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Aberrant histone H4 acetylation in dead somatic cell-cloned calves

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Abstract

In somatic cell-cloned animals, inefficient epigenetic reprogramming can result in an inappropriate gene expression and histone H4 acetylation is one of the key epigenetic modifications regulating gene expression. In this study, we investigated the levels of histone H4 acetylation of 11 development-related genes and expression levels of 19 genes in lungs of three normal control calves and nine aberrant somatic cell-cloned calves. The results showed that nine studied genes had decreased acetylation levels in aberrant clones ($p < 0.05$) and two genes had no significant variations ($p > 0.05$). Whereas 13 genes had significantly decreased expression ($p < 0.05$) in aberrant clones, five genes showed no significant differences between controls and clones ($p > 0.05$), and only one gene had higher expression level in clones ($p < 0.05$). Furthermore, FGFR, GHR, HGFR and IGF1 genes showed lowered levels of both histone H4 acetylation and expression in aberrant clones than in controls, and the level of histone H4 acetylation was even more lowered in aberrant clones than those in controls. It was suggested that the lower levels of histone H4 acetylation in aberrant clones caused by the previous memory of cell differentiation might not support enough chromatin reprogramming, thus affecting appropriate gene expressions, and growth and development of the cloned calves. To our knowledge, this is the first study on how histone H4 acetylation affects gene expression in organs of somatic cell-cloned calves.

Keywords: Somatic cell-cloned calves; Histone H4 acetylation; Gene expression; Lungs

1. Introduction

Although some mammalian species have been successfully cloned by somatic cell nuclear transfer, only a small proportion of embryos (typically between 0% and 4%) reconstructed using adult or fetal somatic cells developed to live young. The low overall success rate is the cumulative result of inefficiencies at each stage of the process, including embryonic, fetal, prenatal and neonatal loss, and production of abnormal offspring. Common anomalies include respiratory distress, increased birth weight and major cardiovascular abnormalities [1] and that the lungs always have the most obvious anomalies in our dead-cloned calves. Recent successes in mammalian cloning with differentiated adult nuclei strongly indicate that the oocyte cytoplasm contains unidentified reprogramming activities with the capacity to erase the previous memory of cell differentiation [2]. Many experiments have demonstrated epigenetic reprogramming in cloned animals, including the changes of chromatin structure, DNA methylation, imprinting, telomere length adjustment, and X chromosome inactivation. Thus, inefficient epigenetic reprogramming could result in inappropriate gene expression. Embryos may die at various stages of pre- or postnatal development depending on a certain threshold for faulty expression of the particular genes affected in a given clone or because of the random dysregulation of a key gene(s) crucial for a specific developmental stage [3].

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Histone acetylation is one key epigenetic modification to regulate gene activation. Four decades ago, Allfrey and his colleagues found a positive correlation between histone acetylation and gene transcription of RNA [4]. The amino termini of histones extend from the nucleosomal core and are modified by histones acetyltransferases (HATs) and histone deacetylases (HDACs) during the cell cycle. As acetylation neutralizes the positively charged lysine residues of the histone N termini, this decreases their affinity for DNA, and might allow the termini to be displaced from the nucleosome, causing the nucleosomes to unfold and increasing access to transcription factors [5]. Generally, actively transcribed chromatin regions have been associated with hyperacetylation and histone acetyltransferase recruitment, but histone deacetylases are recruited to accessible chromatin as well. HDAC-mediated deacetylation is thought to promote the return to a repressive, higher-order chromatin structure. This balance between acetylation and deacetylation is an important factor in regulating gene expression and is thus linked to the control of cell fate. As a consequence, hyperacetylation of normally silenced regions or deacetylation of normally actively transcribed region can lead to various disorders, including developmental and proliferative diseases [6]. A higher level of histone acetylation was observed in swamp buffalo-cloned embryo as compared with *in vitro* fertilized embryos at 4- and 8-cell stages [7] and it was speculated that abnormal epigenetic reprogramming in cloned embryos might be caused by a memory mechanism [8]. The expression of many important genes has been examined in somatic cell-cloned animals including preimplanted embryos [9–13] and neonatal dead calves [14,15].

In this study, we investigated the level of histone H4 acetylation and gene expressions in lungs of nine aberrant somatic cell-cloned calves.

