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Current Status and Prospects of Somatic Cell Nuclear Transfer and Cloning

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ABSTRACT

Somatic cell nuclear transfer is an efficient technique for the multiplication of elite livestock, engineering of transgenic animals, cell therapy and xenotransplantation, and analyzing the interactions between nucleus and cytoplasm, for various agricultural, biomedical and research purposes. Since the first somatic cell clone lamb was born, tremendous progress has been made toward developing technology for animal cloning. Viable farm animals and mice have now been produced by nuclear transfer using various fetal and adult somatic cells as nuclei donors. Transgenic clones were also produced from nuclear transfer of transfected somatic cells. In the future, somatic cell nuclear transfer will provide more numerous opportunities, both in basic and appled research as well as immediate uses in the generations of superior clone and transgenic animals. However, further technology refinement and improved understanding of the process are essential for commercial and basic research applications.

Ⅰ. INTRODUCTION

The ability to recreate an individual by cloning has long been the substance of science fiction. The birth of two lambs, Megan and Morag, produced by nuclear transfer from a culture differentiated cell held out the prospect that somatic cells from an adult animal could be used successfully as donors of genetic material in the cloning procedure (Campbell et al., 1996). Subsequently these experiments were repeated and extended, resulting in the successful birth of lambs created using donor cells derived from fetal and adult tissue (Wilmut et al., 1997). The birth of 'Dolly' gave rise to many scientific and ethical debates as the role and use of technology in medicine, agriculture, biological research and human reproduction. Nevertheless, the cloning process has great promise for potential benefits in agriculture and biomedicine, such as multiplication of elite livestock, production of transgenic animals, salvaging of endangered animals, xenotransplantation and human cell therapy. In this paper, recent advances and future prospects of somatic cell cloning in mammals are discussed.

Ⅱ. CLONING TECHNOLOGY

1. Status of Animal Cloning

Since the first somatic cell clone lamb was born (Wilmut et al., 1997), tremendous progress has been made toward developing technology

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for animal cloning. Viable lambs (Wilmut et al., 1997; Schnieke et al., 1997), calves (Cibelli et al., 1998; Kato et al., 1998; Wells et al., 1998, 1999; Vignon et al., 1999; Zakhartchenko et al., 1999), goats (Baguisi et al., 1999) and mice (Wakayama et al., 1998, 1999) have now been produced by nuclear transfer using fetal and adult somatic cells as nuclei donors. In the mice cloning, Wakayama et al. (1998, 1999) produced both male and female clone mice by directly injecting of nuclei donors into the cytoplasm of recipient cells without cell fusion. It was also reported unofficially that tens of clone cattles were produced from somatic cell nuclear transfer in several countries. Recently, clone cattles were also produced following transfer of fetal (Cheong et al., unpublished) and adult somatic cells (Hwang et al., unpublished) into the enucleated oocytes in our country. On the other hand, birth of clone piglet has not been reported. The development of cloning techniques for pigs would have the greatest impact on biotechnology. The pig has proven itself to be very valuable of choice for xenotransplantation but is also useful for the development of disease models.

2. Cell Cycle

Somatic cell cloning is different from the embryonic cell cloning in a view of the nucleus from a differentiated donor cell to direct development to term. In this procedure, it has been suggested that the quiescent state of donor cells is a very important factor for the reprogramming of donor cell in the oocyte cytoplasm (Campbell et al., 1996; Wilmut et al., 1997). In the majority of somatic cell cloning reports, primary cell populations have been established in culture and induced to enter quiescence prior to use as nuclear donors (Wilmut et al., 1997; Kato et al., 1998; Wells et al., 1999). Naturally quiescent cells also used for nuclear donor. Mouse brain neural cells and Sertoli cells were transferred into enucleated recipients, but could not develop to term. Only cumulus cells that were mostly arrested in G0/G1 developed to term after cytoplasmic injection (Wakayama et al., 1998). On the other hand, nonquiescent proliferating bovine fetal fibroblast cells could develop to term (Cibelli et al., 1998), which suggesting that intentionally induced quiescence is not essential for term development.

3. Totipotency

Variety of fetal and adult somatic cell types were used as nuclei donors to investigate their totipotencies. At present, it was confirmed that fetal fibroblast, fetal skin and muscle cells, adult mammary gland, skin, muscle, cumulus, granulosa and oviduct cells have totipotencies in sheep, bovine, mouse or goat. In sheep, clones were produced from fetal fibroblast cells and adult mammary gland cell (Wilmut et al., 1997). In bovine, clones were obtained from more various cells, such as fetal cells (Zakhartchenko et al., 1999), cumulus cells (Kato et al., 1998), granulosa cells (Wells et al., 1999), oviduct cells (Kato et al., 1998), skin and muscle cells (Vignon et al., 1999). In mouse, clones were produced from cumulus cells (Wakayama et al., 1998) and tail-tip cells (Wakayama et al., 1999), but not from brain and Sertoli cells after direct inject of donor nuclei into the oocyte cytoplasm.

On the other hand, Kato et al. (1999) reported that mouse cumulus and follicular cells have very limited developmental potentials *in vitro* and *in vivo* following conventional nuclear transfer. Developmental potentials *in vitro* varied according to the cell types and researchers. It was suggested that the fetal derived-cells had higher developmental potential than adult cells, However, some kinds of adult cells such as cum-

ulus cells (Kato et al., 1998) and granulosa cells (Wells et al., 1999) more reliably results in blastocyst development and live offspring than fetal cells after nuclear transfer.

