Available online at www.sciencedirect.com
ScienceDirect
Theriogenology 71 (2009) 1245–1252
Theriogenology
www.theriojournal.com

Effect of trychostatin A treatment on gene expression in cloned mouse embryos

G.-B. Shao [a,b], H.-M. Ding [a], W.-L. Gao [a], S.-H. Li [a], C.-F. Wu [a], Y.-X. Xu [a], H.-L. Liu [a,*]

[a] College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, People's Republic of China
[b] School of Medicine, Jiangsu University, Zhenjiang 212013, People's Republic of China

Received 20 May 2008; received in revised form 7 January 2009; accepted 8 January 2009

Abstract

Histone deacetylation occurs upon the transfer of somatic nuclei into enucleated oocytes, but its role in reprogramming somatic chromatin to the totipotent state is unknown. To investigate the importance of histone deacetylation in reprogramming, we constructed embryos by electrofusing breast cancer cells with enucleated mouse oocytes. The reconstructed embryos were then cultured before and/or after activation for 6 h in the presence of trychostatin A (TSA), a potent inhibitor of histone deacetylase. Total RNA was isolated from these TSA-treated and untreated embryos and real-time reverse transcription PCR was conducted to monitor transcription of *ErbB2*, *Muc1*, *eIF-4C*, *MuERV-L*, and *c-mos* genes. The nuclear-cytoplasmic interaction inhibited typical expression of *ErbB2* and *Muc1* in the somatic cells. Moreover, the inhibition of histone deacetylation prior to activation did not increase the levels of *eIF-4C*, *MuERV-L*, and *c-mos* expression in the nuclear transfer (NT) embryos ($P > 0.05$), whereas additional treatment with 100 nM TSA beyond the activation point improved expression of these genes ($P < 0.05$). Trychostatin A treatment also improved the development rates of NT embryos at the 2-cell, 4-cell, and blastocyst stages (78.6% vs. 90.2%, 45.2% vs. 68.9%, and 16.7% vs. 30.3%, respectively, $P < 0.05$). We hypothesized that the reprogramming of gene expression in NT embryos is independent of somatic histone deacetylation, and that hyperacetylation may have a positive effect on NT embryo development.

Keywords: Breast cancer cell; Gene expression; Histone acetylation; Nuclear transfer; Trychostatin A

1. Introduction

The success of somatic cell cloning in mice has led to such applications as species preservation, livestock propagation, and cell therapy for medical treatment [1,2], but the efficiency of animal cloning is still very low. Moreover, several clinical anomalies, including high-abortion rates, increased body weight, and early death, have been reported [3–5]. Recent molecular analyses of cloned embryos have revealed abnormal epigenetic modifications such as DNA methylation and histone modifications [6,7]. In addition, aberrant gene expression has been detected in the placentas of clones [8,9]. Thus, prevention of abnormal gene expression and epigenetic modifications is necessary to improve the success rate of animal cloning.

Zygotic gene activation (ZGA) is a critical event in the onset of transcription during early embryonic development. In mice, zygotic gene activation occurs at the 2-cell stage [10], but transcription actually launches

* Corresponding author at: Laboratory of Animal Genetics and Breeding, College of Animal Science and Technology, Nanjing Agricultural University, WeiGang, Nanjing 210095, People's Republic of China. Tel.: +86 25 84395278; fax: +86 25 84395314.
E-mail address: liuhonglin@263.net (H.L. Liu).

0093-691X/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2009.01.004

at the late S phase in 1-cell embryo [11,12]. Recently, Nervi et al. [13] found that histone hyperacetylation induced by trychostatin A (TSA), a potent inhibitor of histone deacetylase, increased embryonic gene expression during mouse embryogenesis. A similar study found that treatment of *Entamoeba histolytica* with TSA affected both overall gene expression and stage conversion [14]. These findings clearly showed that histone acetylation is involved in embryonic gene expression.

