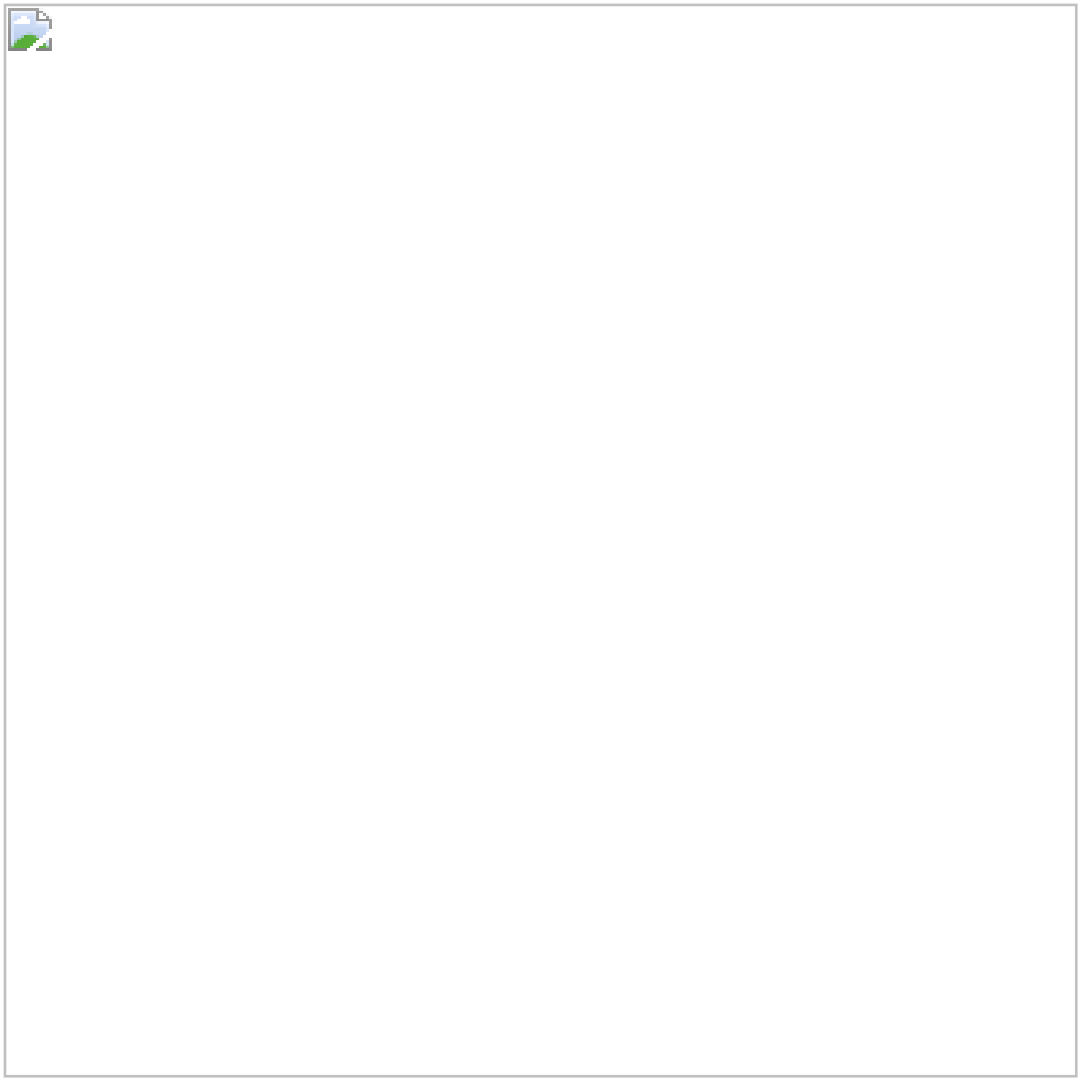

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Source: Biology of Reproduction, 81(3) : 525-530

