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Synergistic effect of trichostatin A and scriptaid on the development of cloned rabbit embryos

C.H. Chen [a,1,2], F. Du [b,c,1], J. Xu [d], W.F. Chang [a], C.C. Liu [a], H.Y. Su [a], T.A. Lin [e], J.C. Ju [e,f], W.T.K. Cheng [a,g], S.C. Wu [a,g], Y.E. Chen [d], L.Y. Sung [a,h,*]

[a] Institute of Biotechnology, National Taiwan University, Taipei, Taiwan
[b] College of Life Sciences, Nanjing Normal University, Nanjing, Jiangsu, China
[c] Renova Life Inc., University of Maryland, TAP Program, College Park, Maryland, USA
[d] Cardiovascular Center, University of Michigan Medical Center, Ann Arbor, Michigan, USA
[e] Department of Animal Science, National Chung-Hsing University, Taichung, Taiwan
[f] Agricultural Biotechnology Center, National Chung Hsing University, Taichung, Taiwan
[g] Institute and Department of Animal Science and Technology, National Taiwan University, Taipei, Taiwan
[h] Agricultural Biotechnology Research Center, Academia Sinica, Taipei, Taiwan

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ABSTRACT

The first successful rabbit SCNT was achieved more than one decade ago, yet rabbits remain one of the most difficult species to clone. The present study was designed to evaluate the effects of two histone deacetylase inhibitors (HDACis), namely trichostatin A (TSA) and scriptaid (SCP), on cloning efficiency in rabbits. The *in vitro* development, acetylation levels of histone H4 lysine 5 (H4K5), and octamer-binding transcription factor 4 (Oct-4) expression patterns of cloned embryos were systemically examined after various HDACi treatments. Supplementation of TSA (50 nM) or SCP (250 nM) in the culture medium for 6 hours improved blastocyst development rates of cloned embryos compared with the treatment without HDACi. The combined treatment with TSA (50 nM) and SCP (250 nM) further enhanced morula (58.6%) and blastocyst (49.4%) rates *in vitro*. More importantly, compared with single HDACi treatments, embryos with the combined treatment had a higher level of H4K5 and an increased total cell number (203.7 ± 14.4 vs. 158.9 ± 9.0 or 162.1 ± 8.2; $P < 0.05$) with a better Oct-4 expression pattern in hatching blastocysts, indicating substantially improved embryo quality. This was apparently the first report regarding Oct-4 expression in cloned rabbit embryos. We inferred that most cloned rabbit embryos had an aberrant inner cell mass (ICM) structure accompanied with abnormal spatial distribution of Oct-4 signals. This study demonstrated a synergistic effect of TSA and SCP treatments on cloned rabbit embryos, which might be useful to improve cloning efficiency in rabbits.

1. Introduction

Somatic cell nuclear transfer (SCNT) technology has been a useful tool for reprogramming terminally differentiated cells in agriculture, and in research in biomedicine and regenerative biology. Using this technology, cloned animals have been born in many species; however, the success rates remain low. Improper gene expression patterns, likely from the inheritance of epigenetic marks of differentiated donor nuclei, can lead to developmental arrest or abnormalities [1–6]. Recently, a key enzyme, oxidase tet methylcytosine dioxygenase 3 in oocytes, was reported to promote active demethylation of the paternal genome via a base excision repair pathway

* Corresponding author. Tel.: +011 886 2 3366 6015; fax: +011 886 2 3366 6001.
E-mail address: liyingsung@ntu.edu.tw (L.Y. Sung).
[1] These authors contributed equally to the study.
[2] Present address: Division of Biotechnology, Animal Technology Institute Taiwan, Miaoli, Taiwan.

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[7–10]. In addition, tet methylcytosine dioxygenase 3-mediated DNA demethylation was also reported in cloned embryos [9,10]. However, it is noted that the somatic genome is much different from the sperm, in which high acetylation levels and lack of normal repressive marks on histone tails are observed after protamine–histone exchange [11]. This discrepancy likely resulted in inefficient demethylation in SCNT embryos and produced a methylation pattern more similar to the donor cell rather than a zygote [12]. Treatments of chromatin modifiers to moderate epigenetic settings are thus suggested to improve SCNT efficiency [13]. Among these approaches, the use of a histone deacetylation inhibitor (HDACi) to alter histone acetylation patterns greatly improve nuclear reprogramming, gene expression, blastocyst quality, and full-term development in various species [14–19].

Applying trichostatin A (TSA), a HDACi, on cloned rabbit embryos has advantageous effects on histone modification patterns, cell number, and embryo development during preimplantation stages in rabbits [20,21]. Notably, however, Meng et al. reported that TSA was not helpful with regard to term development and might have caused neonatal death of cloned rabbits [22].

