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PXD101 significantly improves nuclear reprogramming and the *in vitro* developmental competence of porcine SCNT embryos

Jun-Xue Jin [1], Jin-Dan Kang [1], Suo Li, Long Jin, Hai-Ying Zhu, Qing Guo, Qing-Shan Gao, Chang-Guo Yan, Xi-Jun Yin *

Department of Animal Science, College of Agriculture, Yanbian University, Yanji 133000, China

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ABSTRACT

In this study, we investigated the effects of the histone deacetylase inhibitor PXD101 (belinostat) on the preimplantation development of porcine somatic cell nuclear transfer (SCNT) embryos and their expression of the epigenetic markers histone H3 acetylated at lysine 9 (AcH3K9). We compared the *in vitro* developmental competence of SCNT embryos treated with various concentrations of PXD101 for 24 h. Treatment with 0.5 μM PXD101 significantly increased the proportion of SCNT embryos that reached the blastocyst stage, in comparison to the control group (23.3% vs. 11.5%, $P < 0.05$). We tested the *in vitro* developmental competence of SCNT embryos treated with 0.5 μM PXD101 for various amounts of times following activation. Treatment for 24 h significantly improved the development of porcine SCNT embryos, with a significantly higher proportion of embryos reaching the blastocyst stage in comparison to the control group (25.7% vs. 10.6%, $P < 0.05$). PXD101-treated SCNT embryos were transferred into two surrogate sows, one of whom became pregnant and four fetuses developed. PXD101 treatment significantly increased the fluorescence intensity of immunostaining for AcH3K9 in embryos at the pseudo-pronuclear and 2-cell stages. At these stages, the fluorescence intensities of immunostaining for AcH3K9 were significantly higher in PXD101-treated embryos than in control untreated embryos. In conclusion, this study demonstrates that PXD101 can significantly improve the *in vitro* and *in vivo* developmental competence of porcine SCNT embryos and can enhance their nuclear reprogramming.

1. Introduction

Animal cloning using somatic cell nuclear transfer (SCNT) is a powerful technique to preserve a unique diploid genome. However, the applications of SCNT are limited because it has a low success rate and animals produced via this technique exhibit substantial phenotypic variation [1]. For SCNT embryos to have sufficient developmental competence to develop to term, the differentiated cell nuclei that are transferred into oocytes must be subjected to epigenetic reprogramming processes, including chromatin remodeling and suppression of DNA remethylation. The aforementioned limitations of SCNT and its low efficiency are assumed to be due to incomplete reprogramming of SCNT embryos [2]. Abnormal epigenetic modifications, such as histone modification and DNA methylation, occur in SCNT embryos during early development [3–5].

Histone modifications are important epigenetic modifications and include acetylation, phosphorylation, methylation, and ubiquitination [6]. Hyperacetylation of histones increases the access of some transcription factors to nucleosomes [7,8]. Histone deacetylase inhibitors (HDACi) can induce epigenetic reprogramming modifications during the activation and culture of reconstructed oocytes. Recently, various reagents have been used to regulate histone acetylation, such as trichostatin A (TSA) [9], scriptaid [10], valproic acid [11], sodium butyrate [12], suberoylanilide hydroxamic acid (SAHA) [13], m-carboxycinnamic acid bishydroxamide [14], oxamflatin [15], LBH589 [16], and CUDC-101 [17].

PXD101 (belinostat, N-hydroxy-3-(phenylsulphamoylphenyl) acryl amide) is a HDACi. These inhibitors, in conjunction with histone acetyltransferases, reciprocally regulate acetylation of lysine residues in the positively charged histone tails of nucleosomes [18]. PXD101, like other hydroxamic acid analogues, is a low molecular weight HDACi that inhibits class I and II histone deacetylases and induces the acetylation of histones H3 and H4 in a

* Corresponding author at: Department of Animal Science, College of Agriculture, Yanbian University, 977 Gongyuan Street, Yanji City, Jilin Pro, China. Fax: +86 0433 2435622.
E-mail address: yinxj33@msn.com (X.-J. Yin).
[1] These authors contributed equally to this study.

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concentration-dependent manner [19,20]. However, it is not known whether PXD101 can improve the developmental competence of porcine SCNT embryos, and its mechanisms of action have not been investigated.