Published By: Society for the Study of Reproduction

URL: https://doi.org/10.1095/biolreprod.109.077016

BIOLOGY OF REPRODUCTION **81,** 525–530 (2009)
Published online before print 22 April 2009.
DOI 10.1095/biolreprod.109.077016

Significant Improvement in Cloning Efficiency of an Inbred Miniature Pig by Histone Deacetylase Inhibitor Treatment after Somatic Cell Nuclear Transfer[1]

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ABSTRACT

The National Institutes of Health (NIH) miniature pig was developed specifically for xenotransplantation and has been extensively used as a large-animal model in many other biomedical experiments. However, the cloning efficiency of this pig is very low (<0.2%), and this has been an obstacle to the promising application of these inbred swine genetics for biomedical research. It has been demonstrated that increased histone acetylation in somatic cell nuclear transfer (SCNT) embryos, by applying a histone deacetylase (HDAC) inhibitor such as trichostatin A (TSA), significantly enhances the developmental competence in several species. However, some researchers also reported that TSA treatment had various detrimental effects on the in vitro and in vivo development of the SCNT embryos. Herein, we report that treatment with 500 nM 6-(1,3-dioxo-1H, 3H-benzo[de]isoquinolin-2-yl)-hexanoic acid hydroxyamide (termed *scriptaid*), a novel HDAC inhibitor, significantly enhanced the development of SCNT embryos to the blastocyst stage when NIH inbred fetal fibroblast cells (FFCs) were used as donors compared with the untreated group (21% vs. 9%, $P < 0.05$). Scriptaid treatment resulted in eight pregnancies from 10 embryo transfers (ETs) and 14 healthy NIH miniature pigs from eight litters, while no viable piglets (only three mummies) were obtained from nine ETs in the untreated group. Thus, scriptaid dramatically increased the cloning efficiency when using inbred genetics from 0.0% to 1.3%. In contrast, scriptaid treatment decreased the blastocyst rate in in vitro fertilization embryos (from 37% to 26%, $P < 0.05$). In conclusion, the extremely low cloning efficiency in the NIH miniature pig may be caused by its inbred genetic background and can be improved by alteration of genomic histone acetylation patterns.

developmental biology, early development, embryo, HDAC inhibitor, histone acetylation, inbred, SCNT

INTRODUCTION

Because of their similar anatomy and physiology, swine have been used in biomedical applications for decades as a model for human diseases, as a genetically defined model for surgery and xenotransplantation, and as a source of human disease therapeutics [1]. By combining the technology for homologous recombination in somatic cells with that of somatic cell nuclear transfer (SCNT), it is possible to create specific modifications to the pig genome [2]. Several pig models that have potential applications in basic life science research or in the study of human diseases have been made such as enhanced green fluorescent protein-expressing pigs [3], alpha-1,3-galactosyltransferase knockout pigs [4], and cystic fibrosis transmembrane conductance regulator knockout pigs [5, 6]. However the widespread application of porcine SCNT to biomedical research is being hampered by the large adult size (300–600 lb) of the commercial breeds commonly used for SCNT [7], as well as the low cloning efficiency. The miniature pig also shares many physiological similarities with humans and offers several breeding and handling advantages (compared with nonhuman primates), making it an optimal species for preclinical experimentation [8].