The effects of HDACi agents other than TSA on rabbit cloning have apparently not been reported. For example, scriptaid (SCP), a novel HDACi, is potent to enhance transcriptional activity and protein expression in mammalian cells [23]. In SCNT, SCP treatment is of low toxicity for embryo development, and improves cloning efficiency by modifying histone acetylation patterns and alleviating aberrant gene expression, as demonstrated in mice, pigs, and cattle [18,19,24]. However, there are apparently no reports regarding the use of SCP in culture of cloned rabbit embryos.

Octamer-binding transcription factor 4 (Oct-4) is the most important transcription factor required for pluripotent lineage formation and germ cell development in mice [25,26]. Inefficient reprogramming using SCNT was associated with abnormal Oct-4 reactivation [27]. Moreover, the acetylation level of H4K5 is an indicator of global gene activation. Previously, we documented Oct-4 expression patterns and level of H4K5 acetylation (H4K5ac) in normally fertilized embryos [28].

The present study aimed to assess effects of a combination of TSA and SCP on *in vitro* developmental competence of cloned rabbit embryos. We first examined the effects of TSA or SCP and determined the optimal concentration of the individual HDACi. Development of cloned embryos treated with a single HDACi and the combined treatment were compared, and total cell numbers, levels of H4K5ac, and Oct-4 expression patterns of hatching blastocysts were compared with those in normal embryos.

2. Materials and methods

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA) unless otherwise indicated. The media were prepared as described [29]. Briefly, Dulbecco's phosphate-buffered saline (DPBS; 15240-013; Gibco, Grand Island, NY, USA) containing 0.1% polyvinyl alcohol (PVA) was used for flushing oocytes from oviducts (PBS-PVA) and collecting cumulus–oocyte complexes from ovarian follicles. Tissue and cell cultures were maintained in Dulbecco's minimum Eagle's medium (DMEM; 11995-065; Gibco). Medium 199, which contains Earle's salts, L-glutamine, 2.2 g/L sodium bicarbonate, and 25 mM HEPES (12340-014; Gibco), was supplemented with 10% fetal bovine serum (FBS; SH0070.03; Hyclone, Logan, UT, USA) and used as the standard manipulation medium. Rabbit oocytes and embryos were cultured in B2 medium (Laboratories CCD, Paris, France) containing 2.5% FBS at 38.5 °C in 5% CO_2 and humidified air.

2.1. Preparation of donor animals

Sexually mature (6- to 18-month-old) New Zealand White (NZW) or hybrid strain (NZW female mating with Rex male) female rabbits (oocyte donors) were superovulated as described [29]. Briefly, the present study applied two 3-mg, two 4-mg, and two 5-mg treatments of Folltropin-V (Bioniche Animal Health Canada, Belleville, Ontario, Canada) im at 12-hour intervals. To induce ovulation, 200 IU of human chorionic gonadotropin (hCG; Chorulon, animal use; Intervet Inc., Millsboro, DE, USA) iv was used.

The animal maintenance, care, and use procedures were reviewed and approved by the Institutional Animal Care and Use Committee of National Taiwan University. The surgical procedures in this study were performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals [30].

2.2. Collection of follicular oocytes

The reproductive tracts including ovaries were excised from euthanized donor rabbits using midventral laparotomy for oocyte collection. Cumulus–oocyte complexes were either collected from the top of the ovulation site, the ovarian Graafian follicles, or flushed from the oviducts at 10 to 12 hours after hCG treatment (hpi). Rabbit cumulus–oocyte complexes were treated with 0.5 mg/mL hyaluronidase in PBS-PVA for 1 minute, and cumulus cells were completely removed from the oocytes by careful pipetting with a fine bore glass pipette. The mature oocytes with first polar body examined under a stereomicroscope were selected for nuclear recipients.

2.3. Preparation of donor cells from rabbit tissues

The skin notches were taken from the ear of an adult NZW rabbit. Cell culture was performed as described [29]. Briefly, a skin sample with a diameter of 0.5 cm was cut into four or five pieces, washed, placed in a Falcon cell culture dish (Falcon 3001; Becton Dickinson Labware, Oxnard, CA, USA) containing 10% FBS DMEM, and cultured at 37 °C in 5% CO_2 humidified air. Fibroblast monolayers formed around the tissue explants in 5 to 7 days. Fibroblasts were cultured until confluence was reached. During passaging, cultured cells were thoroughly washed with DPBS and then gently digested with 0.05% trypsin-EDTA for 3 minutes at 37 °C. Trypsinization was terminated by adding 10% FBS in DMEM and washing. The cell suspension was then centrifuged at