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# Kdm6a overexpression improves the development of cloned mouse embryos

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## Summary

Somatic cell nuclear transfer (SCNT) is an important technique for life science research. However, most SCNT embryos fail to develop to term due to undefined reprogramming defects. Here, we show that abnormal Xi occurs in somatic cell NT blastocysts, whereas in female blastocysts derived from cumulus cell nuclear transfer, both X chromosomes were inactive. H3K27me3 removal by *Kdm6a* mRNA overexpression could significantly improve preimplantation development of NT embryos, and even reached a 70.2% blastocyst rate of cleaved embryos compared with the 38.5% rate of the control. H3K27me3 levels were significantly reduced in blastomeres from cloned blastocysts after overexpression of Kdm6a. qPCR indicated that rDNA transcription increased in both NT embryos and 293T cells after overexpression of *Kdm6a*. Our findings demonstrate that overexpression of *Kdm6a* improved the development of cloned mouse embryos by reducing H3K27me3 and increasing rDNA transcription.

Keywords: H3K27me3, Kdm6a, Mouse, rDNA, SCNT

## Introduction

Somatic cell nuclear transfer is an important technique for life science research that has been widely applied in animal husbandry and breeding, in the protection of endangered animal species, and in medical research on stem cells and organs for tissue reconstruction. Despite the widespread use of this technique, however, its efficiency is still very low. Many studies have revealed large differences in gene expression between nuclear transfer (NT) embryos and normal fertilized embryos, especially during the early stages of development and for X chromosome-linked genes (Suzuki *et al*., 2006; Vassena *et al*., 2007; Matoba *et al*., 2014). Inoue *et al*. (2010) revealed significant down-regulation of genes on the X chromosome in cloned embryos and also demonstrated that the X-inactivation-specific transcript (*Xist*) was ectopically expressed on the active X chromosome in female and male cloned embryos. Consequently, the single X chromosome in male cloned embryos and both X chromosomes in female cloned embryos were abnormally inactivated (Matoba *et al*., 2011; Oikawa *et al*., 2013). Subsequently, Ogura's group achieved considerable development of cloned embryos through knocking out or knocking down *Xist* in donor cells (Inoue *et al*., 2010; Matoba *et al*., 2011).

Tri-methylation of lysine residue 27 of histone H3 (H3K27me3) is a marker for an inactivated X chromosome, and also plays a major role in gene repression (Welstead *et al*., 2012; Shpargel *et al*., 2014; Liu *et al*., 2016). Histone demethylase KDM6A (also known as UTX) is responsible for removing a methyl group from H3K27. KDM6A contains a tetratricopeptide motif predicted to mediate protein–protein interactions (Swigut & Wysocka, 2007), and is a member of a stable multi-protein complex that not only de-methylates H3K27me3 but also methylates lysine 4 at histone H3 to facilitate gene expression (Cho *et al*., 2007; Issaeva *et al*., 2007). KDM6A mutations have been discovered in patients with Kabuki syndrome, a rare syndrome associated with distinct facial features,

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intellectual disability, growth retardation, and skeletal anomalies (Van Laarhoven *et al.*, 2015; Bogershausen *et al.*, 2016).

In a previous study, we demonstrated that the efficiency of ribosomal DNA (rDNA) reprogramming in NT embryos is determined by rDNA activity in the donor cells from which they are derived. DNA methylation of rDNA promoters is not fully reprogrammed in oocytes (Zheng *et al.*, 2012). Heterochromatin needs to be remodelled during the reprogramming of somatic cells in NT. Abnormal gene reactivation can be partly rescued by the demethylation of histone H3 lysine 9 trimethylation (H3K9me3) using Kdm4d (Matoba *et al.*, 2014) or treatment with histone deacetylase inhibitors. Given the similar function of H3K9me3 and K3K27me3 in controlling gene activity, we proposed that reduced H3K27me3 may also improve the development of cloned mouse embryos.

## Materials and Methods

### Animals

B6D2F1 (C57BL/6 × DBA/2) female and male mice were obtained at 8 to 10 weeks of age from Vital River (Beijing, China). All animal experiments were performed according to the guidelines of our Institutional Animal Care and Use Committee (IACUC) and were approved by the Harbin Medicine University Ethics Committees.