The National Institutes of Health (NIH) miniature pig was developed by Sachs et al. [9] more than 30 yr ago specifically for xenotransplantation. Three sublines with divergent genotypes at the swine leukocyte antigen (SLA^{aa}, SLA^{cc}, and SLA^{dd}) were derived from two matings of the same two animals and are now highly inbred [10]. Possibly because these animals are highly inbred, their reproductive characteristics are lower than those of domestic pigs [11, 12]. Although several pig breeds have been successfully cloned, the remodeling and reprogramming of differentiated somatic nuclei into a totipotent embryonic state by SCNT are not efficient, and the mechanism by which this remodeling occurs is not known. This results in an overall low cloning efficiency. In most mammalian species studied thus far, the survival rate to birth for cloned blastocysts is only about 1%–5% compared with a 30%–60% birthrate for in vitro fertilization (IVF) blastocysts [13]. Our inventory of NIH miniature pigs consisted of two SLA^{cc} animals (one male and one female). When we tried to clone them from adult ear fibroblast cells (EFCs), none of six embryo transfers (ETs) established a pregnancy (J. Zhao and R.S. Prather, unpublished results), and a previous study [4] showed a cloning efficiency for SLA^{dd} animals of only 0.2%. The lower cloning efficiency has been an obstacle to the application of these inbred swine genetics for biomedical research. The HDAC inhibitor trichostatin A (TSA) can improve the efficiency of cloning mice in both reproductive cloning [14, 15] and SCNT embryonic stem cell derivation [14]. Adult male and female outbred mice (ICR) were successfully cloned only when TSA was applied [16]. The SCNT embryos treated with TSA resulted in higher preim-

[1]Supported by the National Institutes of Health National Center for Research Resources RR018877 and RR013438, and Food for the 21st Century.
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Received: 17 February 2009.
First decision: 4 March 2009.
Accepted: 7 April 2009.

eISSN: 1259-7268 http://www.biolreprod.org
ISSN: 0006-3363

plantation embryonic development in pigs [17, 18], cattle [19, 20], and rabbits [21]. Although TSA application resulted in great improvement in somatic cloning in mice, the effects of TSA treatment on cloning efficiency are controversial in several species, and some groups have reported that TSA treatment had various detrimental effects on the in vitro and in vivo development of the SCNT embryos [22–25]. To our knowledge, the effects of histone deacetylase (HDAC) inhibitor treatment on full-term development have not been determined in any species other than mice. Therefore, we determined if HDAC inhibitor treatment had beneficial effects on SCNT in the pig. Considering the effects of TSA treatment on SCNT, we investigated the effect of 6-(1,3-dioxo-1H, 3H-benzo[de]isoquinolin-2-yl)-hexanoic acid hydroxyamide (termed *scriptaid*), an HDAC inhibitor with low toxicity that enhances transcriptional activity and protein expression [26], on SCNT efficiency of NIH miniature pigs. The objectives of this study were 1) to investigate the effect of scriptaid treatment on the developmental competence of somatic nuclei following SCNT using NIH miniature inbred donor cells in vitro and in vivo and 2) to determine if scriptaid affects the development of in vitro-produced embryos.

MATERIALS AND METHODS

Animals were treated according to a preapproved protocol. University of Missouri institutional animal care and use guidelines were followed.

Media and Reagents

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise stated. All of the following solutions and media were filtered using a 0.22-μm filter. The cell culture medium was Dulbecco modified Eagle medium supplemented with 15% (v/v) fetal bovine serum (catalog No. SH30071.03, lot No. ASM31113; Hyclone, Logan, UT) and antibiotics. The oocyte in vitro maturation (IVM) medium was TCM 199 (Gibco BRL, Grand Island, NY) supplemented with 0.1% polyvinylalcohol (PVA) (w/v), 3.05 mM D-glucose, 0.91 mM sodium pyruvate, 1 μg/ml gentamicin, 0.57 mM cysteine, 0.5 μg/ml luteinizing hormone, 0.5 μg/ml follicle-stimulating hormone, and 10 ng/ml epidermal growth factor. Medium used for IVF was a modified Tris-buffered medium (mTBM) containing 2 mg/ml bovine serum albumin (BSA) and 2 mM caffeine. Sperm washing medium was Dulbecco PBS (DPBS) (Gibco BRL) supplemented with 1 mg/ml BSA (pH 7.3). The embryo culture medium was porcine zygote medium 3 (PZM3), pH 7.3, supplemented with 3 mg/ml BSA. Stock solutions of TSA and scriptaid were dissolved in dimethyl sulfoxide at 100 nM and 1 mM, respectively, and stored at −20°C. They were added to the embryo culture medium in specific amounts according to the protocol of each experiment.