The objective of this study was to determine the effect of PXD101 on the development of porcine SCNT embryos *in vitro* and *in vivo*, and to assess the effects of PXD101 treatment on the expression of the epigenetic markers AcH3K9.

2. Materials and methods

This research was carried out in accordance with the guidelines of the Ethics Committee of Yanbian University. All chemicals used in this study were purchased from Sigma Chemical Company (St. Louis, MO, USA), unless otherwise noted. PXD101 was purchased from Selleck Chemicals (Houston, TX). The experimental procedures were approved by the Animal Care and Use Committee of Jilin University and were in accordance with the animal welfare guidelines of the U.S. National Institutes of Health. The permit number is SYXK (Ji) 2010-0029.

2.1. Oocyte collection and maturation

Ovaries were obtained from prepubertal gilts at a local slaughterhouse and transported to the laboratory at 35 °C. The contents of follicles (3–6 mm in diameter) were recovered by aspiration with an 18-gauge needle. Cumulus-oocyte complexes (COCs) were pooled and washed three times with HEPES-buffered North Carolina State University (NCSU)-37 medium [21] containing 0.1% polyvinyl alcohol (v:v). Only COCs possessing a compact cumulus mass and evenly granulated ooplasm were selected. COCs were cultured for 20–22 h at 38.5 °C in an atmosphere of 5% CO_2 in four-well plates (Nunc, Roskilde, Denmark) under mineral oil, with each well containing 500 μL of maturation medium. The maturation medium was comprised of NCSU-37 medium supplemented with 10% pig follicular fluid (v:v), 0.6 mM cysteine, 1 mM dibutyryl cyclic adenosine monophosphate, and 0.1 IU/mL human menopausal gonadotropin (Teikokuzoki, Tokyo, Japan). Thereafter, COCs were cultured without dibutyryl cyclic adenosine monophosphate and human menopausal gonadotropin for another 18–24 h.

2.2. Donor cell preparation

Fetal fibroblasts were obtained from a hybrid pig on Day 30 of pregnancy. Tissues were cut into small pieces and cultured at 38 °C in an atmosphere of 5% CO_2 in Dulbecco's modified Eagle medium containing 10% fetal bovine serum (FBS) (v:v), 1 mM sodium pyruvate, and 100 U/mL each of penicillin and streptomycin. When fibroblasts were 90% confluent, they were trypsinized, rinsed, and sub-cultured into two 25-cm^2 cell culture flasks (Corning, Kennebunk, MA, USA) for further passaging. Donor cells for SCNT were derived from passages 4–8 and cultured in serum-starved medium (0.5% FBS [v:v]) for 3–4 days.

2.3. Nuclear transfer

Nuclear transfer was performed as described by Yin et al. [22]. *In vitro*-matured eggs with the first polar body were cultured in medium supplemented with 0.05 mol/L sucrose and 0.4 μg/mL demecolcine for 1 h. Sucrose was used to enlarge the perivitelline space of oocytes. Treated oocytes with a protruding membrane were transferred to medium supplemented with 5 μg/mL cytochalasin B (CB) and 0.4 μg/mL demecolcine. Protrusions were then removed by aspiration with a glass pipette that had an inner diameter of 15 μm. A single donor cell was inserted into the perivitelline space of each oocyte, and then couplets were electrically fused using two direct pulses of 150 V/mm for 50 μs in 0.28 mol/L mannitol supplemented with 0.1 mM $MgSO_4$ and 0.01% polyvinyl alcohol (v:v). Fused oocytes were cultured for 1 h in medium containing 0.4 μg/mL demecolcine before electro-activation and then cultured for 4 h in medium supplemented with 5 μg/mL CB. The reconstructed oocytes were activated by two direct pulses of 100 V/mm for 20 μs in 0.28 mol/L mannitol supplemented with 0.1 mM $MgSO_4$ and 0.05 mM $CaCl_2$. Activated oocytes were cultured in this medium for 6 days in an atmosphere of 5% CO_2 and 95% air at 38.5 °C. At the end of the culture, blastocysts were washed three times in phosphate-buffered saline (PBS) and placed onto slides in a drop of mounting medium (mixture of glycerol and PBS (9:1) containing 25 μg/mL of Hoechst 33342). A coverslip was placed on top of the blastocysts, the edge was sealed with nail polish, and the number of nuclei was counted under ultraviolet light.