While histone deacetylation in somatic nuclei occurs upon the transfer of nuclei into metaphase II oocytes [15], the biological consequence of this event has yet to be explored. This study was designed to gain insight into the effect of histone acetylation on the regulation of gene expression in cloned mouse embryos. We treated cloned embryos with TSA and examined their transcription profiles. Furthermore, we investigated the role of histone acetylation in the development of cloned embryos.

In the present study, the genes chosen for analysis were of three types. The first type was represented by two embryonic genes shown to be transcriptionally active during ZGA: murine endogenous retrovirus-like (*MuERV-L*), one of the first genes transcribed in mouse 1-cell embryos [16] that is highly transcribed in 2-cell embryos but only weakly transcribed at the blastocyst stage [16,17], and eukaryotic translation initiation factor-4C (*eIF-4C*), which is predominantly expressed in mouse 2-cell embryos [18]. The second type included two genes that are specifically expressed by donor cells and highly expressed in breast cancer cells: erythroblastic leukemia viral oncogene homolog 2 (*ErbB2*) and mucin 1 (*Muc1*) [19–21]. The third type was *c-mos*, an oocyte-specific gene [22].

The objectives of the current study were to determine the effects of histone acetylation on the expression of developmentally important genes in cloned mouse embryos and the development of cloned embryos.

2. Materials and methods

2.1. Materials

All reagents and chemicals used in this study were purchased from Sigma–Aldrich Inc. (St. Louis, MO, USA) unless otherwise noted. Modified Whitten medium (mWM) [23] and potassium simplex optimized medium (KSOM) [24] were prepared for the handling and culture of the mouse oocytes and embryos, respectively.

2.2. Animals

Kunming (KM) female mice (3–4 wk old) and ICR male mice (12–16 wk old) were obtained from the Experimental Animal Breeding Center of Nanjing Medicinal University. The donor mammary tumor cells (RIII mice C127) were purchased from the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. All experimental protocols were approved by the Animal Ethics Committee of Nanjing Agricultural University. All animals were maintained in accordance with the Animal Experimental Standards of Nanjing Agricultural University.

2.3. In vitro embryo production

Female mice were treated with 10 IU of pregnant mares' serum gonadotrophin (MSG; Tianjin Animal Hormone Factory, Tianjin, China) followed by 10 IU of human chorionic gonadotropin (hCG; Tianjin Animal Hormone Factory) 48 h later. Mature oocytes were collected from the oviducts of the mice 14–16 h after hCG injection, placed in mWM, and treated with 0.1% hyaluronidase until the cumulus cells dispersed. During *in vitro* fertilization (IVF), the metaphase II oocytes were inseminated with capacitated sperm from an ICR male mouse. Two hours after insemination, fertilized eggs were washed with KSOM, then cultured in fresh KSOM supplemented with 3 mg/mL bovine serum albumin (BSA) at 37 °C in an atmosphere of 5% CO_2 and 95% air.

2.4. Nuclear transfer

Enucleation and cell transfer were carried out at 37 °C on the heated stage (controlled by a constant temperature device) of an inverted microscope (IX71; Olympus, Tokyo, Japan) using a piezo micromanipulator controller (PMAS-CT 150; Prime Tech, Ibaraki, Japan). Self-fabricated holding pipettes (80–100 μm in diameter), enucleation pipettes (10–14 μm in diameter), and injection pipettes (12–15 μm in diameter) were sterilized by exposure to ultraviolet light. Approximately 10–15 oocytes were placed into a drop of HEPES-buffered KSOM containing 5 μg/mL cytochalasin B (CB) under mineral oil. Those oocytes with a metaphase plate were identified by inverted microscopy. Using a few piezo pulses (speed 2–3, intensity 2–4) to advance the enucleation pipette while applying very slight negative pressure, the chromosomes were gently aspirated. After removal of the CB, the oocytes were cultured in fresh KSOM.