2. Materials and methods

2.1. Animals

The normal controls were Holstein calves produced by artificial insemination (AI) and were slaughtered within 12 h after birth. The somatic cell-cloned Holstein calves were produced by nuclear transfer [16] in our laboratory. The nine aberrant-calves (Table 1) were of prenatal loss (C1–C5, 250–291 days fetus) and neonatal deaths (C6–C9, 3–7 days). The lungs showed obvious morphological abnormalities including pulmonary hemorrhage, inflammation, congestion and thickening of the lung alveolar wall. Especially, all the four neonatally died cloned calves suffered respiratory distress. All major internal organs were dissected immediately after the death and were collected and frozen in liquid nitrogen, then kept at −70 °C.

2.2. Acetyl-histone H4 immunoprecipitation (ChIP) assay

ChIP is a very useful method to test the level of histone H4 acetylation [17–19]. In the experiment, approximately 1 mg of the lung tissue ($\approx 1 \times 10^6$ cells) was homogenized in 1 ml of 1% formaldehyde. After 30 min incubation on ice for cross-linking histone to DNA, an acetyl-histone H4 Immunoprecipitation (ChIP) Assay Kit was used to prepare immunoprecipitated DNA (Upstate Biotechnology, Lake Placid, USA). In these processes, the protease inhibitors (phenylmethylsulfonyl fluoride, aprotinin and pepstatin A) (Roche, Mannheim, Germany) were always used. The DNA was recovered and purified by the Wizard DNA Clean-Up System (Promega, Madison, USA). This assay was repeated three times for each sample and then samples were combined.

2.3. RNA isolation and cDNA preparation

Total RNA was extracted from the lung tissue using the TRIZOL RNA Isolation Kit (Invitrogen, Carlsbad, USA). The RNA preparations were treated with RNase-free DNase I to remove a possible contamination of DNA and stored at −70 °C. Reverse transcription was conducted using a Reverse Transcription Kit (Promega) and 1 μg RNA in a total volume of 20 μl following manufacturer's guidelines.

2.4. Primer design

Primers for PCR amplifications were designed using the Oligo 6 software (Table 2). With these primers the PCR products were obtained from the gene promoter region and the upstream region of the promoters. The PCR

Table 1
Summary of the lung pathologies in cloned fetuses and offsprings

Clones	Loss or died days	Pathologic description
C1	Gestation 250 days	Atelectasis, congestion
C2	Gestation 252 days	Atelectasis, thicken of alveolar wall
C3	Gestation 252 days	Atelectasis, thicken of alveolar wall
C4	Gestation 274 days	Pulmonary aplasia
C5	Gestation 291 days	Atelectasis, congestion
C6	Died 3 days after birth	Atelectasis, congestion, six lung lobes not connection
C7	Died 3 days after birth	Respiratory distress, one additional lung lobe, inflammation
C8	Died 3 days after birth	Respiratory distress
C9	Died 7 days after birth	Respiratory distress