2.4. Transfer of embryos and pregnancy determination

Cloned embryos at the 1-cell stage following fusion or at the 2–4-cell stage after a day of culture were transferred to the oviducts of naturally cycling gilts on the first day of standing estrus. The pregnancy status of recipients was determined by ultrasonography between day 25 and day 26 post-transfer, and fetuses were recovered at day 26 post-transfer.

2.5. Immunodetection of AcH3K9

To stain for AcH3K9, embryos were rinsed three times in PBS and fixed for 30 min in PBS containing 4% paraformaldehyde (w:v). Embryos were then transferred to PBS containing 1% Triton X-100 (v:v) at 37 °C for 30 min. After blocking non-specific sites with PBS containing 2% bovine serum albumin (w:v) overnight at 4 °C, embryos were incubated with primary antibodies against AcH3K9 (1:200; Upstate Biotechnology, Lake Placid, NY, USA) at 37 °C for 3 h. Goat anti-rabbit fluorescein isothiocyanate (FITC)-conjugated secondary antibody (1:200; Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA) was then applied for 3 h at room temperature. After washing three times in PBS, DNA was counterstained with 25 μg/mL propidium iodide for 5 min. Stained embryos were mounted beneath a coverslip using antifade mounting medium to retard photobleaching. Each experiment was repeated at least three times, and at least five randomly selected reconstructed embryos were examined each time. Slides were scanned using an epifluorescence microscope (IX71 Olympus) and a filter to detect the emission wavelength of FITC.

2.6. Experimental design and statistical analysis

Experiment 1: To determine the concentration of PXD101 that most effectively improves development, porcine SCNT embryos were treated with various concentrations of PXD101 for 24 h after activation, and the developmental rates of the various groups were compared.

Experiment 2: To determine the treatment time of PXD101 that most effectively improves development, porcine SCNT embryos were treated with 0.5 μM PXD101 for various amounts of time after activation, and the developmental rates of the various groups were compared.

Experiment 3: PXD101-treated (0.5 μM for 24 h) and untreated porcine SCNT embryos were transferred into the oviducts of surrogates on the day on which estrus began or 1 day after.

Experiment 4: The level of AcH3K9 was determined in porcine SCNT embryos treated with or without 0.5 μM PXD101 for 24 h at various developmental stages.

2.7. Statistical analysis

Each experiment was repeated at least three times. Data expressed as percentages were analyzed using the chi-square test. Numbers of nuclei were analyzed using an analysis of variance. Levels of AcH3K9 were analyzed using the independent samples *T*-test. Statistical analyses were performed using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). *P* values < 0.05 were regarded as statistically significant.

3. Results

3.1. Experiment 1: Effect of PXD101 treatment concentration on the in vitro development of porcine SCNT embryos

Porcine SCNT embryos were treated with 0.5, 1, 2.5, or 5 μM PXD101 for 24 h, or were not treated. The proportion of embryos that developed to the blastocyst stage (Fig. 1A) was significantly higher in the group treated with 0.5 μM PXD101 than in the control untreated group (23.3% vs. 11.5%, $P < 0.05$; Table 1). However, treatment with 0.5 μM PXD101 had no effect on blastocyst quality, as determined by the mean number of cells per blastocyst (36.00 ± 4.062 vs. 42.33 ± 12.307).

3.2. Experiment 2: Effect of PXD101 treatment time on the in vitro development of SCNT embryos

Porcine SCNT embryos were treated with 0.5 μM PXD101 for 6, 12, 24, or 48 h, or were not treated. Neither the percentage of embryos that underwent cleavage nor the mean number of cells per blastocyst significantly differed among the groups (Table 2). However, the percentage of embryos that reached the blastocyst stage was significantly higher in the group treated with PXD101 for 24 h than in the untreated control group (25.7% vs. 10.6%, $P < 0.05$).