### Cell preparation

Cumulus cells (CCs) were obtained during oocyte collection, then washed in HEPES-buffered CZB medium (HEPES-CZB) several times, resuspended in HEPES-CZB containing 3% PVP (polyvinylpyrrolidone, Sigma, PVP360) and used as donor cells (G0/G1) for NT directly. HEK293T cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Gibco), 50 U/ml penicillin and 50 μg/ml streptomycin, in 5% $CO_2$ in air at 37°C. Cells were transfected with lipofectamine® LTX (Invitrogen) according to manufacturer's instructions.

### Oocyte recovery and spermatozoa preparation

Female B6D2F1 mice were superovulated via intraperitoneal injection of 5 IU pregnant mare serum gonadotropin (NSH; China) followed 48 h later with 5 IU of human chorionic gonadotropin (NSH, China). Oocytes were collected from the oviducts 14 h after human chorionic gonadotropin injection. Cumulus cells were removed from the oocytes with 300 μg/ml hyaluronidase (Sigma, H4272) in HEPES-CZB by pipetting. Denuded oocytes with homogeneous ooplasm were selected and kept in new droplets of CZB medium containing 5.6 mM glucose (CZBG), covered with sterile mineral oil (Fisher, O121-20), then cultured at 37°C in a 5% $CO_2$ in air atmosphere until use. Spermatozoa were collected from the cauda epididymis of 8–12-week-old B6D2F1 males, then kept in CZB-HEPES medium and prepared for injection.

### Generation of intracytoplasmic sperm injection and nuclear transfer embryos

Intracytoplasmic sperm injection (ICSI) was carried out by a piezo-driven unit using methods described elsewhere (Bai *et al.*, 2017), except that our experiment was performed in HEPES-CZB containing 5 μg/ml cytochalasin B (Sigma, C6762) at room temperature. Only the sperm head was injected into the oocyte. After 30 min of recovery, the ICSI-generated embryos were washed several times and cultured in K-modified simplex optimized medium (KSOM) at 37°C in a 5% $CO_2$ in air atmosphere. To eliminate any possible effects attributed to the NT methods from the following investigation, we adopted a one-step micromanipulation technique to reconstruct CC NT embryos, as described previously with modifications. The outer diameter of the injection pipettes used for CC injection was 9 μm. Briefly, the donor cell membrane was disrupted with several piezo pulses, and four to seven cells were then sucked into the injection pipette. The oocyte metaphase II (MII) spindle was adjusted to 8 to 10 o'clock, and one donor cell was injected into the nearby plasma. The spindle was immediately aspirated into the injection pipette and removed from the oocyte. One hour after NT, the reconstructed CC NT embryos were activated with 5 mM $SrCl_2$ (Sigma, 439665) in $Ca^{2+}$-free CZB medium containing 5 μg/ml cytochalasin-B (CB) for 6 h. Then, the embryos were washed in KSOM and cultured under the same conditions as ICSI embryos.

### Plasmids and antibodies

The plasmids used were as followed: psp73-KDM6A and GFP-C1-KDM6A were cloned from the cDNA of mouse ES cell line that established in our laboratory (Bai *et al.*, 2016). The antibodies covered were as followed: monoclonal anti-B23 antibody (Sigma, FC82291), monoclonal anti-UBF antibody (Sigma, WH00007343M1), polyclonal rabbit anti-H3K27me3 (Millipore, 07-449), monoclonal rabbit anti-PARP1 (Abcam, ab32138), monoclonal mouse anti-GAPDH (CW0266A, CWBiotech).

### *In vitro* transcription of mRNA and mRNA injection

Kdm6a cDNAs were cloned into T7-driven vectors and synthesized with the mMESSAGE mMACHINE T7 Ultra Kit (Life Technologies, Grand Island, NY,

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USA) according to the manufacturer's instructions. The storage concentration of mRNA was normalized to 1000 ng/μl. The integrity of manufactured mRNA was confirmed by electrophoresis with formaldehyde gels. Enucleated oocytes or 5-h post-activation somatic cell nuclear transfer (SCNT) embryos were injected with approximately 10 pl of mRNA using a Piezodriven micromanipulator. Water was used as a control.

