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Dynamics and regulation of lysine-acetylation during one-cell stage mouse embryos

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ABSTRACT

Previous studies show that treatment of zygotes with trichostatin A (TSA), a histone deacetylase inhibitor (HDACi), impacts the subsequent development to a blastocyst as well as full-term development. To reveal the dynamics of protein acetylation, with and without TSA treatment during one-cell stage, we examined oocytes and zygotes by immunofluorescence and Western Blot analyses using anti-acetylated lysine and acetylated α-tubulin antibodies. In unfertilized oocytes, lysine acetylation level was extremely low over all but faintly detected in the spindle. Once oocyte activation occurs, a dramatic increase of lysine acetylation signal was observed mostly in the pronuclei and a fiber-like structure, the so called midbody, suggesting activation coupled up-regulation of lysine acetylation presumably in histones and α-tubulin. TSA treatment resulted in significantly more hyperacetylation not only in the midbody structure and pronuclei but also in the whole cytoplasm. Consistently, Western Blot analysis revealed that acetylation of proteins about 53 kDa and 11 kDa in size, corresponding to α-tubulin and histone H4 sizes respectively, were increased mainly after oocyte activation and exclusively enhanced by TSA treatment in zygotes. To confirm this behavior of acetylated nonhistone proteins, acetylated α-tubulin was examined and found to be faintly detected in the spindle of MII oocytes but later in whole in the cell of zygotes including the midbody, which was enhanced by TSA treatment. To elucidate the mechanism underlying up-regulation of lysine acetylation following oocyte activation, we assayed the HDAC activity, and found significant reduction of HDAC activity from MII to zygotic stages. Taken together, our data indicate that HDACs play an important role in maintaining low acetylated status in a MII oocyte. However, once an oocyte has been activated, histone and nonhistone proteins including α-tubulin are hyperacetylated partly due to a reduction of HDAC activity. TSA treatment of zygotes enhances their acetylation, which could affect subsequent embryonic development.

1. Introduction

Lysine acetylation is one type of reversible posttranslational protein modification and plays important roles in regulating protein function including gene expression for a wide range of cellular processes [1]. Lysine residues are acetylated by histone acetyltransferases (HATs) and deacetylated by histone deacetylases (HDACs), also called lysine deacetylases (KDACs). The modification change of core histone tails by HATs and HDACs contribute to the regulation of gene expression and works as epigenetic memory [2]. In addition to histones, other substrates for these enzymes include nonhistone proteins which are divided into two groups, nuclear and nonnuclear proteins represented by p53 and α-tubulin, respectively [1]. Treatment of cells with HDAC inhibitors (HDACi) such as trichostatin A (TSA) resulted in hyperacetylation of various proteins, suggesting a dynamic equilibrium of lysine acetylation *in vivo*.

In preimplantation embryos, it was suggested that the regulation of lysine acetylation through HDAC activity plays a pivotal role in the subsequent embryonic development rates [3]. Actually, treatment of the fertilized embryos with TSA leads to significant reduction of blastocyst rates [4]. In contrast, the treatment of parthenogenetic or round spermatid-injected embryos increased those rates [4]. More strikingly, treatment of the somatic cloned embryos with HDACi including TSA resulted in a more efficient *in vitro* development to the blastocyst stage from 2- to 5-fold [5]. Thus, the effects, harmful or not, of TSA treatment on embryonic development depend on their nuclear derivations [4]. In general, these effects of HDACi on embryonic development are supposed