3.3. Experiment 3: Transfer of untreated and PXD101-treated porcine SCNT embryos into surrogate sows

PXD101-treated (0.5 μM for 24 h) porcine SCNT embryos were transferred into two surrogate sows, one of whom became pregnant. Four fetuses were obtained from this sow (Fig. 2). Untreated SCNT embryos were transferred into three surrogate sows, one of

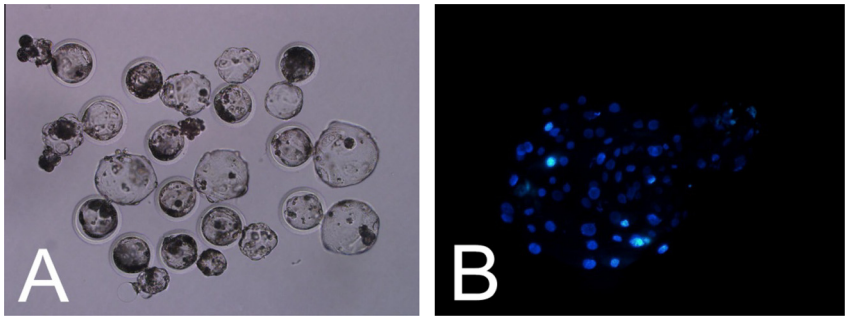

Fig. 1. PXD101-treated porcine embryos at the blastocyst stage and day 7. (A) Blastocysts derived from PXD101-treated porcine SCNT embryos. (B) Hoechst 33342 staining of a PXD101-treated porcine SCNT embryo at day 7.

Table 1
Concentration-dependent effect of PXD101 treatment on the *in vitro* development of porcine SCNT embryos.

Concentration of PXD101 (μM)	No. of embryos cultured	No. of embryos that reached the 2–4-cell stage (%)	No. of embryos that reached the blastocyst stage (%)	Mean no. ± SEM of cells per blastocyst (n)
0	131	122 (85.5)	15 (11.5)[b]	42.33 ± 12.307 (6)
0.5	133	112 (87.2)	31 (23.3)[a]	36.00 ± 4.062 (5)
1	145	128 (88.3)	27 (18.6)[a,b]	38.33 ± 8.406 (6)
2.5	134	114 (85.1)	24 (17.9)[a,b]	42.40 ± 6.841 (5)
5	133	112 (84.2)	24 (18.0)[a,b]	39.62 ± 7.927 (8)

[a,b] Values with different superscripts in the same column are significantly different ($P < 0.05$).

Table 2
Time-dependent effect of PXD101 treatment on the *in vitro* development of porcine SCNT embryos.

Duration of PXD101 treatment (h)	No. of embryos cultured	No. of embryos that reached the 2–4-cell stage (%)	No. of embryos that reached the blastocyst stage (%)	Mean no. ± SEM of cells per blastocyst (n)
0	133	101 (89.4)	12 (10.6)[b]	40.83 ± 7.782 (6)
6	112	99 (88.4)	12 (10.7)[b]	38.86 ± 17.373 (7)
12	133	101 (89.4)	21 (18.6)[a,b]	36.57 ± 10.114 (7)
24	133	95 (84.1)	29 (25.7)[a]	43.00 ± 16.116 (8)
48	133	98 (86.7)	27 (23.9)[a]	45.88 ± 9.598 (8)

[a,b] Values with different superscripts in the same column are significantly different ($P < 0.05$).

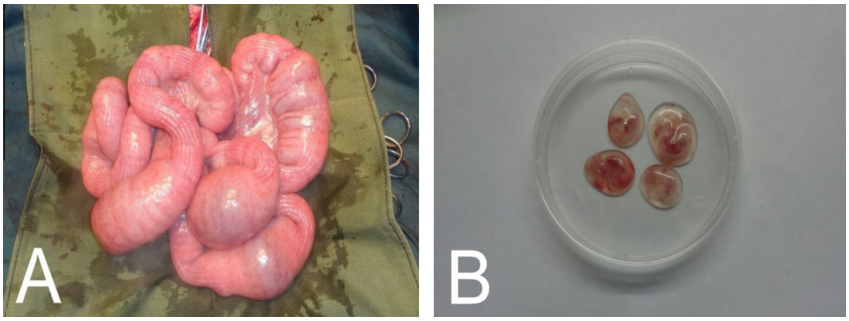

Fig. 2. Transfer of PXD101-treated porcine SCNT embryos into surrogate sows. PXD101-treated porcine SCNT embryos were transferred into a sow. The uterus of the sow (A) and the resulting fetuses (B) are shown at day 26 of gestation.

Table 3
In vivo development of untreated and PXD101-treated porcine SCNT embryos.