### Immunofluorescent detection of H3K27me3

Embryos at 72 h post-activation (hpa) were collected. They were fixed in 4% (m/v) paraformaldehyde for 30 min and permeabilized with 1% (v/v) Triton X-100 for 50 min. After blocking with 1% (m/v) bovine serum albumin (Sigma, A9418) in PBS for 1 h, the embryos were incubated with an anti-H3K27me3 antibody (Millipore, 1:100) overnight at 4°C, followed by Alexa Fluor 488 mouse anti-rabbit IgG (Invitrogen, 1:100) incubation for 1 h. After the nuclei were stained with 10 μg/ml Hoechst 33342, the embryos were mounted on slides with DABCO (1,4-diazabicyclo-(2.2.2) octane; Beyotime, P0126) and observed under a microscope (Nikon, TE2000-U).

### Western blotting

Western blot analysis of 293T cells was performed as described previously (Zhao *et al*., 2016).

### qPCR Evaluation of X chromosome-linked and rDNA gene expression

Embryos were collected at blastocyst stages. RNA extraction and qPCR evaluation were performed as previously described with some modifications (Bai *et al*., 2017). Total RNA was extracted from embryos using an RNeasy Mini Kit (Qiagen, 74104) according to the manufacturer's instructions. Total RNA was extracted from 293T cells with TRIzol reagent (Invitrogen) according to the manufacturer's protocol. cDNA was synthesized from total RNA with a High-Capacity cDNA Reverse Transcription Kit (ABI, 4368814), the total volume was 20 μl (2 μl 10× RT buffer, 0.8 μl 25× dNTP Mix, 1 μl MultiScribe reverse transcriptase, 1 μl oligo dT primer, 2 μl of 10× RT Random Primers, 1 μg of RNA, and up to 20 μl of RNase-free $dH_2O$). Quantitative polymerase chain reaction (qPCR) was performed using a 1 μl cDNA sample, 10 μl of TransStart™ Top Green Q-PCR SuperMix (TransGen, AQ131), and gene-specific primers in a 20-μl reaction system in a CFX96 Real-time System (Bio-Rad). Thermal cycling conditions were 95°C for 3 min, followed by 42 PCR cycles for 10 s at 95°C for DNA denaturation, 10 s at 60°C for primer annealing and 30 s at 72°C for primer extension. The melting protocol was from 65°C to 95°C (increment: 0.5°C/5 s). The threshold cycle ($C_t$) value represents the cycle number at which the fluorescence of the sample is significantly higher than the background. Reactions were conducted according to the protocol provided with the Haigene SYBR green quantitative PCR kit. The PCR products were analysed by generating a melting curve. The relative amount of gene expression was analysed via the $2\text{-}\Delta\Delta C_t$ method, which is a convenient method for analysing relative changes in gene expression in real-time quantitative PCR experiments. The amplification specificity of each qPCR assay was confirmed by melting curve analysis to verify that the primers amplified could form only one specific PCR product. The amplification efficiencies were calculated according to the formula: efficiency (%) $= (3^{(-1/\text{slope})-1}) \times 100$. The amplification efficiencies of all the tested genes ranged from 94% to 110%, with all correlation coefficients $> 0.99$. These results demonstrated that the synthesized primer sequences were accurate and suitable for the experiments (Table S1). The assay included a no-template control (NTC), which was detected and indicated no amplification. All qPCR reactions were carried out biologically and technically in triplicate, 10 blastocysts from each group were used as one repeat. The specificity of the qPCR reaction was confirmed by single peaks in the melt curves. *H2afz* and *Cyca* were employed as candidate references for embryos, and *Gapdh* was used for cells.

### Experimental design and statistical analysis

Each experiment was repeated at least three times. All embryos were allocated randomly to each treatment group. Blastocyst formation rate was analysed with the $\chi^2$ test. More than five embryos were selected from each group for observations of H3K27me3 using a confocal microscope. In qPCR experiments, 10 blastocysts from each group were used as one repeat. Statistical comparisons were analysed by one-way analysis of variance (ANOVA). The relative abundances of gene transcripts were established by testing the data for normality and equal variance using the Levene median test, ANOVA, and followed multiple pairwise comparisons using the Tukey's test. Differences in $P$-value $< 0.05$ were considered to be significant. The difference in mRNA expression was analysed using SPSS 19.0 software.