4. Postnatal characteristics

It is now well established that a significant proportion of bovine offspring produced by nuclear transfer exhibit abnormal phenotypes at birth. When embryonic cells are utilized as nuclei donors, the primary abnormality appears to be high birth weights, and the physiological problems associated with this which lead to low survival rate (Willadsen et al., 1991; Wilson et al., 1995; Garry et al., 1996). In somatic cell cloning, similar abnormalities have been observed and many times lead to death at the time of birth or shortly thereafter (Schnieke et al., 1997; Wilmut et al., 1997; Cibelli et al., 1998; Wells et al., 1999). These include high birth weight, abnormal placentation, pulmonary hypertension leading to insufficient pulmonary perfusion and respiratory distress syndrome, enlarged/dilated right ventricles and patent ductus arteriosus. Besides the developmental abnormalities observed at the time of birth, it is clear that embryos produced by nuclear transfer also result in decreased pregnancy rates following embryo transfer and fetal losses are significantly higher.

Ⅲ. TRANSGENIC CLONING

Somatic cells nuclear transfer can provide advantages for making transgenic animals. 1) When a transfected cell line is used as the nuclear donor, all animals will be transgenic. Currently success rates in making transgenic livestock are lower than 10% using pronuclear microinjection. 2) It is possible that the best protein expression levels will be obtained by targeting inserted genes to sites of high expression. 3) The ability to produce numbers of genetically identical transgenic livestock animals in the first generation will significantly reduce the time to production of protein or peptide. 4) It is possible that the selective removal of gene(s), replacing them with the desired human equivalents. Gene targeting could be used to remove/inactivate unwanted genes(e.g., prion gene in cows, or α-galactosidase in pigs for xenotransplantation), or to add genes to, or modify genes at specific sites. 5) It is possible that the cellular-level analysis of clones allowing pre-selection for optimal protein expression.

Schnieke et al. (1997) produced transgenic clone lambs by introducing a foreign gene(human clotting factor Ⅸ gene) into fibroblast subsequently used for nuclear transfer, which requires fewer embryos than direct DNA microinjection into zygotes. Transgenic bovine clones also produced by the same approach (Cibelli et al., 1998), in which three transgenic clone calves having β-galactosidase-neomycin resistance fusion gene were born from nuclear transfer of transfected fetal fibroblast cells. Another approach was attempted to make transgenic clone goats, in which the cells derived from transgenic fetus were transferred into enucleated oocytes, and obtained three offsprings (Baguisi et al., 1999).

Ⅳ. INTERSPECIES NUCLEAR TRANSFER

Interspecies nuclear transfers were attempted to investigate the interaction between nuclear and cytoplasm derived from different species, and the possibilities of using for salvaging endangered species or human cell therapies (Dominko et al., 1999; White et al., 1999). Dominko et al. (1999) reported that although no pregnancies have been carried to term after transfer of em-

bryos into surrogate animals, bovine oocyte cytoplasm can support the introduced, differentiated nucleus regardless of chromosome number, species, or age of the donor fibroblast. The interspecies nuclear transfer technology has potential application in the production of primitive stem cells genetically identical to a patient. In this technology, human body cells are transferred to enucleated animal oocyte cytoplasm, to produce cells capable of forming virtually any cell type, such as bone marrow cells, nerve cells, heart muscle cells, pancreatic islet cells and so on.

Ⅴ. CELL THERAPY AND XENOTRANSPLANTATION

The somatic cell cloning technology has potential applications in the production of cloned transgenic animals to provide cells for use in treating neurodegenerative diseases such as Parkinson's disease, diseases of the endocrine system such as juvenile-onset diabetes, arthritis and many others. A potential treatment option for neurodegenerative disease involves replacing damaged brain cells with genetically engineered animal neural cells. Other potential applications include development of cloned transgenic animals for use in xenotransplantation.

Zawada and Colleagues (1998) use xenotransplants of cloned, transgenic bovine neurons to treat Parkinson's disease in rats. The Zawada's research demonstrates the combination of three independent technologies: 1) bovine cloning by nuclear transfer from a cultured fetal cells, 2) transgenesis of the cultured cells prior to animal production and 3) tissue xenotransplantation for the treatment of Parkinson's disease. Zawada et al.(1998) produced the cloned transgenic embryos by transferring *lacZ* gene-transfected fetal bovine fibroblasts into enucleated bovine oocytes. Neural cells secreting dopamine were collected from the ventral mesencephalon of 50-day -old cloned, transgenic bovine fetuses, and were transferred into the striatum of immunosuppressed rats. Xenotransplanted rats showed some improvements in motor activity compared to controls.

Establishment of human pluripotent stem cell (Thomson et al., 1998), which can give rise to many tissues of the body, greatly improved the human cell therapies.

Ⅵ. PROSPECTS AND CONCLUSION

In the future, somatic cell nuclear transfer will provide more numerous opportunities, both in basic and appled research as well as immediate uses in the generations of superior clone and transgenic animals. The use of somatic cell cloning technique hold tremendous promise for enhancing the quality of products in dairy and beef industries. Transgenic cloning process will be improved by development of gene targeting and multiple gene insertion techniques. Interspecies nuclear transfer will be established and greatly facilitate the salvaging of endangered animal species and the human cell therapy. Development of the pig as a xenograft donor will be greatly accelerated by development of nuclear transfer procedures using modifiable somatic cells. Further technology refinement and improved understanding of the process are essential, if the promise of nuclear transfer for commercial and basic research applications is to be fulfilled.

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