PXD101 treatment	Recipient no.	No. of embryos transferred	Pregnancy status	No. of fetuses recovered (fetus collection day)
Untreated	1	253	+	2 (day 26)
	2	240	–	
	3	261	–	
PXD101-treated	4	260	+	4 (day 26)
	5	237	–	

whom became pregnant. Two fetuses were obtained from this sow (see Table 3).

3.4. Experiment 4: Detection of AcH3K9 in PXD101-treated SCNT embryos at various developmental stages

Fig. 3 shows immunostaining for AcH3K9 in porcine SCNT embryos at the pseudo-pronuclear, 2-cell, 4-cell, morula, and blastocyst stages. At the pseudo-pronuclear and 2-cell stages, the fluorescence intensity of AcH3K9 staining was significantly higher in PXD101-treated embryos than in untreated embryos. However, at the other developmental stages, the fluorescence intensity of AcH3K9 staining did not significantly differ between the two groups.

4. Discussion

SCNT is a useful tool to study cell reprogramming and to clone animals. SCNT technology could be used for pet or domestic animal cloning, preservation of endangered species, and cell therapy using autologous embryonic stem cells [23]. However, the efficiency with which porcine SCNT embryos give rise to healthy offspring remains low, similar to SCNT embryos of other species. This low cloning success rate of SCNT is believed to be associated with epigenetic errors, including abnormal DNA hypermethylation and histone hypoacetylation. Recently, investigations have focused on the ability of HDACi to improve the cloning efficiency of SCNT. However, studies exploring the mechanism by which HDACi enhance epigenetic remodeling of somatic cell nuclei in SCNT embryos are scarce [24].

Histone acetylation is extremely important for nucleosome assembly and chromatin folding. Acetylation favors an open chromatin structure by interfering with interactions between nucleosomes and inducing the release of histone tails from linker DNA [25]. HDACi positively or negatively regulate gene expression by inducing the acetylation of histones, transcription factors, or other proteins. HDACi compounds can be classified into four categories according to their chemical properties: hydroxamic acids, such as TSA and SAHA; carboxyl acids, such as butyrate and sodium valproate; benzamides; and aliphatic acids [26]. Acetylation of nuclear core histones is thought to play an important role in the retention of functional genomics information during the mitotic phase, thereby allowing this information to be propagated from one cell generation to the next [27].

The current study sought to improve the developmental competence of porcine SCNT embryos. To this end, we investigated the PXD101 treatment conditions that most effectively improve the development of these embryos. PXD101 treatment significantly increased levels of AcH3K9 during early developmental stages and significantly improved the developmental capacity of these embryos. Furthermore, we demonstrated that during the *in vitro* development of porcine SCNT embryos, the level of histone acetylation was an important factor at the pseudo-pronuclear and 2-cell stages, but not at any other developmental stage. These findings indicate that a high level of histone acetylation during the pseudo-pronuclear and 2-cell stages promotes reprogramming of the transferred donor nucleus and thereby enhances the development of porcine SCNT embryos.

The percentage of porcine SCNT embryos that developed to the blastocyst stage was 2-fold higher in the PXD101-treated group than in the control untreated group. Embryo quality, as judged by the total number of cells per blastocyst, did not significantly differ between the two groups. Of the conditions tested in this study, treatment with 0.5 μM PXD101 for 24 h improved the development of porcine SCNT embryos most effectively. Furthermore, when PXD101-treated porcine SCNT embryos were transferred into two surrogate sows, one sow became pregnant and four fetuses developed.

Next, we investigated the levels of acetylated histone H3 in porcine SCNT embryos. Histone H3 is hyperacetylated in active genes, whereas they are underacetylated in silent genes. Histone H4 is initially acetylated at lysine 16, then at lysine 8 or lysine 12, and ultimately at lysine 5 [28]. When the gene is activated, the lysine 9 and lysine 13 residues of histone H3 are also acetylated in the promoter region [29]. Therefore, we analyzed the level of AcH3K9 as an indicator of epigenetic reprogramming. At the pseudo-pronuclear and 2-cell stages, the level of AcH3K9 was significantly higher in PXD101-treated embryos than in control untreated embryos. These results suggest that histone deacetylation is involved in the reprogramming of gene expression at the pseudo-pronuclear and 2-cell stages. Although the detailed mechanism by which PXD101 treatment improves the developmental competence of porcine SCNT embryos is unclear, hyperacetylation of histones facilitates the access of some factors to nucleosomes.