## Results

### Both X chromosomes in cumulus NT embryos are inactive

In mammals, the difference in the chromosome complement between XY males and XX females is

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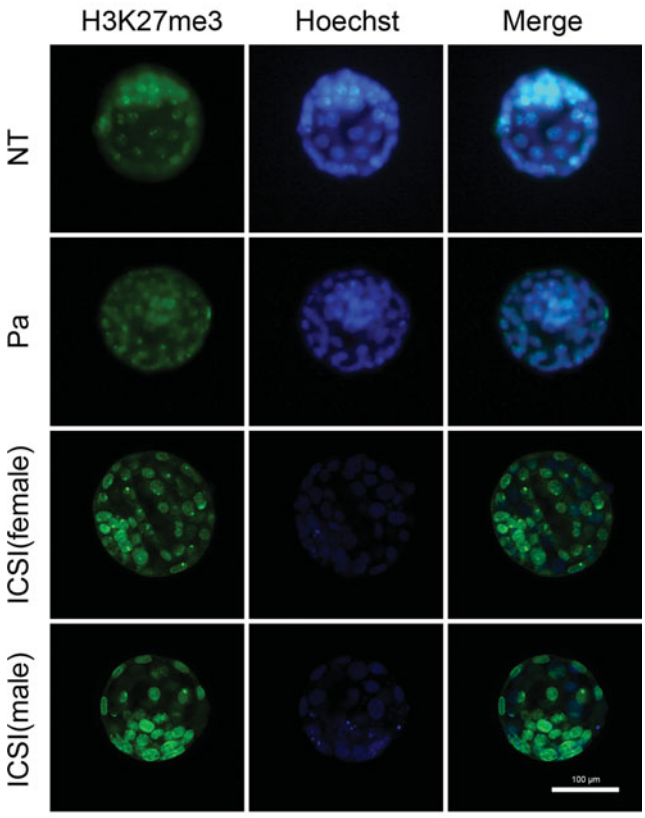


**Figure 1** Distribution of H3K27me3 in ICSI and NT blastocysts. ICSI female and male, NT, and parthenogenetic blastocysts. ICSI: intracytoplasmic sperm injection, NT: CCs as the donor for nuclear transfer, Pa: parthenogenesis. H3K27me3 is presented in green, whereas Hoechst is presented in blue. The merged photographs show both H3K27me3 and Hoechst.

compensated by transcriptional silencing of the genes on one of the two X chromosomes in females. Thus, in both male and female cells, only a single copy of X-linked genes is active. This situation is in contrast with autosomal genes, which are expressed from two homologous chromosomes. The *Xist* gene is exclusively expressed from the inactive X chromosome (Xi) and has been suggested to act as a non-coding RNA based on the convincing argument that the majority of *Xist* RNA localizes to the nucleus and, more specifically, accumulates within the territory of the Xi. The Ogura group confirmed that the localization of trimethylated histone H3 at lysine 27 (H3K27me3) is responsible for the repressive chromatin state in the inactive X chromosome. Thus, we used H3K27me3 as a marker for Xi and identified one inactive X chromosome per blastomere in female ICSI embryos or parthenogenetic embryos and no inactive X chromosomes in males (Fig. 1). These results confirmed that H3K27me3 is an effective marker of Xi. Then, we detected the fluorescent signal of H3K27me3 in NT embryos and found two inactive X chromosomes in each blastomere of NT blastocysts (Fig. 1). These observations suggest that abnormal Xi occurred in the NT embryos, and this reason may account for its impaired development.

### Overexpression of *Kdm6a* mRNA greatly improves the development of NT embryos

In recent studies, H3K9me3 was found to be a barrier for efficient reprogramming by SCNT. It had been reported that overexpression of Kdm4d, the H3K9me3 demethylase, improved SCNT efficiency (Matoba *et al*., 2014). As H3K9me3 and H3K27me3 are both repressed markers during early embryonic development and H3K27me3 also indicates the inactive X chromatin, we wondered if the removal of H3K27me3 could also improve the efficiency of NT. KDM6A is the H3K27me3 demethylase. Some X chromosome-linked genes are controlled by KDM6A, and KDM6A also affects embryo development (Berletch *et al*., 2013; Shpargel *et al*., 2014; Welstead *et al*., 2012). We overexpressed *Kdm6a* by mRNA injection into the 1-cell stage of CCNT embryos, and then compared the developmental potential of *Kdm6a* mRNA overexpression NT embryos with the controls. In control NT embryos, the developmental rate reached 38.5% of cleaved embryos successfully developing to the blastocyst stage after 96 h of culture (Fig. 2*A*, *B* and Table 1). Strikingly, NT embryos with *Kdm6a* overexpression rarely arrested during 2- to 4-cell and 4-cell to morula stage transition and developed to the blastocyst stage with high efficiency (70.2%; Fig. 2*A*, *B* and Table 1). Also, it was found that the number of blastomeres containing two-point signals of H3K27me3 significantly reduced, while the number of blastomere containing one point increased (Fig. 2*C*, *D*). These results demonstrated that H3K27me3 removal by *Kdm6a* mRNA overexpression can significantly improve the preimplantation development of NT embryos.