Primary Cell Establishment and Donor Cell Preparation

Adult EFCs were established from our two NIH miniature SLA^{cc} animals and then used for SCNT and ETs to produce pregnancies for making fetal fibroblast cells (FFCs). At Day 35, the female was euthanized, and FFCs were derived from two fetuses (one male and one female) and established as previously described [27]. Briefly, the fetuses were recovered and rinsed three times with DPBS. After removal of head, intestine, liver, limbs, and heart, the remaining tissues were finely minced into pieces (1 mm^3) using scissors in DPBS. Minced tissue pieces were digested with collagenase (200 μ/ml) and DNase I (25 U/ml) in cell culture medium for 4–5 h at 38.5°C and 5% CO_2 in air. The digestion was rinsed in cell culture medium by centrifugation at 500 × *g* for 5 min to remove the residual enzyme. The cell pellet was resuspended in the cell culture medium, seeded in a 75-cm^2 culture flask, and left to culture until confluent.

For SCNT, the cells were thawed, cultured, and subsequently used between passages three and five. A suspension of single cells was prepared by trypsinization of the cultured cells, followed by resuspension in manipulation medium (25 mM Hepes-buffered TCM199 with 3 mg/ml BSA) before SCNT.

In Vitro Maturation

Ovaries were collected from prepubertal gilts at a local slaughterhouse and transported to the laboratory at 37°C. Follicles between 3 and 6 mm in diameter were aspirated with an 18-gauge needle attached to a 10-cc syringe. The cumulus-oocyte complexes (COCs) in the follicular fluid were allowed to settle by gravity. The COCs were rinsed in Hepes-buffered Tyrode medium containing 0.01% PVA three times [28]. Only the COCs with multiple layers of intact cumulus cells and uniform ooplasm were selected for IVM. After washing three times in IVM medium, a group of 70–80 COCs was placed into each well of four-well cell culture plates (Nunc, Roskilde, Denmark) containing 500 μl of IVM medium. The COCs were matured for 44 h (if used for IVF) or 40 h (if used for SCNT) at 38.5°C and 5% CO_2 in air (100% humidity). Matured COCs were then vortexed in 0.1% hyaluronidase in Hepes-buffered Tyrode medium containing 0.01% PVA for 4 min to remove the cumulus cells. Only the matured oocytes having an extruded first polar body (PB) with uniform cytoplasm were used for the in vitro development of SCNT or IVF embryos. For in vivo development of SCNT embryos, maturing oocytes from sows were purchased from ART Inc. (Madison, WI) and shipped overnight in their commercial maturation medium No. 1 [29]. Twenty-four hours after being placed in the maturation medium No. 1, the oocytes were moved to medium No. 2. After a total of 40 h of maturation, the cumulus cells were removed as already described and used for SCNT.

Somatic Cell Nuclear Transfer

Matured oocytes were used for SCNT in manipulation medium supplemented with 7.5 μg/ml cytochalasin B, which was overlaid with warm mineral oil. Oocytes were enucleated by aspirating the PB and metaphase II chromosomes and a small amount of surrounding cytoplasm using a beveled glass pipette with an inner diameter of 17–20 μm. A single intact donor cell was injected into the perivitelline space and placed adjacent to the recipient cytoplasm. Karyoplast-cytoplast complexes (KCCs) were placed into embryo culture medium until fusion and activation. The fusion and activation of the KCCs were accomplished with two direct current pulses (1-sec interval) of 1.2 kV/cm for 30 microseconds provided by a BTX Electro-cell Manipulator 200 (BTX, San Diego, CA) in fusion medium (0.3 M mannitol, 1.0 mM $CaCl_2$, 0.1 mM $MgCl_2$, and 0.5 mM Hepes [pH adjusted to 7.0–7.4]). Oocytes were then incubated for 20 min in PZM3 and evaluated for fusion under a stereomicroscope. Only the fused embryos were placed into four-well cell culture plates (Nunc) containing 500 μl of PZM3 at 38.5°C and 5% CO_2 in humidified air.