In conclusion, we determined the PXD101 treatment conditions (0.5 μM for 24 h) that most effectively improve the *in vitro*

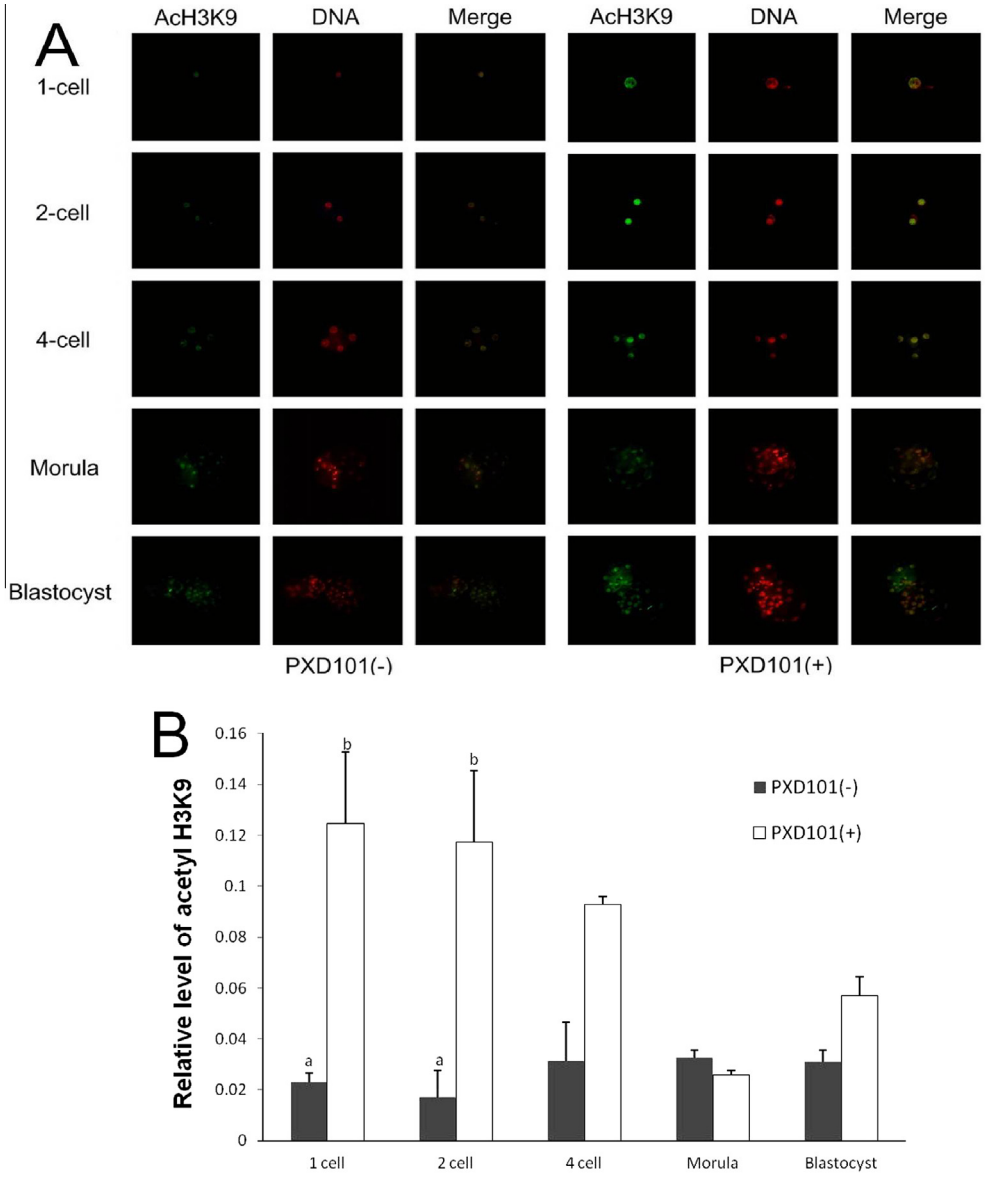

Fig. 3. The global AcH3K9 levels in pseudo-pronuclear to blastocyst stages embryos. (A) Pocrine SCNT embryos at the pseudo-pronuclear, 2-cell, 4-cell, morula, and blastocyst stages were not treated (PXD101(−)) or treated with PXD101 (PXD101(+)) and then labeled for AcH3K9 (green) and DNA (red). Merged images of AcH3K9 and DNA staining are shown. The merged images of AcH3K9 and DNA are yellow. Original magnification ×200. (B) Fluorescence intensity was measured using Image-Pro Plus 6.0. Bars and error bars show mean values and SEM, respectively. Different superscripts above columns of the same developmental stage indicate statistically significant differences ($P < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

developmental capacity of porcine SCNT embryos. Furthermore, enhancement of the levels of AcH3K9 at the pseudo-pronuclear and 2-cell stages can improve the development of porcine SCNT embryos. This improvement in development following PXD101 treatment may be due to enhanced hyperacetylation in somatic cell nuclei and upregulation of pluripotency genes.

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References

[1] S. Kishigami, S. Wakayama, Y. Hosoi, A. Iritani, T. Wakayama, Somatic cell nuclear transfer: infinite reproduction of a unique diploid genome, Exp. Cell Res. 314 (2008) 1945–1950.