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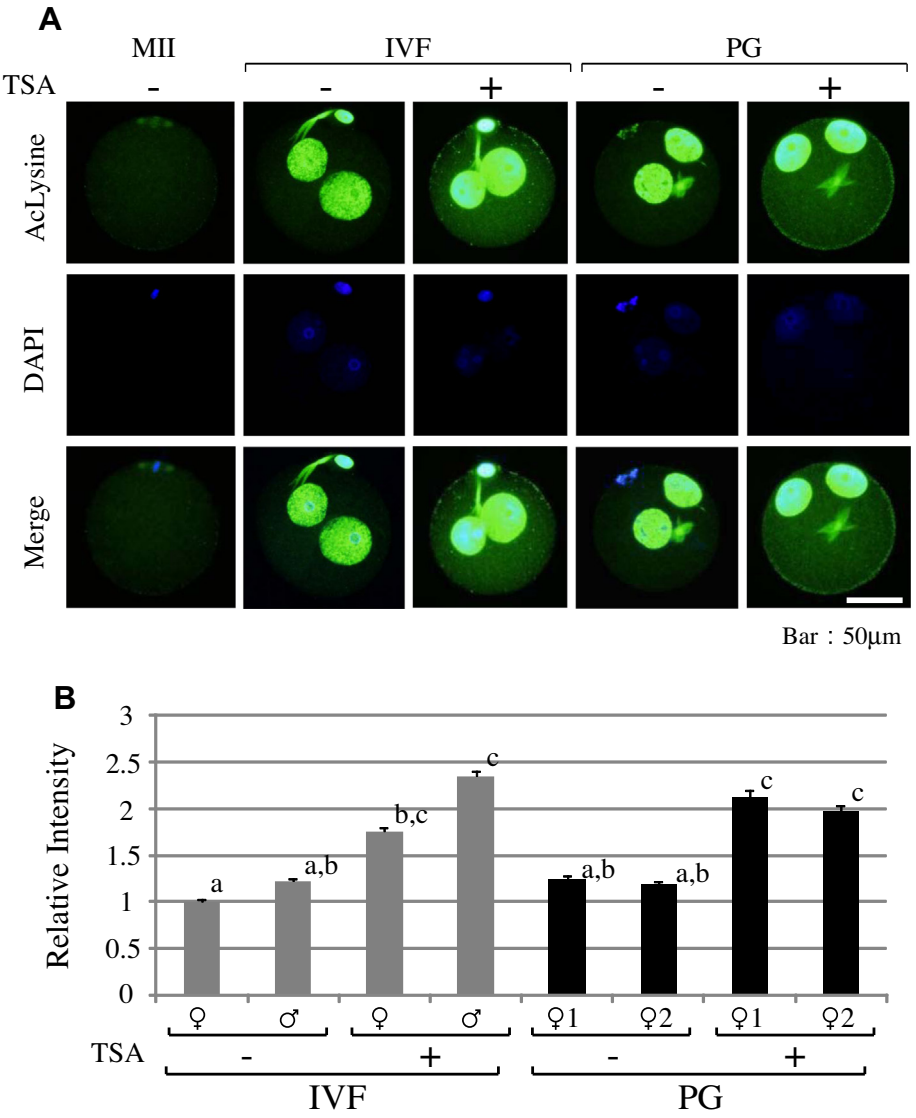

Fig. 1. Dynamics of lysine acetylation in MII oocytes, IVF and PG embryos with and without TSA treatment. (A) Embryos collected 10 h after oocyte activation or IVF were immunostained with anti-acetylated lysine antibody. Acetylated lysine (AcLysine) is shown in green. The DNA was counterstained with DAPI. Scale bar = 50 um (B) Quantification of acetylation in pronuclei in both IVF and PG embryos. In a PG embryo, the pronucleus which showed higher intensity is ♀1. Each value is shown after normalization by the mean value of these intensities female pronuclei in IVF embryos. These data are presented as the mean ±SEM. Values with different superscripts are significantly different at $P < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

to be due to the status of histone acetylation. However, the specific targets of HDACi remain unclear and little is known about details of the impact on nonhistone proteins by HDACi treatment other than histones.

Based on previous cell culture experiments, HDACi treatment of embryos is expected to result in hyperacetylation of a variety of proteins including histone and nonhistone proteins. In this study, we focus on the dynamics of lysine acetylation at one-cell stage and its regulation, and also the impact of TSA treatment on lysine acetylation and α-tubulin as a nonhistone protein.

2. Materials and Methods

2.1. Animals and collection of oocytes-cumulus complexes

B6D2F1 (C57BL/6 X DBA/2) mice were obtained at 7–8 weeks of age from SLC (Hamamatsu, Japan). All procedures involving animal conformed to the Kinki University Guidelines for the Care and Use of Laboratory Animals. To superovulate, B6D2F1 mice were injected with 7 IU pregnant mare serum gonadotropin (PMSG) and 7 IU human chorionic gonadotropin (hCG), which were given 48 h apart. Fourteen to sixteen hours after hCG injection, oocyte-cumulus complexes (OCCs) were collected from the oviducts.

2.2. Collection of sperms and in vitro fertilization

Sperms were collected from caudal epididymis for male B6D2F1 and cultured for 1 h in Human Tubal Fluid (HTF) medium containing 0.3% bovine serum albumin (BSA). After 1 h, collected OCCs were cultured with the sperms for 6 h in HTF medium containing 0.3% BSA. After 6 h, fertilized oocytes were transferred to KSOM AA medium and cultured for 4 h. These embryos were cultured with and without TSA treatment for a total of 10 h. The culture condition was at 37 °C in an atmosphere of 5% CO_2 in air.