In Vitro Fertilization

The IVF was performed as previously described [30]. Briefly, oocytes with a PB at 44 h of IVM were washed three times in mTBM medium. Approximately 30–35 oocytes were transferred into 50-μl droplets of IVF medium covered with mineral oil that had been equilibrated for 4 h at 38.5°C in 5% CO_2 in air. A 0.1-ml frozen semen pellet was thawed at 38.5°C in 10 ml of sperm washing medium. After washing twice by centrifugation (1900 × *g* for 4 min), cryopreserved ejaculated spermatozoa were resuspended with fertilization medium to a concentration of 1×10^6 cells/ml. Fifty microliters of the sperm sample was added to the fertilization droplets containing the oocytes, giving a final sperm concentration of 0.5×10^6 cells/ml. Oocytes were coincubated with sperm for 4–6 h. After fertilization, oocytes were washed three times and cultured in 500 μl of PZM3 in four-well Nunclon dishes (Nunc) at 38.5°C in 5% CO_2 in air.

Postactivation Treatment and Embryo Culture

In a preliminary study, SCNT embryos derived from Landrace FFCs treated with 500 nM scriptaid showed higher developmental competence than controls (Zhao and Prather, unpublished results). Therefore, following electrical activation or fertilization, the SCNT (males were used as donor cells) or IVF embryos were treated with 500 nM scriptaid for 14–16 h. After treatment, embryos were washed three times in fresh PZM3 medium, transferred into a four-well cell culture plate containing 500 μl of PZM3 under mineral oil, and then cultured at 38.5°C in 5% CO_2 in humidified air for 6 days. Cleavage and blastocyst formation were evaluated on Days 2 and 6, respectively, with the day of SCNT or IVF designated as Day 0. Day 2 cleavage and Day 6 blastocyst rates were used to evaluate in vitro development.

Embryo Transfer

Day 1 SCNT zygotes (>100) were transferred to the oviducts of surrogates on the day of or 1 day after the onset of estrus. Embryo transfers alternated between treated and control donor cells, with the male donor cell ETs completed first, and then the female cell ETs were completed about 6 mo later. Pregnancy was diagnosed on Day 25 (Day 0 was the day of SCNT) and then

TABLE 1. Effect of scriptaid treatment on the developmental competence of SCNT (male donor cells) and IVF embryos using NIH mini FFCs as donors.

Treatment (nM)	No. of embryos	No. of replications	No. of cleaved embryos (%)* 24 h	No. of cleaved embryos (%)* 48 h	No. of blastocysts (%)† 144 h	Total cell no. in blastocyst‡
SCNT (0)	171	3	92 (54)[a]	148 (87)[a]	16 (9)[d]	36.8 ± 4.4[a]
SCNT (500)	155	3	81 (52)[a]	134 (86)[a]	33 (21)[c]	31.8 ± 2.3[a]
IVF (0)	169	3	110 (65)[a]	134 (79)[a]	63 (37)[a]	47.8 ± 3.3[a]
IVF (500)	183	3	120 (66)[a]	142 (78)[a]	47 (26)[b]	55.5 ± 4.6[a]

* Percentage is no. of embryos cleaved/no. of embryos cultured.
† Percentage is no. of blastocysts/no. of embryos cultured.
‡ Data are mean ± SEM.
[a–d] Values with different superscript letters within a column are significantly different ($^{a,b}P < 0.05$; $^{c,d}P < 0.05$): developmental competence of embryos from different concentrations in nuclear transfer and IVF groups were compared individually.

was checked regularly at 2-wk intervals by ultrasound examination. The cloned piglets were delivered by cesarean section on Day 117 of gestation if they did not start to farrow on their own and were hand raised.