Table 2
Primers for real-time PCR analysis of ChIP DNA and cDNA

Gene	Forward primers (5′→3′)	Reverse primers (5′→3′)	PCR product (bp)	Tm (°C)	GenBank No.
ChIP DNA primers					
β-Actin	GCTCGCCATCAGTTACAAG	GGACACACAGACCCGAGAGT	231	56	AY293622
EGF	CCCCAGGGACCCAGTTCATC	CGTCGGCACTCATCAATATC	220	62	AY192564
FGF	GAGAAACCACGACCTTCACG	CTTCTCCTGTCCCTGAGTAAG	212	61	M13439
FGFBP	CTGGCTGTTCCGATGCTC	GCCAATTTGTTTCCAATAGG	321	58	AY642852
FGFR	GGGCCGCTCTATGTCATCG	GCCATCTGCTCCTCGGGAAC	125	62	AY64283
GHR	GCCCA GTAAGCGACATTAC	GTCTACCTCGCAGAAGTAAGC	156	60	AF140284
Gli2	TGGCCCGCCTCCACTCCG	CTCGCCCGAGAAGTGCACGC	122	67	AY642854
HGFR	GAAGTATCAGCTTCCCAACTTC	AAACAATCTGGGTGTTCC	171	56.5	AY642856
IGF1	CTCTTTCCAGGTGACCCAATG	AATGCCTTGGCTCCCTAGATG	164	61	Y18831
RAR	GGGAAGGGTTCACCGAAAGTTC	TCCTACCCCGACGGTGCCCAGA	215	64	AY642857
Shh	TGCTGCTGCTGGCGAGATGT	TCTCCGCCACGTTGGGGAT	159	56	AY642859
cDNA primers					
IGF1	AGCAGTCTTCCAACCCAATTA	TGGCAGAGCTGGTGAAGG	130	58	AY277405
FGFBP	CTCCTCCTGGCTGTTCCGATG	GGGTAATTGGTTGGCTGGCTC	140	61	AF271896
FGFR	TGGCCGTGAAGATGTTGAAAG	CGCAGGTATTCCCGAAGGTTG	190	56.5	Z68150
GAPDH	GCACAGTCAAGGCAGAGAAC	CTTATGACCACTGTCCACGC	420	58	BTU85042
VEGF	GAACTTTCTGCTCTCTTGGG	CTGGCTTTGGTGAGGTTTGA	340	61	NM_174216
FGF10	AACTCCTCTTCTTCTTCTTCCTC	TTCACAGCAACAACTCCGAT	239	58	AY183659
BMP4	CTTTTCGTTTCCTCTTTAACC	TCCCACCGTGTCACATTGT	229	54.5	AF136233
SP-A	CCAGGAAGAGATGGGAGAGA	GATATGCTGGAAACCCTGGA	184	60	AY486457
GHR	CGGCTCGCAGGTCCTACA GGT	GGTGAACGGCACTTGGTGAAT	197	61	NM_176608
PDGFRa	GAGTGAAGTGAGCTGGCAGT	TCCGTCATTCCTAGAGGTAC	249	62	NM_033023
PCAF	TTTCTGTCAGCACACTCGGC	CAAGGGTTTTGTGTTTCGGG	220	61	NM003884
Hsp70	TACAAAGGGGAGACCAAGGC	TTCCTCTTGAACTCCTCCAC	425	62	BtU09861
HGFR	GAAGTATCAGCTTCCCAACTTC	AAACAATCTGGGTGTTCC	171	56.5	NM_000245
EGFR	FTAACAAGCTCACGCAGTTGG	GTTGAGGGCAATGAGGACAT	178	60	U48722
RAR	GGAAGGGTTCACCGAAAGTT	TAGACCCTCCTGCCTCTGAA	105	60	AY642857
RABP	CCCACTTGGGAGAATGAGAA	GCCTTCATTCCCGAACATAA	166	54.5	M17253
IGFBP	AATGGCAGTGAGTCGGAAGA	AAGTTCTGGGTGTCTGTGCT	194	60	M76478
HGF	GATGTCCATGGGAGAGGAGA	TCAGGAATTGTGCACCCATA	170	60	AB110822
Gli2	GATAGTCCCTCTCACCCAGT	GCGAACTTACCATCATTTCA	176	58	L12259
Shh	CTGAACGCCTTAGCCATATC	GTGTGCCTTGGATTCGTAG	225	58	AF144100

annealing temperatures of primer pairs were determined using Mastercycler Gradient (Eppendorf, Hamburg, Germany).

2.5. Real-time quantitative PCR (Q-PCR)

Real-time PCR was carried out using the SYBR PCR Reagents Kit and the ABI Prims 7900HT Sequence Detection System (Applied Biosystems, USA). The 20 μl reaction mixture contained 1× SYBR Green I PCR Mastermix, 0.1 μM of each primer, and 1 μl of the template. The PCR reaction started at 95 °C for 10 min followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at the temperature for the corresponding primers (Table 2) for 30 s, and extension at 72 °C for 1 min. A standard curve was produced by a 10-fold dilution of the DNA template in the PCR system and the correlation was at least 0.995. Negative control was the PCR without DNA template. Each sample was tested by PCR for 4 times, and the standard curve was produced 3 times. The samples with C_T values >35 were treated as negative. In real-time PCR analysis of ChIP DNA, a house-keeping gene β-actin was used to quantitate the amount of DNA present in different samples. In real-time PCR analysis of cDNA, a house-keeping gene GAPDH was used as an internal control to quantitate the amount of cDNA present in different samples.

