Transgenic Animal Model in Reproductive Medicine

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Summary

Transgenic animal technology has provided new opportunities in many aspects of biotechnology and medicine during two decades. Several gene delivery systems including pronuclear injection, retroviral vectors, sperm vectors, and somatic cell cloning have been tried to generate new functional animals. In the future somatic cell cloning technology will be a major method in the transgenic animal production. Many factors enhancing overall transgenic efficiency should be overcome to facilitate the industrial applications of transgenic technology. Transgenic animal technology has settled down in some areas of the medicine, especially the mass production of pharmaceutical proteins and xenotransplantation. Thus, animal biotechnology will contribute to welfare of human being.

INTRODUCTION

Transgenic animals have been defined as animals that have integrated foreign DNA into their germline as a consequence of experimental introduction of DNA (Palmiter & Brinster, 1985). Production of transgenic animals using various gene delivery systems has been demonstrated. The most commonly used methods include pronuclear injection, retroviral vector, use of embryonic stem (ES) cells, sperm vector and somatic cell nuclear transfer. Transgenic animals from various mammals including mice, rabbits, goats, sheep, pigs and cattle have been produced. However, the efficiency often varies between species and methodology, although numerous approaches have been used to improve transgenic efficiency. Nuclear transfer technology using mammalian somatic cells will greatly extend the efficiency of transgenic animals. A variety of transgenic animals including mice, cattle and Korean Native goats have been generated and nuclear transfer technology using transformed somatic cells is under development in our laboratory. Here the methods of gene transfer and applications of transgenic animals in the medicine are described.

Generation of transgenic livestock

Several methods have been developed to introduce genetic materials into the developing mammalian embryo in such a way that it may become stably integrated into chromosomes of the animal.

Pronuclear Injection

Pronuclear injection is to introduce DNA into one of the pronuclei of a zygote by using a micromanipulator. Pronuclear injection is the predominant method for creation of transgenic animals, although it is labor intensive and requires a high level of technical skills and experience in embryo manipulation. The process of making transgenic farm animals by pronuclear injection is almost exactly the same as that used to produce transgenic mice. However, the major problem of pronuclear injection is the low transgenic rate in large animals as compared to mice (Wall, 1996). The efficiency in producing transgenic cattle is at least 40-fold lower than that of mice. Another problem of pronuclear injection is mosaicism of transgenic founders. The number of mosaic transgenic mice represents approximately 15% of the total, but this may be an underestimate since many investigators have not reported frequencies of mosaicism among their transgenic mice. After microinjection of DNA, a high percentage of mosaic mouse embryos is detected in early cleavage stages (Burdon & Wall, 1992). Whitelaw et al. (1993) demonstrated that at least 62% of integrations following DNA microinjection resulted in mosaic embryos. Eyestone (1999) reported that 7 of 8 transgenic founder cattle passed their transgenes to the embryos at low transmission rates of less than 30%, showing varying degrees of mosaicism. Mosaic transgenesis is common due to late integration after the first round of zygotic DNA synthesis. In bovine zygotes, pronuclei do not become visible by Normarski optics 16 to 18 h post-insemination when DNA replication is already in progress (Wall, 1996). Therefore, most of the zygotes in the present work are injected during DNA synthesis, increasing the probability that the resulting transgenic offspring will be mosaic and transmit their transgenes only to 25% of their offspring. Despite these limitations, pronuclear injection has several advantages over other gene delivery systems. It is considered a safe and reliable method for gene transfer. Transgenic animals have been consistently produced by pronuclear injection in a variety of species, although there is a great variation in the transgenesis frequency between species. Schedl et al. (1993) reported the production of transgenic mice carrying a yeast artificial chromosome (YAC) ranging in size 350 to 460 kb. Thus, there is no size limitation of DNA fragment being injected (Brem et al., 1996). Another advantage is that the integration efficiency has no apparent correlation with DNA length (Brinster et al., 1985).

Retroviral Vectors

Retroviruses have a single-stranded RNA genome, which after infection of the host cell is reverse-transcribed into DNA and integrated into a host chromosome in a singe copy. Retroviruses can be genetically modified to act as vectors allowing the infection of embryos with exogenous DNA (Jaenisch et al., 1975). Superficially, this is an attractive option as the process is relatively efficient. The major physical limitation of retroviral vectors is their size. A maximum of 10 kb of retroviral sequences can be efficiently reverse-transcribed and encapsidated to form an infectious particle. Another drawback is that transgenic mice produced by retroviral infection are almost mosaic because infection occurs after several cleavages. Kim et al. (1993) demonstrated successful infection of bovine

preimplantation embryos by replacing the endogenous MoMLV envelope glycoprotein with that of the gibbon ape leukemia virus. When retroviral vectors were infected into bovine oocytes arrested at metaphase II (MII) of the second meiotic division before fertilization, all the offspring born from infected oocytes were transgenic (Chan et al., 1998). These transgenic animals showed germline transmission and transgene expression. Thus, retroviral vector systems provide a very promising method for producing genetically modified animals.

Sperm Vectors

The use of spermatozoa as noninvasive delivery vesicles to transfer exogenous DNA into oocytes during in vitro fertilization has provided an ideal alternative in generation of transgenic animals (Lavitrano et al., 1989; Spadafora, 1998). Due to its relative simplicity compared to pronuclear injection, many researchers have attempted to create transgenic animals by sperm vector. So far, offspring have been produced in several species including mice (Lavitrano et al., 1989; Maione et al., 1998), pigs (Sperandio et al., 1996), fish (Khoo et al., 1992) and cattle (Schellander et al., 1995) by using this method. The success of sperm transformation depends on the binding efficiency of DNA and its subsequent internalization into sperm nuclei. To improve the binding and internalization of DNA, different methods including the use of liposome (Bachiller et al., 1991), the direct injection of DNA complex into seminiferous tubules or vas deferens (Kim et al., 1997; Huguet & Esponda, 1998), and the electroporation of spermatozoa (Gagne et al., 1991). Interestingly, Perry et al. (1999) reported an adaptable method of transgenesis that exogenous DNA could reproducibly be delivered into an oocyte by microinjected spermatozoa. Although only limited success has been demonstrated, sperm vectors still hold great promise for future transgenic technology.