Number of Nuclei in Blastocysts

For embryos derived from IVF, zonae pellucidae were removed by pronase treatment to eliminate the attached sperm. Expanded Day 6 blastocysts derived from IVF or SCNT were selected for cell number comparison. After being fixed in 4% paraformaldehyde in PBS for 15 min at room temperature, embryos were mounted on slides in mounting medium containing 4,6-diamidino-2-phenylindole. At least 10 oocytes or embryos were processed for each separate sample, and the experiments were replicated three times. Slides were analyzed under an epifluorescent microscope (Nikon, Natick, MA) equipped with a digital camera. Images were captured and processed using Nikon NIS-Elements software.

Statistical Analysis

Experiments were repeated at least three times. Data expressed as proportions (percentages) were analyzed using chi-square test, and numbers of nuclei were analyzed by ANOVA using SAS 6.12 (SAS Institute, Cary, NC).

RESULTS

Effect of Scriptaid on the In Vitro Developmental Competence of SCNT Embryos Reconstructed Using NIH Miniature Inbred FFCs as Donor Cells

The SCNT embryos derived from the NIH miniature inbred donor cells were treated with 500 nM scriptaid for 14–16 h after activation. Development to the blastocyst stage was increased when SCNT embryos were treated with scriptaid (21% vs. 9%, $P < 0.05$) (Table 1). However, scriptaid treatment had no effect on percentage cleavage at 24 h and 48 h and blastocyst quality as determined by the total cell number.

Effect of Scriptaid on the Full-Term Developmental Competence of SCNT Embryos Reconstructed Using NIH Miniature Inbred FFCs as Donor Cells

The SCNT embryos were transferred to the surrogates to test the effect of scriptaid treatment on full-term development. Scriptaid treatment resulted in a higher percentage of full-term development in the treatment group compared with the untreated group. Eight of 10 surrogates (80%) became pregnant and went to term in the scriptaid treatment group, while only one pregnancy was obtained in the untreated group from nine ETs (Table 2). Twenty-one live piglets were born from eight litters, accompanied by a total of seven mummified fetuses. Two of the live piglets died soon after birth, and two piglets with deformed legs were euthanized. This resulted in an overall cloning efficiency of 1.3%. However, there was just the sole pregnancy in the untreated group, and it contained only three mummies and no viable piglets.

Effect of Scriptaid on the Development of IVF-Derived Embryos

The IVF embryos were treated with 500 nM scriptaid for 14–16 h after fertilization. Scriptaid treatment decreased development to the blastocyst stage (37% vs. 26%, $P < 0.01$) (Table 1) but had no effect on cleavage at 24 h or 48 h.

DISCUSSION

Reprogramming events following the transfer of somatic nuclei into oocyte cytoplasm occurs at the epigenetic level [15]. Accumulating evidence suggests that epigenetic reprogramming in the SCNT embryo is defective, and abnormal epigenetic modifications such as DNA methylation and histone modifications occur [31–34]. These abnormal epigenetic modifications are likely associated with the overall low success rate of cloning [14].

