인간 난포자극호르몬 형질전환 젖소의 생산을 위한 체세포복제기술의 이용

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Use of Somatic Cell Nuclear Transfer Technology to Produce Human Follicle-Stimulating Hormone (hFSH) Transgenic Cows

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Introduction

For past several years, cloning and transgenic animal production have been greatly enhanced by the development of nuclear transfer (NT) technology. Multiplication of animals with high genetic value can be achieved by these techniques. Moreover it became feasible to create domestic animals with precise genetic modifications by introduction of transgene into somatic cells prior to nuclear transfer. Various cell types have been successfully used as donors to create clone animals. Both cell fusion and microinjection have successfully been used to create these animals. Although for the most part gene expression is reprogrammed in nuclear transfer embryos, all structural changes may not be correctly achieved. This often causes poor success rates and developmental anomalies in clone animals and limits wide and efficient use of somatic cell nuclear transfer procedures in the production of transgenic animals. This review discusses the development of nuclear transfer technology and its application focused on the production of human follicle-stimulating hormone transgenic cows.

Optimization of Nuclear Transfer Procedures

In nuclear transfer procedures, compatibility of cell cycle between donor cell nuclei and

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metaphase II oocytes is an important factor for reprogramming of transferred nuclei and for development of reconstituted embryos. Offspring could produced from nuclei of somatic cells in G0 and G1/S stage (Wilmut et al. 1997; Cibelli et al 1998). To find the most appropriate stage of donor cell cycle in somatic cell NT, the effect of cell cycle stage of donor cells on development of NT embryos was tested. The results suggest that an exit from the normal cell cycle for karyoplasts was not an absolute requirement to reprogram nuclei of fibroblasts for preimplantation development. Furthermore, synchronization of donor cells to G1/S stage even enhanced the development of NT embryos (Shim et al., 1999). Introduction of transgene into nuclear donor cells showed no influence on normal blastocyst development. However, using donor cells from early passages of culture increased blastocyst formation (Roh et al., 2000).

Cloning of Korean Native Cattle with High Genetic Value

Reproductive cloning could be employed in the context of animal husbandry as a convenient, efficient method to upgrade the quality of a herd by, for instance, cloning an animal noted for its exceptional milk production or wool quality. Nucleus from fibroblast cells taken from Korean bull with high genetic value were transferred into enucleated oocytes (Park et al., 2001). Less than 2 months after the transfer of NT embryos to recipients, most of transferred embryos were lost. Develpmental potential of cloned embryos to term was extremely low. One live offspring was born, and microsatellite profile of the clone animal matched to the karyoplast donor. The results indicated that multiplication of animals using somatic cell NT was feasible, and rapid propagation of superior genetic traits may be achieved along with progress of NT technology to overcome current low efficiency of reproductive cloning.

Nuclear Transfer Using Fibroblasts Transfected With Single-Chain Human Follicle-Stimulating Hormone Gene

Human follicle-stimulating hormone (hFSH) is a pituitary glycoprotein that plays an essential role in the regulation of follicular development and ovulation. Clinicaly, hFSH has been used to induce follicular growth in infertile women. The hormone was composed of heterodimers including a common α subunit among gonadotropin family and hormone-specific β subunit. Since assembly of the heterodimer is often a rate-limiting step in the

production of functional hormone from cultured cells and transgenic animals, nuclear transfer was attempted using fibroblasts carrying single-chain hFSH transgene (Kwon et al., 2003; Yoon et al., 2003). Genes encoding α and β subunit of hFSH were linked using C-termial peptide (CTP) sequence from β subunit of human chorionic gonadotropin. To test a secretion of hFSH prior to NT experiment, hFSH gene under the regulation of rat β actin promoter was introduced into Chinese hamster ovary (CHO) cells. In subsequent enzyme-linked immunosorbent assay, production of hFSH from CHO cells was verified. Bovine fibroblasts were transfected with a gene construct including β casein promoter and single-chain hFSH sequences. Fibroblasts carrying hFSH transgene were transferred to enucleated oocytes, and an existence of transgene in NT embryos was confirmed by polymerize chain reaction. Transfers of NT embryos to recipients to produce cloned calves carrying single-chain hFSH are currently in progress.

Conclusion

Remarkable progress has recently been made in mammalian cloning using nuclear transfer. Until the announcement of the successful cloning of sheep from adult mammary gland cells (Wilmut et al., 1997), dogma that cloning by nuclear transfer could only be accomplished with relatively undifferentiated embryonic cells had not been challenged. Since one way path of differentiation that once considered irreversible now can be reversed, reprogramming of somatic cell nuclei by nuclear transfer is an interesting issue in basic biology. Not only it is important in science, but cloning becomes an useful tool in multiplication of animals with high genetic value as well as transgenic animal production. Results from the experiment carried out in our laboratory suggest that multiplication of Korean Native Cattle with high genetic value and production of hFSH trasgenic embryos are feasible. Although an efficiency of cloning is still low and postnatal lethality and abnormality are not fully solved, the nuclear transfer procedure remains as robust tools to modify animal genome for both agricultural and biomedical use. Scientific challenges will be continued to overcome current technical problems of nuclear transfer and to find better and wider use of this fascinating technology.

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