As KDM6A is the demethylase of H3K27me3 that indicates Xi, we next detected the representative X chromosome-linked genes (Inoue *et al*., 2010; Matoba *et al*., 2011) *Magea6*, *Xlr4*, *Msn* and *Plac1* in NT blastocysts with *Kdm6a* overexpression. We found that *Xlr4* and *Plac1* increased in NT blastocysts with *Kdm6a* overexpression compared with the control (Fig. 2*E*). EZH2, the methylase of H3K27me3, owns the antagonistic function of KDM6A, which is significantly decreased after overexpression of *Kdm6a* in NT blastocysts. This result was similar to the finding that EZH2 protein levels were mildly upregulated in Kdm6 mutant MEFs (Shpargel *et al*., 2014). Embryonic ectoderm development (EED) shares the same function of EZH2, but did not decrease significantly (Fig. 2*E*). rDNA activity was also essential for SCNT

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**Table 1** The development rate of nuclear transfer embryos after *Kdm6a* overexpression

| Groups | Embryos | 2-cell (%) | 4-cell (%) | Morula (%) | Blastocysts (%) |
|---|---|---|---|---|---|
| Control | 63 | 52 (83.30 ± 6.61)[a] | 40 (77.46 ± 9.58)[a] | 30 (57.53 ± 7.36)[a] | 20 (38.51 ± 2.34)[a] |
| Kdm6a | 68 | 58 (85.44 ± 1.74)[a] | 52 (85.70 ± 1.53)[a] | 49 (81.85 ± 4.76)[a] | 41 (70.20 ± 6.80)[b] |

The experiment was repeated three times.
[a]Values with different superscript symbols in the same column differ significantly ($P < 0.05$).
[b]Values with different superscript symbols in the same column differ significantly ($P < 0.05$).