[2] K.E. Latham, Early and delayed aspects of nuclear reprogramming during cloning, Biol. Cell 97 (2005) 119–132.
[3] Y.K. Kang, D.B. Koo, J.S. Park, Y.H. Choi, A.S. Chung, K.K. Lee, Y.M. Han, Aberrant methylation of donor genome in cloned bovine embryos, Nat. Genet. 28 (2001) 173–177.
[4] W. Xu, Y. Wang, Y. Li, L. Wang, X. Xiong, J. Su, Y. Zhang, Valproic acid improves the in vitro development competence of bovine somatic cell nuclear transfer embryos, Cell Reprogram 14 (2012) 138–145.
[5] G. Wee, D.B. Koo, B.S. Song, J.S. Kim, M.J. Kang, S.J. Moon, Y.K. Kang, K.K. Lee, Y.M. Han, Inheritable histone H4 acetylation of somatic chromatins in cloned embryos, J. Biol. Chem. 281 (2006) 6048–6057.
[6] W. Fischle, Y. Wang, C.D. Allis, Histone and chromatin cross-talk, Curr. Opin. Cell Biol. 15 (2003) 172–183.
[7] D.Y. Lee, J.J. Hayes, D. Pruss, A.P. Wolffe, A positive role for histone acetylation in transcription factor access to nucleosomal DNA, Cell 72 (1993) 73–84.
[8] E. Li, Chromatin modification and epigenetic reprogramming in mammalian development, Nat. Rev. Genet. 3 (2002) 662–673.
[9] K. Yamanaka, S. Sugimura, T. Wakai, M. Kawahara, E. Sato, Acetylation level of histone H3 in early embryonic stages affects subsequent development of miniature pig somatic cell nuclear transfer embryos, J. Reprod. Dev. 55 (2009) 638–644.