2.6. Statistical analysis

All data were analyzed by ANOVA using the SPSS software (Version 10.0, USA). An independent sample *T*-test was used to compare the differences in histones H4 acetylation and gene expression assayed by quantitative PCR between normal controls and aberrant-cloned calves. Differences of $p < 0.05$ were considered to be significant.

3. Results and discussion

In examination of the histone H4 acetylation levels of 11 development-related genes in lungs of aberrant-cloned calves, we found that most of the genes (9/11) in aberrant clones had a decreased acetylation level ($p < 0.05$) to different extent, compared to normal controls from 1.8- to 34.3-fold, namely Gli2 (34.3-fold), HGFR (28.0-fold), EGF (20.5-fold), β-actin (10.8-fold), Shh (5.8-fold), FGFR (5.2-fold), GHR (3.3-fold), FGF (3.3-fold) and IGF1 (1.8-fold). However, FGFBP and RAR genes showed no significant differences in expression levels ($p > 0.05$)

Table 3
T-test of differences between normal controls and aberrant clones of histone H4 acetylation

Gene	Normal controls	Aberrant clones	*p*	Variations
β-Actin	1.00 ± 0.25	0.09 ± 0.01	<0.0001	Down
EGF	1.00 ± 0.14	0.05 ± 0.01	<0.0001	Down
FGF	1.00 ± 0.24	0.30 ± 0.03	<0.0001	Down
FGFBP	1.00 ± 0.22	1.28 ± 0.13	0.3222	
FGFR	1.00 ± 0.18	0.19 ± 0.03	<0.0001	Down
GHR	1.00 ± 0.33	0.29 ± 0.03	0.0003	Down
Gli2	1.00 ± 0.17	0.03	<0.0001	Down
HGFR	1.00 ± 0.15	0.04	<0.0001	Down
IGF1	1.00 ± 0.22	0.55 ± 0.04	0.0028	Down
RAR	1.00 ± 0.12	1.21 ± 0.11	0.2958	
Shh	1.00 ± 0.25	0.17 ± 0.01	<0.0001	Down

Data are shown as means ± standard error. Sample size in normal controls and aberrant clones is 3 and 9, respectively. Differences of $p < 0.05$ were considered to be significant. Down denotes a significantly lower levels of histone H4 acetylation in aberrant clones than in normal controls.

between normal controls and aberrant clones (Table 3). In examination of the expression levels of 19 development-related genes in lungs of aberrant-cloned calves. We found that 13 out of 19 genes had a significantly repressed expression level ($p < 0.05$), ranging from 1.4- to 4.0-fold, such as IGF1 (4.0-fold), RAR (2.9-fold), PDGFR (2.5-fold), EGFR (2.4-fold), HGFR (2.3-fold), FGFBP (2.2-fold), HGF (2.2-fold), VEGF (2.1-fold), GHR (2.0-fold), SP-A (2.0-fold), FGFR (1.9-fold), FGF10 (1.5-fold) and RABP (1.4-fold). The other five genes (BMP4, IGFBP3, Gli2, PCAF and Shh) had no significant differences in gene expression between controls and clones ($p > 0.05$). Only Hsp70 showed an increased expression levels (2.7-fold) in cloned calves ($p < 0.05$) (Table 4). We considered that the inefficient histone H4 acetylation levels are probably due to the inappropriate epigenetic reprogramming of the transferred nucleus in the oocyte cytoplasm, and the inefficient activities of histones acetyltransferases in the oocyte cytoplasm would result in incompetence of histone acetylation in the whole or a part of chromatin. This inefficient histone H4 acetylation would have some effects on the appropriate gene expression. This may also have some implications for nuclear transfer in animal cloning, such as using some deacetylation inhibitors or acetylation activators to increase acetylation levels in the reconstruction of embryos, so as to improve the success rate of cloning [20,21].

