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Epigenetic Reprogramming and Somatic Cell Cloning

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A viable animal clone derived from somatic cell indicates that a differentiated cell nucleus is recapitulated to an embryonic totipotent state by complex epigenetic reprogramming processes (Kang et al., 2003). However, molecular events regarding epigenetic reprogramming of somatic chromatinsin the early-stage embryos are poorly understood. Here we provide an insight that the somatic chromatins are unyielding to epigenetic during early embryogenesis. Understanding the epigenetic reprogramming processes of donor genome will clearly define the faulty development of cloned embryos.

I. Introduction

Despite successful production of clones from somatic cells has been achieved in various species such as sheep (Wilmut et al., 1997), cattle (Cibelli et al., 1998), mice (Wakayama et al., 1998), goats (Baguisi et al., 1999), pigs (Onishi et al., 2000; Polejaeva et al., 2000), cats (Shin et al., 2002), rabbits (Chesne et al., 2002), mule (Woods et al., 2003) and rats (Zhou et al., 2003), till now its efficiency is very low in that only less than 1% of the reconstructed embryos give rise to live-born animals. Moreover, the nuclear transfer has raised severe developmental problems including high rate of abortion during early gestation and increased perinatal death (Heyman et al., 2002; Hill et al., 2000; Hill et al., 1999). It is uncertain whether these developmental failures of cloned embryos are due to the incomplete nuclear reprogramming or the cloning procedure itself. Nuclear transfer involves a series of complex procedures including culture of donor cells, *in vitro* maturation of oocytes, enucleation, cell or nucleus injection, fusion, activation, *in vitro* culture of reconstructed embryos and embryo transfer. If any part of the nuclear transfer procedures is improperly performed, the production of cloned embryos or animals can be tackled. Although cloned animals have been successfully generated from many research groups worldwide, we still have limited information about nuclear reprogramming of cloned embryos. Here, epigenetic reprogramming, or DNA methylation state of cloned embryos during early development is described in details.

Ⅱ. Epigenetic Reprogramming in Early Embryos

In mammals, epigenetic modifications such as DNA methylation and histone modifications play important roles in the regulation of gene expression and are essential for normal embryonic development. The most dramatic changes in the methylation state occur during gametogenesis and early embryonic development (Monk et al., 1987). During early embryonic development, the overall methylation level sharply decreases and reaches to low point at the blastocyst stage (Monk et al., 1987; Razin and Shemer, 1995). The process of epigenetic reprogramming in early embryos erases gamete-specific methylation patterns inherited from the parents (Howlett and Reik, 1991; Monk et al., 1987; Oswald et al., 2000). This genome-wide demethylation process may be crucial for the formation of pluripotent stem cells that are important for the later development. During post-implantation development, a wave of *de novo* methylation takes place and most of the genomic DNAs are methylated at defined developmental timepoints. Another demethylation/remethylation event occurs during gametogenesis and is necessary for resetting of genomic imprinting. Therefore, dynamic epigenetic changes of the genomic DNAs appear to be essential and unique during embryonic development in mammals. Recently, we have foundthat the specific genomic regions or sequences have spatially/temporally their unique characteristics in the reprogramming of the DNA methylation during preimplantation development, suggesting that the epigenetic reprogramming occurs independently on the distinct genomic regions by different mechanisms (Kim et al., 2004).

Ⅲ. Abnormal Epigenetic Reprogramming in Cloned Embryos

Successful cloning of animals by nuclear transfer requires epigenetic reprogramming of the differentiated state of the donor cell to a totipotent, embryonic ground state (Gurdon, 1999). It means that the donor nuclei must cease its own program of gene expression and restore a particular program of the embryonic expression necessary for normal development. Epigenetic reprogramming processes after somatic cell nuclear transfer include remodeling of chromatin structure, global changes in DNA methylation, expression of imprinted genes, restoration of telomere length, X chromosome inactivation and other events during early embryonic development. Recently, some interesting results on epigenetic reprogramming of the donor genome have been taken out in cloned embryos and cloned animals.

In cloned bovine embryos, the epigenetic reprogramming mechanism represented by the methylation/demethylation process probably is not working well (Bourc'his et al., 2001; Kang et al., 2001). Various repetitive genomic sequences such as satellite I, satellite II, 18S rDNA, LINE and art-2 SINE sequences showed aberrant methylation status in cloned embryos (Kang et al., 2001). The methylation patterns of cloned embryos were quite different from those of normal embryos produced *in vitro* or *in vivo*, but closely resembled those of donor cells in the overall genomic methylation status. Immunostainning results also showed differences in the dynamics of chromosome methylation between cloned and normal embryos at the

preimplantation stages (Bourc'his et al., 2001).