[10] K.M. Whitworth, J. Zhao, L.D. Spate, R. Li, R.S. Prather, Scriptaid corrects gene expression of a few aberrantly reprogrammed transcripts in nuclear transfer pig blastocyst stage embryos, Cell Reprogram 13 (2011) 191–204.
[11] J.D. Kang, S. Li, Y. Lu, W. Wang, S. Liang, X. Liu, J.X. Jin, Y. Hong, C.G. Yan, X.J. Yin, Valproic acid improved in vitro development of pig cloning embryos but did not improve survival of cloned pigs to adulthood, Theriogenology 79 (2013) 306–311. e301.
[12] Z.C. Das, M.K. Gupta, S.J. Uhm, H.T. Lee, Increasing histone acetylation of cloned embryos, but not donor cells, by sodium butyrate improves their in vitro development in pigs, Cell Reprogram 12 (2010) 95–104.
[13] T. Ono, C. Li, E. Mizutani, Y. Terashita, K. Yamagata, T. Wakayama, Inhibition of class IIb histone deacetylase significantly improves cloning efficiency in mice, Biol. Reprod. 83 (2010) 929–937.
[14] X. Dai, J. Hao, X.J. Hou, T. Hai, Y. Fan, Y. Yu, A. Jouneau, L. Wang, Q. Zhou, Somatic nucleus reprogramming is significantly improved by m-carboxycinnamic acid bishydroxamide, a histone deacetylase inhibitor, J. Biol. Chem. 285 (2010) 31002–31010.
[15] S.J. Park, H.J. Park, O.J. Koo, W.J. Choi, J.H. Moon, D.K. Kwon, J.T. Kang, S. Kim, J.Y. Choi, G. Jang, B.C. Lee, Oxamflatin improves developmental competence of porcine somatic cell nuclear transfer embryos, Cell Reprogram 14 (2012) 398–406.
[16] J.X. Jin, S. Li, Q.S. Gao, Y. Hong, L. Jin, H.Y. Zhu, C.G. Yan, J.D. Kang, X.J. Yin, Significant improvement of pig cloning efficiency by treatment with LBH589 after somatic cell nuclear transfer, Theriogenology 80 (2013) 630–635.
[17] J.X. Jin, S. Li, Y. Hong, L. Jin, H.Y. Zhu, Q. Guo, Q.S. Gao, C.G. Yan, J.D. Kang, X.J. Yin, CUDC-101, a histone deacetylase inhibitor, improves the in vitro and in vivo developmental competence of somatic cell nuclear transfer pig embryos, Theriogenology 81 (2014) 572–578.
[18] Y. Dai, S. Chen, L.B. Kramer, V.L. Funk, P. Dent, S. Grant, Interactions between bortezomib and romidepsin and belinostat in chronic lymphocytic leukemia cells, Clin. Cancer Res. 14 (2008) 549–558.
[19] R. Feng, A. Oton, M.Y. Mapara, G. Anderson, C. Belani, S. Lentzsch, The histone deacetylase inhibitor, PXD101, potentiates bortezomib-induced anti-multiple myeloma effect by induction of oxidative stress and DNA damage, Br. J. Haematol. 139 (2007) 385–397.
[20] J.A. Plumb, P.W. Finn, R.J. Williams, M.J. Bandara, M.R. Romero, C.J. Watkins, N.B. La Thangue, R. Brown, Pharmacodynamic response and inhibition of growth of human tumor xenografts by the novel histone deacetylase inhibitor PXD101, Mol. Cancer Ther. 2 (2003) 721–728.
[21] R.M. Petters, K.D. Wells, Culture of pig embryos, J. Reprod. Fertil. Suppl. 48 (1993) 61–73.
[22] X.J. Yin, T. Tani, I. Yonemura, M. Kawakami, K. Miyamoto, R. Hasegawa, Y. Kato, Y. Tsunoda, Production of cloned pigs from adult somatic cells by chemically assisted removal of maternal chromosomes, Biol. Reprod. 67 (2002) 442–446.
[23] H. Kang, S. Roh, Extended exposure to trichostatin A after activation alters the expression of genes important for early development in nuclear transfer murine embryos, J. Vet. Med. Sci. 73 (2011) 623–631.
[24] J. Su, Y. Wang, Y. Li, R. Li, Q. Li, Y. Wu, F. Quan, J. Liu, Z. Guo, Y. Zhang, Oxamflatin significantly improves nuclear reprogramming, blastocyst quality, and in vitro development of bovine SCNT embryos, PLoS One 6 (2011) e23805.
[25] A. Kretsovali, C. Hadjimichael, N. Charmpilas, Histone deacetylase inhibitors in cell pluripotency, differentiation, and reprogramming, Stem Cells Int. 2012 (2012) 184154.
[26] M. Esteller, Epigenetics provides a new generation of oncogenes and tumour-suppressor genes, Br. J. Cancer 94 (2006) 179–183.
[27] J.M. Kim, H. Liu, M. Tazaki, M. Nagata, F. Aoki, Changes in histone acetylation during mouse oocyte meiosis, J. Cell Biol. 162 (2003) 37–46.
[28] A.W. Thorne, D. Kmiciek, K. Mitchelson, P. Sautiere, C. Crane-Robinson, Patterns of histone acetylation, Eur. J. Biochem. 193 (1990) 701–713.
[29] T. Agalioti, G. Chen, D. Thanos, Deciphering the transcriptional histone acetylation code for a human gene, Cell 111 (2002) 381–392.