Table 4
T-test of differences between controls and clones in gene expression

Gene	Controls	Clones	*p*	Variations
BMP4	1.00 ± 0.26	1.06 ± 1.07	0.7826	Down
EGFR	1.00 ± 0.21	0.41 ± 0.26	<.0001	Down
FGF10	1.00 ± 0.33	0.68 ± 0.53	0.0429	Down
FGFBP	1.00 ± 0.63	0.47 ± 0.42	0.0406	Down
FGFR	1.00 ± 0.23	0.52 ± 0.45	0.0004	Down
GHR	1.00 ± 0.28	0.51 ± 0.45	0.0008	Down
Gli2	1.00 ± 0.36	0.88 ± 0.95	0.5629	
HGF	1.00 ± 0.11	0.46 ± 0.29	<.0001	Down
HGFR	1.00 ± 0.47	0.43 ± 0.72	0.0255	
Hsp70	1.00 ± 0.36	2.67 ± 2.36	0.0013	Up
IGF1	1.00 ± 0.55	0.25 ± 0.35	0.00057	Down
IGFBP3	1.00 ± 0.14	1.16 ± 0.69	0.2013	
PCAF	1.00 ± 0.38	0.92 ± 0.71	0.673	
PDGFR	1.00 ± 0.39	0.41 ± 0.38	0.0015	Down
RABP	1.00 ± 0.20	0.69 ± 0.44	0.0054	Down
RAR	1.00 ± 0.20	0.35 ± 0.21	<.0001	Down
Shh	1.00 ± 0.82	1.31 ± 2.22	0.5132	
SP-A	1.00 ± 0.15	0.49 ± 0.29	<.0001	Down
VEGF	1.00 ± 0.25	0.47 ± 0.49	0.0003	Down

Data are shown as least square means ± standard error. Sample size in controls and aberrant clones is 3 and 9, respectively. Differences of $p < 0.05$ were considered to be significant. Up denotes a significantly higher of expression levels in aberrant clones than in normal controls. Down denotes a significantly lower of expression levels in aberrant clones than in normal controls.

When comparing the histone H4 acetylation level with the expression level of genes FGFBP, FGFR, GHR, Gli2, HGFR, IGF1, RAR and Shh, we found that genes FGFR, GHR, HGFR and IGF1 showed lowered levels of both histone H4 acetylation and expression in aberrant clones than in controls, and histone H4 acetylation was even more obviously lowered in aberrant clones than those in controls (Table 5). From other reports that have demonstrated that histone acetylation is involved in the acquisition or maintenance of the open chromatin conformation associated with transcriptional competence and the modification plays an 'enabling' role in transcriptional activation [22–24] and from the knowledge that histone acetylation of the promoter precedes the activation of many genes and is thought to establish a chromatin environment suitable for the assembly of the transcriptional complex [18]. Thus, the more varying range of histone H4 acetylation may cause cells to conveniently achieve the primary regulation for other regulation and the gene expression, and the gene expression can directly and exactly affect development and growth of animals. Interestingly, FGFBP and RAR showed no significant changes of histone H4 acetylation levels but significantly decreased expression levels, whereas Gli2 and Shh showed significantly decreased histone H4 acetylation levels but no significantly varied expression levels. Therefore some other regulating mechanisms might exist and need to be uncovered.

Table 5
Variation of the levels between histone H4 acetylation and gene expression

Gene	Histone H4 acetylation		Gene expression	
	Fold	Variation	Fold	Variation
FGFBP			2.8	Down
FGFR	5.2	Down	1.9	Down
GHR	3.5	Down	2.0	Down
Gli2	34.3	Down		
HGFR	28.0	Down	2.3	Down
IGF1	1.8	Down	4.0	Down
RAR			2.9	Down
Shh	5.8	Down		

Fold denotes the decreased fold compared between normal controls and aberrant clones. Down denotes a significantly lower level in aberrant clones than in normal controls.

