2. Materials and methods

Unless otherwise specified, all chemicals used in this study were purchased from Sigma Company (St. Louis, MO, USA). All culture media were sterilized by passing through a 0.22-μm filter (Millipore).

2.1. Maturation and collection of oocytes

Chinese swamp buffalo ovaries were obtained from a local slaughterhouse. Ovaries were excised within 20 to 30 minutes of slaughter and transported to the laboratory within 4 hours in a thermos containing PBS at 30 °C to 37 °C. Cumulus–oocyte complexes were recovered by aspiration of follicles in diameter of 2 to 6 mm using a 10-mL syringe with a 12-ga needle. Cumulus–oocyte complexes with intact cumulus cells were isolated from cellular debris and rinsed three times with a washing medium (TCM-199 [Gibco BRL] + 5.0-mM $NaHCO_3$ + 5-mM HEPES + 2% fetal bovine serum [FBS]). After washing, cumulus–oocyte complexes were matured in a10-mm glass dish containing 1.5-mL maturation medium (TCM-199 + 26.2-mM $NaHCO_3$ + 5-mM HEPES + 0.1 μg/mL FSH + 5% FBS + 60 mg/L penicillin G + 100 mg/L streptomycin sulfate) supplemented with or without 500-nM scriptaid for 22 hours under a humidified 5% CO_2 in air atmosphere at 38.5 °C. Scriptaid was dissolved in DMSO at 5 mM and then added to the maturation medium resulting in a working concentration at 500 nM (10,000 times diluted) in which the concentration of DMSO was 0.01%. After IVM of 22 hours, oocytes that extruded the first polar body were selected to enucleate and used for the recipient cytoplasm.

2.2. Immunohistochemistry analysis

The oocytes at the GV, GVBD, MI, and MII stage were selected for fixation at 0, 9, 15, and 22 hours after IVM according to the observation in bovine meiotic progression [27]. The surrounding cumulus cells of oocytes were completely removed manually by pipetting in the presence of 0.1% hyaluronidase. After washing twice in PBS supplemented with 0.01% Triton X-100 and 0.3% BSA (TBP), the denuded oocytes were fixed in 4% paraformaldehyde in PBS for 30 minutes at room temperature and then stored in 4% paraformaldehyde of PBS at 4 °C until immunohistochemical analysis. The fixed oocytes were permeabilized in 1% Triton X-100 in PBS for 10 minutes at room temperature and incubated with 1% BSA in PBS for 1 hour. After washing three times in TBP (5 minutes for each time), they were incubated with rabbit antibody against acetylated lysine at the 18th position of histone H3 (H3K18, 1:100 dilution; Upstate Biotechnology, USA) overnight at 4 °C. After washing in TBP, the oocytes were incubated with goat antirabbit immunoglobulin G fluorescein isothiocyanate–conjugated antibody (1:200 dilutions; Millipore, USA) for 1.5 hours at room temperature and then washed three times with TBP. The DNA in oocyte was counterstained with 25 μg/mL propidium iodide for 5 minutes. Samples were mounted on slides with a drop of Fluoromount-G (SouthernBiotech, USA) and detected under the LSM 510 META Laser Scanning Microscope (Zeiss, Germany). The nuclear fluorescence intensity was measured by Zeiss imagine software, and three replicates were performed for each stage with 10 oocytes.

2.3. Quantification real-time polymerase chain reaction

Five oocytes were collected at the each meiotic stage (GV, GVBD, MI, or MII) for preparing complementary DNA