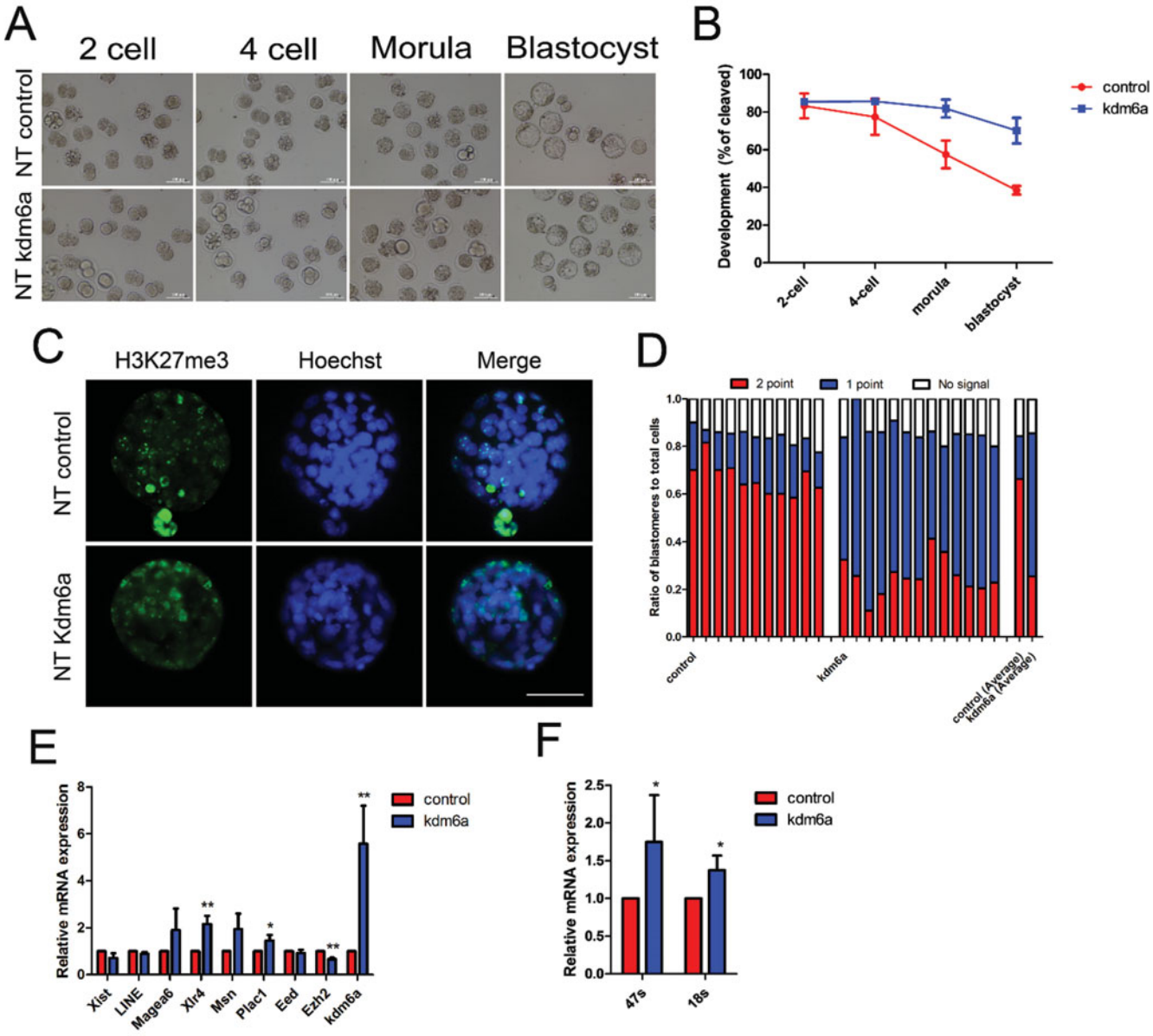


**Figure 2** Overexpression of *Kdm6a* mRNA greatly improves the development of NT embryos. (*A*) Representative images of control and Kdm6a mRNA overexpressed NT embryos at 96 h post-activation. Scale bar represents 100 μm. (*B*) Overexpressing *Kdm6a* greatly improved the preimplantation development rate of NT embryos. Cumulus cells were used as donor cells. The data are represented as the mean ± standard deviation (s.d.) ($n > 3$). (*C*) Distribution of H3K27me3 in NT blastocysts with or without Kdm6a overexpression. (*D*) The ratios of blastomeres classified according to the number of point signals of H2K27me3. (*E*) Expression of X chromosome-related genes in NT blastocysts after overexpressing *Kdm6a*. (*F*) Expression of 47S and 18S in NT blastocysts after overexpression of *Kdm6a*. The expression level of the control was set as 1. *Indicates significant differences at $P < 0.05$.