As described herein, increased histone acetylation by TSA could dramatically enhance the developmental competence of SCNT embryos in mouse, pig, cattle, and rabbit. However, in contrast to these results, Meng et al. [22] reported no differences in the cleavage and blastocyst rates or the blastocyst cell numbers between TSA-treated and untreated SCNT rabbit embryos; furthermore, both the TSA-treated and untreated clones can develop to term in rabbits, but all of the offspring from TSA-treated embryos died within 1 h to 19 days, while four pups of the TSA-untreated group grew into adulthood, and three of them produced progeny. Wu et al. [23] also reported that cells treated with 50 ng/ml TSA resulted in significantly lower blastocyst development (9.9%) than the other experimental and control groups (around 20%) in bovine. Known to be teratogenic [24], TSA results in a significant reduction in the success rates of cloning when the concentration is high or exposure is long. It is possible that an overdose of TSA may cause developmental defects after implantation. The SCNT zygotes treated with TSA for 14 h and 26 h had reduced development to the blastocyst stage, and fetuses were not obtained [25]. The TSA treatment at 500 nM for 10 h or 50 nM for 20 h to SCNT embryos resulted in more severe placentomegaly [14]. Considering the detrimental effects of TSA on the cloning procedure, we herein investigated the effect of an HDAC inhibitor, scriptaid, on the in vitro and in vivo developmental potential of SCNT embryos using NIH miniature inbred somatic cells. Treatment with scriptaid greatly enhanced the developmental potential of reconstructed NIH miniature inbred embryos in vitro and in vivo.

TABLE 2. Full term development of nuclear transfer-derived embryos using NIH miniature FFCs as donors following scriptaid treatment.

ET no.	Donor cells	No. of transferred embryos	Scriptaid treatment*	Day 42 pregnancy status†	No. of piglets born	Cloning efficiency (%)‡
1	Male	190	+	+	1 + 4 Mummies	1.3[a]
2	Male	109	+	−	0	
3	Male	154	+	+	1 + 3 Mummies	
4	Male	174	+	+	2 Live + 2 died after birth	
5	Male	152	+	+	2	
6	Male	190	+	+	2	
7	Female	136	+	+	2 + 1 Died after birth	
8	Female	160	+	+	2	
9	Female	169	+	+	5 + 1 Died after birth	
10	Female	176	+	−	0	
11	Male	140	−	−	0	0[b]
12	Male	163	−	−	0	
13	Male	161	−	−	0	
14	Male	151	−	−	0	
15	Male	151	−	−	0	
16	Female	146	−	−	0	
17	Female	161	−	+	3 Mummies	
18	Female	138	−	−	0	
19	Female	178	−	−	0	

* +, Treated; −, untreated.
† +, Pregnant; −, not pregnant.
‡ No. of piglets/no. of embryos transferred.
[a,b] Values with different superscript letters within a column are significantly different ($P < 0.01$).

Inbred miniature pigs such as the NIH miniature have been extensively used as a large-animal model in many biomedical experiments and may be a source of organs for xenotransplantation to humans. Because we had very low success when we tried to clone an NIH SLA^{cc} miniature pig by adult EFCs, we made FFCs from cloned fetus and then used them as donor cells. To test the effects of increased histone acetylation on the somatic cell cloning efficiency of NIH miniature inbred pig, we treated the reconstructed embryos with 500 nM scriptaid after activation and found that the development to the blastocyst stage was significantly improved compared with that in the untreated group (21% vs. 9%); furthermore, the pregnancy rate of the surrogates was also significantly improved (80% vs. 11%). Fourteen healthy piglets were born from eight litters, which was more than in the untreated group (1.3% vs. 0.0%) ($P < 0.01$). Compared with the cloning efficiency of 0.2% in a previous study [4], the cloning of this inbred pig is a great improvement.