Then, what can viable offsprings really develop from the unlikely cloned embryos showing heavy methylation states? Some clues on this question canbe answered by analyses of individual cloned embryos for the methylation state. Unexpectedly, the methylation level of bovine satellite sequences was greatly varied in individual cloned embryos (Kang et al., 2001). The possibility that individual variation of methylation level shown in cloned embryos may depend upon innate methylation difference among individual donor cells cannot be excluded. Considering the nature of centromeric satellite I DNA such as high copy number and relatively stable epigenetic status, however, it is more likely that methylation variations observed in individual cloned embryos are determined largely by different abilities of the recipient oocytes or reconstituted embryos to modify the epigenetic status of donor genome. In addition, we have demonstrated that epigenetic modification can take place differentially in the cloned embryos (Kang et al., 2001; Kang et al., 2002). In contrast to the genomic repeats, the promoter sequences of tissue-specific genes such as bovine epidermal cytokeratin gene and beta-lactoglobulin gene were completely demethylatedat the blastocyst stage of cloned embryos. Another sigle-copy gene, bovine galanin gene, in the cloned embryos showed a normal methylation process during preimplantion development (Kang et al., 2003). These results indicate the possibility that single-copy genes that are important for embryonic development can be selectively demethylated in cloned embryos, like tissue-specific genes.

The phenomenon of differential demethylation appears not only among different genomic sequences, but also between the different regions of cloned embryos at the blastocyst stage. Aberrant allocations of inner cell mass (ICM) cells and trophectoderm (TE) cells were observed in cloned bovine blastocysts (Koo et al., 2002), showing a higherproportion of ICM in cloned embryos as compared with normal embryos produced *in vitro* and *in vivo*. It then was postulated whether the methylation differences in cloned embryos might be derived from epigenetic difference between ICM and TE genomes. We observed in cloned blastocysts that the satellite sequences remain methylated in trophectoderm cells but not in inner cell mass cells (Kang et al., 2002). However, it is unknown that the unequal methylation between ICM and TE cells observed in cloned blastocysts is a general phenomenon that appears also in normal bovine embryos derived by fertilization. Although a similar methylation difference between ICM and TE cells was not detected in IVF-derived embryos, this observation alone cannot exclude the possibility of differential demethylation in normal embryos. Since the satellite sequences maintain low methylation status in IVF-derived embryos throughout preimplantation stage, this makes it difficult for the two distinct lineage cells to manifest the potential ability to modify their epigenetic status differentially. More information is needed on other genomic sequences that remain methylated till the blastocyst, which can be very helpful for elucidating methylation differences between ICM and TE regions.

Whether or not the methylation difference between ICM and TE cells is a unique feature of cloned embryos remains to be elucidated yet, but it is clear that TE lineage cells of the blastocyst clones with normal ICM/TE ratios are aberrantly methylated in the satellite sequences (Kang et al., 2002). Various processes programmed by the TE cells may be

essential to establish and maintain the stable placentation and thereby to support the growth of embryo proper. The functional integrity of these all processes can be guaranteed by the proper methylation status in the corresponding cells. In this line of connection, it is likely that TE cells of cloned blastocysts become strayed out of the developmental orbit since the observation of abnormal DNA methylation of the satellite sequences leads us to easily infer methylation aberrancy in other genomic regions including single-copy genes with important roles in normal development of cloned embryos. Abnormal methylation of unique gene sequences necessarily gives rise to misregulation of gene expressions, and the cumulative action of many abnormally expressed genes may affect subsequent viability of cloned embryos after implantation. Deficient placentation is most frequently observed in dead fetal clones of various mammalian species, and also has been recognized as a potential cause of early fetal loss (Hill et al., 2000) and neonatal mortality (Wakayama et al., 1998) in cloned animals. Although our results did not present direct evidence for this hypothesis and thus appear to be more or less suggestive, it seems natural to believe the correlation of abnormal methylation in TE cells of cloned blastocysts with the placental defects observed in cloned fetuses/animals.

Ⅳ. Conclusions

Poor epigenetic reprogramming in early cleavage embryos may entail aberrant expression of the genes at multiple loci, and then the accumulated actions of many abnormally expressed genes in cloned embryos or fetuses can be detrimental to normal full-term development. However, the precise understanding for the epigenetic anomaly inthe cloned embryos remains mainly unclear. Studies on epigenetic reprogramming at the preimplantation stage contribute to understand the molecular basis for developmental competence of cloned embryos. To improve efficiency of the present cloning system, extensive researches on molecular mechanisms underlying the reprogramming of donor genome are needed.

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