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Development of Transgenic Pigs for Bio-organ

Kwang-Wook PARK

Dept. of Animal Science & Technology, Sunchon National University,

MGEN, Inc., #1101 World Meridian Venture Center, Seoul

Somatic cell nucear transfer (NT) is one of the technologies for producing genetically identical animals. The birth of cloned lamb 'Dolly' (Wilmut et al., 1997) derived from adult cells was reported, and focused a great deal of attention to the field of biotechnology. After the succeed of cloning, more than 10 animals were successfully cloned including cattle, goat, mice, cat, horse, dog, etc. The cloning technology makes possible cloned animals as well as transgenic animals, which are harboring or knocked out some special gene(s). Somatic cell NT makes up deficits of the former transgenesis and increase the transginc efficiency. Until now as transgeinc cloned animals, sheep expressing human clotting factor IX in mammary grand (1997, PPL), cattle expressing b-galactosidase (1998, University of Massachusetts), PrP (prion) gene knockout sheep (2001, Roslin), etc were reported by using NT technology. The first cloned pigs derived from somatic cell NT were made by PPL (Polejaeva et al., 2000). Pig cloning was succeeded latter than other livestock because the research history of pig embryo was shorter than other species, and pigs have a physiological characteristic that at least 4 fetus have to be implanted in uterus to maintain pregnancy. the writer of this article has successfully produced the first transgenic cloned pigs expressing green florescent protein ('golden pigs', Park et al., 2001). The birth of golden pigs open the window to develop special pigs for biomedicine (therapeutic protein) and bio-organ (xenotransplantation, organ donor for human). Here I introduce three tree kinds of transgeinc cloned pigs with special genes; green florescent protein (GFP), human granulocyte-macrophage colony stimulating factor (hGM-CSF), and human leukocyte antigen-G1 (HLA-G1).

Cloned Pigs Expressing Green Florescent Protein (GFP)

Various cell types in higher multicellular organisms are genetically homogenous, but are functionally and morphologically heterogeneous due to the differential expression of genes during development. Here, we show that dynamic changes in histone modifications and DNA methylation in the upstream coding region of a gene containing the transcription initiation site determine the tissue-specific gene expression pattern. These findings indicate that dynamic change of histone modification and DNA methylation is potentially important in the establishment and maintenance of tissue-specific gene expression.

Human Granulocyte-Macrophage Colony Stimulating Factor (hGM-CSF) Transgenic Cloned Pigs

The developmental potential of porcine cloned fetal fibroblast transfected with the hGM-CSF was evaluated. Cloned fetal fibroblasts were isolated from a recipient after transplantation of NT embryos. Two of these recipients delivered seven healthy female piglets. The presence of the hGM-CSF gene was confirmed by polymerase chain reaction and fluorescent in situ hybridization analyses. These results demonstrate that somatic cells derived from a cloned fetus can be used to produce recloned and transgenic pigs.

Transgenic Cloned Pigs with Human Leukocyte Antigen-G1 (HLA-G1)

Natural killer (NK) cells constitute a principal component of the cellular response in xenotransplantation. The expression of HLA-G1, which has been shown to inhibit the cytotoxicity of NK cells, has been proposed as a potential solution for the circumvention of NK cell-mediated xenogeneic cytotoxicity. We stably transfected the HLA-G1 gene into a normal mini-pig fetus cell line, and established HLA-G1-expressing NT-donor cell lines. Expressing transgenic mini-pig fetus and transgenic mini-piglets were generated via nuclear transfer, using NT-donor cell lines. Using FACS and immunohistochemistry, HLA-G1 expression was evaluated in the mini-piglets.

We have generated three kinds of cloned transgenic pigs. The cloning and transgnic technology would be useful to develop special animals for biomedicine and bio-organ as well as human disease models.