Several researchers have reported successful cloning of miniature pigs. Miyoshi et al. [35] reported successful cloning of Clawn miniature pigs, while Li et al. [36] cloned the Chinese Bana miniature pig at a cloning efficiency of 0.1%. Yucatan miniature pigs were cloned successfully by Hao et al. [37] at a cloning efficiency of 0.5% and by Estrada et al. [7]; Kurome et al. [38] showed that NIBS [Nippon Institute for Biological Sciences, Hokuto, Japan] strain miniature pigs can be cloned, and the cloning efficiency ranged from 0.9% to 7.8% when different donor cells and pig breeds were used as surrogates. Wakai et al. [39] reported healthy cloned offspring of Gottingen miniature pigs. However, all of these described miniature pigs are outbred. In the mouse, the success of cloning depends on the genetic background [40, 41]. To date, many of the inbred mouse strains such as C57BL/6 and C3H/He have not been cloned, and while DBA/2 and 129/Sv strains have been cloned, the cloning efficiency of these two inbred strains is extremely low [40, 41]. Therefore, we believe that the extremely low cloning efficiency in the NIH miniature pig may be caused by its inbred genetic background and might be improved by alteration of genomic acetylation patterns. After scriptaid treatment to the reconstructed embryos in the present study, the cloning efficiency in the NIH miniature inbred pigs improved from 0.0% to 1.3%; this is higher than our previous results [4]. Combined with genetic manipulation, scriptaid treatment will greatly facilitate the genetic modification of NIH miniature inbred pigs for biomedical research.

Although the underlying mechanism of how scriptaid treatment improves cloning efficiency remains unknown, it is thought that HDAC inhibitor can induce hyperacetylation of the core histones, resulting in structural changes in chromatin that permit transcription, as well as enhanced DNA demethylation of the somatic cell-derived genome after nuclear transfer [14], which is a necessary part of genetic reprogramming [42, 43]. Increased acetylation of histones leads to relaxed binding of the nucleosome to DNA and/or linker histones, relaxation of chromatin structures, and formation of a transcriptionally permissive state [44–47]. Histone deacetylation, frequently followed by histone methylation, establishes a base for highly repressive chromatin structures such as heterochromatin [48]. Scriptaid treatment-induced hyperacetylation decreases DNA methylation and thus activation of genes that are key for development [49].

When we applied 500 nM scriptaid to the IVF-derived embryos after fertilization, this treatment decreased development to the blastocyst stage in IVF preimplantation embryos. Scriptaid treatment of parthenogenetically activated (PA) embryos also numerically decreased the percentage of blastocysts to 19% compared with 29% in the control group (data not shown). Together, these results suggest that scriptaid treatment negatively affects IVF embryos but is beneficial to SCNT embryos. These results are also similar to previous findings in which TSA caused detrimental effects and teratogenicity on both preimplantation [50] and postimplantation [50, 51] development in normal fertilized mouse embryos but not in PA embryos. This may reflect the difference in chromatin structures between nuclei derived from SCNT, IVF, and PA embryos. Similarly, Kishigami et al. [52] reported that TSA treatment inhibited preimplantation development of intracytoplasmic sperm injection (ICSI) embryos but not round

spermatid injection (ROSI) or PA embryos and decreased the offspring rate for ICSI- but not ROSI-produced embryos. Perhaps the negative effect of HDAC inhibitor is apparent in IVF embryos because chromatin is already in a state suitable for embryonic development and inhibition of histone deacetylation is detrimental. Furthermore, during early development of normal IVF embryos, it is believed that a transcriptionally repressive state is essential for further development in IVF embryos, as relieving this state by inducing histone hyperacetylation with TSA inhibits cleavage of two-cell stage embryos to the four-cell stage [50]. However, the present study showed that, although scriptaid treatment decreased blastocyst development, it did not affect cleavage. The disparate findings may be a result of different durations of exposure between our study (14–16 h) vs. the study by Ma et al. [50] (50 h), species specificity, or the advantages of scriptaid over TSA.

Herein, we focused on the application and optimization of the HDAC inhibitor scriptaid in improving the cloning efficiency of inbred miniature pigs. However, further studies are needed to elucidate which cluster of genes are affected by scriptaid treatment, thus improving the cloning efficiency. In conclusion, the in vitro and in vivo developmental competence of SCNT inbred NIH miniature pigs was dramatically improved by scriptaid treatment.

ACKNOWLEDGMENTS

The authors would like to thank all the members in both Dr. Prather's laboratory and the National Swine Resource and Research Center.

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