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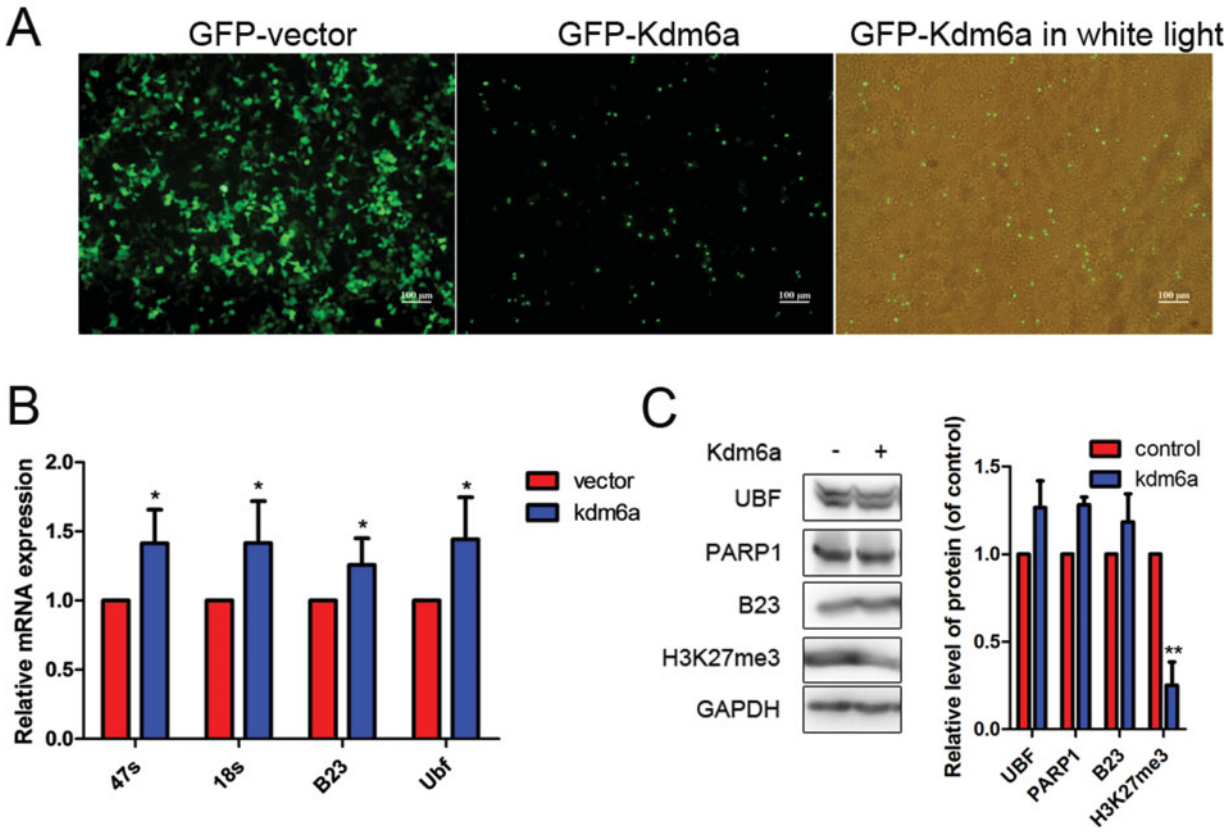


**Figure 3** Reduced H3K27me3 promotes rDNA expression in 293T. (*A*) Expression of GFP–Kdm6a in 293T cells after transfection. (*B*) Expression of rDNA related genes in 293T cells after overexpression of *Kdm6a*. The expression level of vector was set as 1. *Indicates significant differences at $P < 0.05$. (*C*) Detection of rDNA-associated protein expression through western blotting, the right-hand figure indicts greyscale analysis of the western blot.

development (Zheng *et al.*, 2012); the head product and end product of rDNA (47s and 18s) were detected in NT blastocysts. Both 47S and 18S increased in NT blastocysts after overexpression of *Kdm6a* (Fig. 2*F*). These results suggested that Kdm6a overexpression improved the developmental potential of CCNT embryos by reducing H3K27me3 and increasing rDNA transcription in NT embryos. However, not all the X chromosome genes had increased expression in the blastocysts with *Kdm6a* overexpression.

### Reduced H3K27me3 promote the rDNA expression

To confirm rDNA transcription that is improved by reducing H3K27me3, we constructed a GFP–kdm6a overexpression vector and detected rDNA related genes in 293T cells after GFP–kdm6a transfection. We found that Kdm6a was expressed in the nucleus (Fig. 3*A*); qPCR showed that both 47S and 18S increased in 293T cells with Kdm6a overexpression. *Ubf* and *B23* mRNA levels also increased (Fig. 3*B*). UBF (upstream binding factor) is the processing element of rRNA that controls the rDNA promoter, and B23 (nucleophosmin) is the processing element of rRNA (Zhao *et al.*, 2016). Poly(ADP-ribose) polymerases (PARPs) are enzymes that transfer ADP-ribose groups to target proteins and thereby affect various nuclear and cytoplasmic processes. PARP1 plays an important role in the rDNA heterochromosome (Dantzer & Santoro, 2013). However, western blot analysis showed that the protein levels of UBF, B23 and PARP1 did not change, only H3K27me3 protein levels were significantly reduced in the Kdm6a overexpression group (Fig. 3*C*). These results indicated that reduction in H3K27me3 promotes rDNA expression, although possibly not though UBF and B23 pathway. This finding indicates that there may be an alternative way in which H3K27me3 demethylation promotes rDNA expression.