The dysregulation of a key gene(s) crucial for a specific developmental stage could result in the embryo dying at various stages, either during gestation, as neonates, or postnatally. However, the cloned embryos could develop to birth even if there are some dysregulation of gene expression in the lungs [25]. This may explain why the lungs in our dead somatic cell-calves are quite clearly abnormal. Growth factors IGFs with IGFBPs [26,27], GH with GHR [28], EGF [29], HGF with HGFR [30], RA with RAR and RABP [25], SP-A [31,32] and VEGF [33] all play prominent roles in the lung development. The repressed expression of those genes may have some contributions to the lung developmental abnormalities in clones. Particularly, FGF10, FGFR [25] and FGFBP [34] play a critical role in the lung formation and all the three genes have repressed expression in this study. In cloned calf C6, FGF10 had the lowest expression level and FGFR also had very low expression level, however, FGFBP had the highest expression level in clones. Cloned calf C6 had obviously abnormalities: six separate lung lobes and those aberrant expressions might have some effects on C6 lung development. Hsp70 can inhibit apoptosis by neutralizing and interacting with apoptosis-inducing factor [35]. This was the only gene that has increased expressions in clones (2.7-fold) and the increased expressions of Hsp70 may be a counteraction to the aberrant epigenetic environment and expressions of other genes. BMP4 is an important regulation during lung morphogenesis, Shh regulates lung pattern formation of a variety of developing structures and Glis regulates lung growth and pattern formation [25]. We observed that BMP4 and Shh genes in C7 had the highest levels of expression. Cloned calve C7 had special abnormalities with one additional lung lobe and the abnormal expression of BMP4 and Shh may have some contributions to its lung developmental abnormalities. PCAF is a transcriptional co-activator with intrinsic histone acetylase activity and contributes to transcriptional activation by modifying chromatin and transcription [36,37]. This gene had a lowered histone H4 acetylation level in clones than those in controls, but no significant differences between controls and clones in expression level. Thus, we suggest that the aberrant-cloned animals could also be models to investigate genes' function in the process of growth and development of animals.

Taken together, this study demonstrated that the decreased levels of histone H4 acetylation may affect appropriate gene expression, and hence affecting growth and development of the cloned calves. These findings gave us some knowledge on understanding the low efficiency of somatic cell nuclear transfer.

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References

[1] Wilmut I, Beaujean N, de Sousa PA, et al. Somatic cell nuclear transfer. Nature 2002;419:583–6.
[2] Wade PA, Kikyo N. Chromatin remodeling in nuclear cloning. Eur J Biochem 2002;269:284–7.
[3] Rideout WM, Eggan K, Jaenisch R. Nuclear cloning and epigenetic reprogramming of the genome. Science 2001;293:1093–8.
[4] Allfrey VG, Faulkner R, Mirsky AE. Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. Proc Natl Acad Sci USA 1964;51:786–94.
[5] Grunstein M. Histone acetylation in chromatin structure and transcription. Nature 1997;389:349–52.
[6] Timmermann S, Lehrmann H, Polesskaya A, et al. Histone acetylation and disease. Cell Mol Life Sci 2001;58:728–36.
[7] Suteevun T, Parnpai R, Smith SL, et al. Epigenetic characteristics of cloned and in vitro-fertilized swamp buffalo (*Bubalus bubalis*) embryos. J Anim Sci 2006;84:2065–71.
[8] Wee G, Koo DB, Song BS, et al. Inheritable histone H4 acetylation of somatic chromatins in cloned embryos. J Biol Chem 2006;281:6048–57.
[9] Daniels R, Hall V, Trounson AO. Analysis of gene transcription in bovine nuclear transfer embryos reconstructed with granulosa cell nuclei. Biol Reprod 2000;63:1034–40.
[10] Daniels R, Hall VJ, French AJ, et al. Comparison of gene transcription in cloned bovine embryos produced by different nuclear transfer techniques. Mol Reprod Dev 2001;60:281–8.
[11] Wrenzycki C, Wells D, Herrmann D, et al. Nuclear transfer protocol affects messenger RNA expression patterns in cloned bovine blastocysts. Biol Reprod 2001;65:309–17.
[12] Wrenzycki C, Herrmann D, Lucas-Hahn A, et al. Gene expression patterns in in vitro-produced and somatic nuclear transfer-derived preimplantation bovine embryos: relationship to the large offspring syndrome? Anim Reprod Sci 2004;82/83:593–603.
[13] Li X, Amarnath D, Kato Y, et al. Analysis of development-related gene expression in cloned bovine blastocysts with different developmental potential. Cloning Stem Cells 2006;8:41–50.
[14] Li S, Li Y, Du W, et al. Aberrant gene expression in organs of bovine clones that die within two days after birth. Biol Reprod 2005;72:258–65.
[15] Long JE, Cai X. Igf-2r expression regulated by epigenetic modification and the locus of gene imprinting disrupted in cloned cattle. Gene 2007;388:125–34.
[16] Gong G, Dai Y, Fan B, et al. Birth of calves expressing the enhanced green fluorescent protein after transfer of fresh or vitrified/thawed blastocysts produced by somatic cell nuclear transfer. Mol Reprod Dev 2004;69:278–88.
[17] Vogelauer M, Wu J, Suka N, et al. Global histone acetylation and deacetylation in yeast. Nature 2000;408:495–8.
[18] Zhou W, Chang S, Aune TM. Long-range histone acetylation of the Ifng gene is an essential feature of T cell differentiation. Proc Natl Acad Sci USA 2004;101:2440–5.
[19] Yoshioka H, Shirayoshi Y, Oshimura M. A novel in vitro system for analyzing parental allele-specific histone acetylation in genomic imprinting. J Hum Genet 2001;46:626–32.
[20] Enright BP, Kubota C, Yang X, et al. Epigenetic characteristics and development of embryos cloned from donor cells treated by trichostatin A or 5-aza-2′-deoxycytidine. Biol Reprod 2003;69:896–901.
[21] Shi W, Hoeflich A, Flaswinkel H, et al. Induction of a senescent-like phenotype does not confer the ability of bovine immortal cells to support the development of nuclear transfer embryos. Biol Reprod 2003;69:301–9.
[22] Hebbes TR, Clayton AL, Thorne AW, et al. Core histone hyperacetylation co-maps with generalized DNase I sensitivity in the chicken beta-globin chromosomal domain. EMBO J 1994;13:1823–30.

