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Donor cell trichostatin A treatment improves the in vitro development of cloned goat embryos

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ABSTRACT

The low success rate of somatic cell nuclear transfer (SCNT) is primarily due to incomplete reprogramming of the transferred nuclei. As an inhibitor of histone deacetylase, trichostatin A (TSA) might promote the reprogramming process and improve cloned embryo development. In the current study, TSA was investigated in terms of its ability to improve the development of cloned goat embryos, and the appropriate exposure time and concentration were also optimized. The results revealed that donor cells treated with 25 nM TSA for 12 h exhibited significantly increased blastocyst development (34.0% vs. 17.6%, $p < 0.05$) that was accompanied by a moderate increase in the two-cell embryo histone acetylation level and increased blastocyst cell numbers compared to the control group. Donor cells from different individuals were also examined, and consistent results were found in all groups. In conclusion, the treatment of donor cells with TSA improved the development of cloned goat embryos.

1. Introduction

Somatic cell nuclei can be reprogrammed to an embryonic state through nuclear transfer, and oocyte cytoplasm can provide factors that are necessary for conferring pluripotent status on differentiated somatic nuclei (Wilmut et al., 1997; Wakayama et al., 1998, 2001). However, the efficiency of this reprogramming process is low. The low success rate of SCNT is primarily due to incomplete reprogramming and subsequent errors in epigenetic modifications (Jaenisch et al., 2002; Wilmut, 2002) including nuclear histone acetylation (Turner, 2000).

Histones have dual functions in the nucleus as important structural components and gene expression regulators. Increased histone acetylation relaxes the chromatin configuration and confers a transcriptionally permissive state (Lee et al., 1993; Zlatanova et al., 2000; Li, 2002) that improves the process of reprogramming donor chromatin, which might improve the development of cloned embryos (Wilmut et al., 2002). TSA is a potent inhibitor of histone deacetylase and thus might enhance the pool of acetylated histones (Yoshida et al., 1990) and promote the expression of the transcriptionally silent alleles of imprinted genes (Pedone et al., 1999). Studies in bovines have indicated that treatment with TSA can improve in vitro blastocyst production (Enright et al., 2003; Ding et al., 2008; Srirattana et al., 2012; Oh et al., 2012). The treatment of cloned embryos with TSA also results in significant improvements in embryo development in mice (Kishigami et al., 2006; Rybouchkin et al., 2006) and pigs (Zhang et al., 2007; Chawalit et al., 2012). However, the effects of TSA treatment on goat SCNT have not been reported, and the TSA concentration and exposure period also require refinement. In the present study, the effects of TSA treatment of donor cells on

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the development and histone acetylation status of cloned goat embryos in vitro were investigated.

2. Materials and methods

2.1. Preparation of the donor cells for nuclear transfer

Adult fibroblast cells were derived from three newborn Saanen dairy goats. Briefly, ear skin was collected and minced into small pieces (1–2 mm^2) that were cultured for 1 to 2 wk in Dulbecco's Modified Eagle Medium (DMEM, Gibco) containing 10% fetal calf serum (FCS) at 38.5 °C in a humidified atmosphere of 5% CO_2. When a cell monolayer formed, the tissue blocks were removed, and the fibroblast cells were cultured until confluence. The cells at passages three to five times were selected as nuclei donors and induced into the G0/G1 phase using the serum deprivation method. Portions of the donor cells were treated with TSA as explained below.

2.2. Donor cell treatment outline

The donor cell treatment was performed before somatic cell nuclear transfer. The cells were plated in 24-well plates at a density of 1.0×10^5 cells/mL. TSA (Sigma) was dissolved in dimethylsulfoxide (DMSO, Sigma) and prepared in 1000-fold concentrated stock solutions (12.5 μM, 25 μM, 50 μM, 100 μM and 200 μM) according to the following experimental procedure. These TSA stock solutions were aliquoted, frozen at −20 °C and added to the culture media at a 1:1000 dilution immediately before use. An equal volume of vehicle was added to the control group (0 nM TSA).