## Discussion

SCNT-mediated reprogramming can produce blastocysts as well as full-term individuals, with the clear promise for therapeutic applications and animal cloning. Although SCNT technology has progressed

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over the last decade, developmental efficiency remains extremely low, and the molecular mechanism underlying SCNT embryo arrest remains largely undefined. Previous studies have demonstrated that compared with the transcriptome of *in vitro* fertilization (IVF) embryos, 222 genomic regions were identified, termed reprogramming resistant regions (RRRs) containing ribosome DNA genes that are resistant to transcriptional reprogramming in SCNT embryos.

RRRs in somatic cells are enriched for H3K9me3. Therefore, H3K9me3-initiated heterochromatin assembly can prevent access to reprogramming and transcriptional factors and thereby prevent the activation of developmentally important genes in RRRs (Matoba *et al*., 2014). The modification of these histones promotes reprogramming (Yuan *et al*., 2016; Gong *et al*., 2017; He & Kidder, 2017; Hormanseder *et al*., 2017; Jullien *et al*., 2017). H3K27me3 was more concentrated in the inactivated X chromosome (Inoue *et al*., 2010), which may indicate Xi to a certain extent. In our study, reducing H3K27me3 improved the development of cloned mouse embryos. The blastomere containing two-point signals of H3K27me3 changed to a one point signal, indicting that Xi was reactive in most blastomeres. Therefore we proposed that H3K27me3 removal may lead reactive Xi to promote development. Some X chromosome-linked genes were detected in NT blastocysts after overexpressed *Kdm6a* levels increased, but not all the blastomere contained only a one point signal of H3K27me3 in NT blastocysts with Kdm6a overexpression. Reduced *Ezh2* levels indicated that *Kdm6a* is functioning. Interestingly, we found that rDNA expression increased in NT blastocysts after overexpression of *Kdm6a*, which indicated that reduced levels of H3K27me3 in somatic cell reprogramming may promote rDNA expression.

Our previous study showed that levels of most X chromosome-linked genes such as *Magea3*, *Magea6*, *Msn* and *Rhox6* were reduced in NT blastocysts compared with IVF (data not shown). RHOX6 was regulated by the histone demethylase KDM6A (Berletch *et al*., 2013). KDM6A is an H3K27me3-specific histone demethylase that takes part in the inactive X chromosome and has the opposite effect of H3K27me3-specific histone methylase EZH2/EED (Plath *et al*., 2003; Welstead *et al*., 2012; Shpargel *et al*., 2014). Moreover, KDM6A is encoded by an X-linked gene that escapes X inactivation; male-biased mutations in genes that escape X inactivation, such as ATRX, CNKSR2, DDX3X, KDM5C, KDM6A, and MAGEC3, were observed in combined analysis across many cancers and in several individual tumour types, suggesting biallelic expression of EXITS genes in females as a portion of the reduced cancer incidence in females as compared with males across a variety of tumour types (Dunford *et al*., 2016). As Kdm6a is the X-linked gene that escapes X inactivation, its expression would not change even for two inactive X chromosomes in the female NT embryo. Differential expression profiling (DEP) between IVF and NT blastocysts confirmed this (Table S2). In our study, we reduced the level of H3K27me3 by overexpressing Kdm6a during early embryonic development.

H3K27me3 plays an important role in embryonic development (Suzuki *et al*., 2006; Vassena *et al*., 2007; Welstead *et al*., 2012; Shpargel *et al*., 2014; Gong *et al*., 2017; Kuang *et al*., 2017), even though it is the inhibitory marker of gene expression. Some somatic developmental genes, such as the *Hox* gene cluster, did need to be silenced by H3K27me3 for promoting development to next stage (Liu *et al*., 2016; Zhang *et al*., 2016). It has been reported that the loss of Ezh2 in progenitor cells leads to a decrease in the number of neurons observed by long-term tracing. Conditional knockout of Ezh2 ultimately resulted in impairments in spatial learning and memory, contextual fear memory, and pattern separation (Zhang *et al*., 2014). In our study, we found high efficiency in preimplantation development of NT embryos by reducing H3K27me3, but whether the post-implantation development of NT embryos can also be improved is not yet known.

Low efficiency of SCNT has been widely observed and might be conserved in all mammals. Several approaches for histone modification could improve the efficiency of SCNT in other species. In our study, overexpression of *kdm6a* mRNA greatly improves the development of NT embryos and indicated that reduced H3K27me3 levels promoted NT-mediated reprogramming.

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## Supplementary material

To view supplementary material for this article, please visit https://doi.org/10.1017/S0967199417000673

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