[23] Mizzen CA, Allis CD. Linking histone acetylation to transcriptional regulation. Cell Mol Life Sci 1998;54:6–20.
[24] Hebbes TR, Thorne AW, Crane-Robinson C. A direct link between core histone acetylation and transcriptionally active chromatin. EMBO J 1988;7:1395–402.
[25] Cardoso WV. Molecular regulation of lung development. Annu Rev Physiol 2001;63:471–94.
[26] Moats-Staats BM, Price WA, Xu L, et al. Regulation of the insulin-like growth factor system during normal rat lung development. Am J Respir Cell Mol Biol 1995;12:56–64.
[27] Schuller AG, van Neck JW, Beukenholdt RW, et al. IGF, type I IGF receptor and IGF-binding protein mRNA expression in the developing mouse lung. J Mol Endocrinol 1995;14:349–55.
[28] Isaksson OG, Eden S, Jansson JO. Mode of action of pituitary growth hormone on target cells. Annu Rev Physiol 1985;47:483–99.
[29] Miettinen PJ, Warburton D, Bu D, et al. Impaired lung branching morphogenesis in the absence of functional EGF receptor. Dev Biol 1997;186:224–36.
[30] Kolatsi-Joannou M, Moore R, Winyard PJ, et al. Expression of hepatocyte growth factor/scatter factor and its receptor, MET, suggests roles in human embryonic organogenesis. Pediatr Res 1997;41:657–65.
[31] Blau H, Riklis S, Kravtsov V, et al. Secretion of cytokines by rat alveolar epithelial cells: possible regulatory role for SP-A. Am J Physiol 1994;266:L148–55.
[32] Hallman M, Merritt TA, Akino T, et al. Surfactant protein A, phosphatidylcholine, and surfactant inhibitors in epithelial lining fluid. Correlation with surface activity, severity of respiratory distress syndrome, and outcome in small premature infants. Am Rev Respir Dis 1991;144:1376–84.
[33] Kasahara Y, Tuder RM, Taraseviciene-Stewart L, et al. Inhibition of VEGF receptors causes lung cell apoptosis and emphysema. J Clin Invest 2000;106:1311–9.
[34] Kurtz A, Wang HL, Darwiche N, et al. Expression of a binding protein for FGF is associated with epithelial development and skin carcinogenesis. Oncogene 1997;14:2671–81.
[35] Gurbuxani S, Schmitt E, Cande C, et al. Heat shock protein 70 binding inhibits the nuclear import of apoptosis-inducing factor. Oncogene 2003;22:6669–78.
[36] Workman JL, Kingston RE. Alteration of nucleosome structure as a mechanism of transcriptional regulation. Annu Rev Biochem 1998;67:545–79.
[37] Schiltz RL, Nakatani Y. The PCAF acetylase complex as a potential tumor suppressor. Biochim Biophys Acta 2000;1470:M37–53.