To optimize the timing and concentration of the TSA treatment, goat donor cells were treated with 50 nM TSA for different time periods (0 h, 6 h, 12 h, 24 h and 48 h), and the embryo development, blastocyst cell numbers and histone acetylation levels of the cloned embryos were measured as described in a previous study of bovines (Ding et al., 2008). This procedure indicated that 12 h was the optimal treatment time. Next, different concentrations of TSA (0 nM, 12.5 nM, 25 nM, 50 nM, 100 nM and 200 nM) were evaluated in 12 h treatments, and the parameters were measured.

To compare the variations across the different donor cell samples, fibroblast cells derived from another two individuals treated with 25 nM for 12 h, and the numbers of blastocysts were compared across the donor individuals.

2.3. Oocyte collection and in vitro maturation

Ovaries were collected from a local abattoir and transported to our laboratory within 4 h of slaughter. Only healthy-looking cumulus-oocyte complexes (COCs) were chosen and cultured in Tissue Culture Medium-199 (TCM199, Gibco) plus 10% FCS at 38.5 °C in 5% CO_2 for 20–22 h. Thereafter, the cumulus cells were removed by repeated pipetting in PBS containing 1 mg/mL hyaluronidase, and only oocytes with the first polar body were used in the further experiments.

2.4. Somatic cell nuclear transfer

Enucleation of the oocytes was performed in microdrops of PBS supplemented with 7.5 μg/mL cytochalasin B and 10% FCS. Thereafter, TSA-treated and control donor cells were trypsinized using 0.25% trypsin-EDTA, washed by centrifugation, resuspended in PBS, and then transferred to the recipient oocyte perivitelline spaces. After microinjection, the reconstructed embryos were transferred to Zimmermann's fusion medium to equilibrate for 5 min. Fusion was performed with a pair of tip-end microelectrodes using two direct current pulses (32 V for 20 μs) as described by Liu et al. (2007). All fused reconstructed embryos were further activated in 5-μM ionomycin for 5 min and incubated with 1.9-mM 6-dimethylaminopurine for 4 h in synthetic oviduct fluid with amino acids (SOFaa).

2.5. Embryo culture and blastocyst cell number evaluation

After activation, all reconstructed embryos were cultured in SOFaa with 8 mg/mL BSA in a humidified atmosphere of 5% CO_2 at 38.5 °C for 48 h and then transferred to SOFaa supplemented with 10% FCS for an additional 5 days. The subsequent in vitro development of the embryos to the two-cell and blastocyst stages was recorded. More than 10 cloned blastocysts in each group were stained with Hoechst 33342 (10 μg/mL) for 10 min, and the total blastocyst cell numbers were evaluated using fluorescence microscopy.

2.6. Histone acetylation immunodetection in cloned embryos

The immunofluorescence analyses were performed as previously described (Beaujean et al., 2004; Ding et al., 2008). Briefly, the two-cell cloned embryos and blastocysts were fixed in 4% paraformaldehyde for 30 min at room temperature, permeabilized with 0.5% Triton X-100 for 30 min, washed in PBS plus 0.05% Tween-20 and blocked for 1 h in PBS containing 2% BSA. Histone H4 (acetyl lysine 12) primary antibody (rabbit polyclonal antibody against histone H4 acetyl K12, Santa Cruz, 1:200) was used to stain the embryos for 1 h at 37 °C. After washing with PBS (30 min), the embryos were labeled with anti-rabbit IgG FITC-conjugated secondary antibody, and the DNA was counterstained with 10 μg/mL propidium iodide for 30 min. The embryos were mounted on glass slides with one drop of mounting medium and detected with a Carl Zeiss laser-scanning confocal microscope (Oberkochen, Germany). For the quantitative measurements of histone acetylation, the nuclear intensities were measured by manually outlining all nuclei (two-cell embryos) or 25 nuclei/blastocysts (randomly selected), and the total fluorescence intensity emitted by each individual nucleus was measured to create per embryo averages. In each quantification procedure, the value of the control embryos was arbitrarily set to 100%, and the fluorescence intensities observed in the individual samples are presented relative to the controls. Appropriate controls for the