Somatic Cell Cloning

The successful production of offspring derived from nuclear-transferred (NT) embryos using somatic cells in mammals has important implications not only for multiplication of valuable domestic animals, but also for elucidation of genomic totipotency of donor nuclei. Fetal and adult somatic cells have been used to produce animals after nuclear transfer using electrical fusion or intracytoplasmic injection of nuclei into oocytes. Consequently, cloned several species animals including sheep (Wilmut et al., 1997; Schnieke et al., 1997), goat (Baguisi et al., 1999), mouse (Wakayama et al., 1998) and cattle (Cibelli et al., 1998; Kato et al., 1998; Wells et al., 1999; Brink et al., 2000) have been successfully produced by somatic cell nuclear transfer. The major obstacle for the application of somatic cell cloning remains its low efficiency. Intensive studies on remodeling/reprogramming of transferred nuclei, nuclei synchronization of donor cells or oocytes, poor development of NT embryos, low pregnancy and telomere restoration will improve the cloning success. Nonetheless, cloning from transformed cells has other advantages for making transgenic animals (Anderson and Seidel, 1998). The production of transgenic animals by nuclear transfer method using transformed somatic cells showed 100% efficiency (Schnieke et al., 1997; Cibelli et al., 1998; Brink et al., 2000). These results suggest the future place of cloning technology for the production of transgenic animals. Thus, the enhanced transgenic

efficiency must be achieved by cloning technology using somatic cells.

Applications of transgenic animal technology in the medicine

Transgenic technology has the possibility for diverse applications of research and industry. It has been used for studying the mechanisms of developmental regulation of gene expression (Rossant & Joyner, 1989), the identification of new genes (Jaenisch, 1988), the production of animal models of human disease (Hooper, 1990), the production of large amount of specific proteins through mammary gland (Clark et al., 1987), the modification of milk compositions (Wilmut et al., 1990), the enhanced growth performance (Pursel et al., 1989), the development of transgenic animals for xenotransplantation (Hammer et al., 1998) and so on. Here we would like to focus on transgenic animals to produce human pharmaceutical proteins and modify genetically pig organs for xenotransplantation.

Transgenic Animals as Bioreactors

A proposal that mammary specific expression of human genes might be exploited for the production of recombinant proteins in the milk of transgenic animals was first suggested by Clark et al. (1987). To direct tissue specific gene expression, structural genes (cDNA or genomic) should be connected to mammary specific promoter sequences such as caseins, whey acid protein (WAP) and lactoglobulin. Transgenic animals named as animal bioreactors have many benefits as compared to conventional production systems through microorganisms or animal cells (Janne et al., 1992; Bremel, 1996). The advantages include high productivity, low operating costs, appropriate post-translational modification of proteins, and that most transgenic animals can give rise to their transgenic progeny. Since the feasibility of genetically engineering animals to produce human proteins in milk has been established, the generation of transgenic farm animals expressing human proteins have been reported (Ebert et al., 1991; Wall et al., 1991; Wright et al., 1991; Krimpenfort et al., 1991). In 1996 and 1998, we also produced transgenic cattle with human lactoferrin gene and transgenic goats carrying human granulocyte-colony stimulating factor (G-CSF) gene, respectively. However, there is a limiting phenomenon in attaining high expressing transgenic lines for commercial production. The level of transgene expression is mainly affected by integration site into the genome, so-called position effect. Unfortunately, there is no control over the site of integration of an injected gene. Thus, the generation of a high expressing line is basically a numbers game. Products from transgenic milk have made considerable progress towards the market. Now, PPL Therapeutics and Genzyme Transgenic Corporation have recombinant -1-antitrypsin (AAT) and human antithrombin III (ATIII) from transgenic milk in phase III clinical trials, respectively. It is expected that various pharmaceutical proteins such as growth hormones, cytokines and antibodies will be produced in the milk of transgenic animals in the future.

Transgenic Animals for xenotransplantation

The grafting of living organs, tissues or cells between members of different species, whether genetically modified or not, is called xenotransplantation. Such transfer of animal organs to humans appears on the horizon as a last resort to solve the severe shortage of transplants of human origin for patients

suffering from end-stage disease. The remaining alternative is to use an animal similar in size to human, readily available, without the danger of infectious diseases. The domestic pig is found to possess several advantages except immunological and physiological incompatibility. Porcine and human organs have approximately the same size and a similar efficiency. The most serious huddle to xenotransplantation is immediate destruction of xenografts by hyperacute rejection (HAR). The histology of HAR is characterized by extensive intravascular thrombosis and extravascular hemorrhage. The general principles of genetic modification to promote xenotransplantation are to reduce the expression of the additional antigens and to correct the molecular incompatibilities responsible for dysregulation of the immune response. An efficient approach to mitigate HAR is to express human complement regulator proteins such as human decay-accelerating factor (h-DAF) or membrane cofactor protein (MCP) on the surface of the xenogenic donor cells (Rosengard et al., 1995). Orthotopic transplantation of h-DAF transgenic pig hearts to juvenile baboons ended at a maximal time of 10 days (White, 1996). With vigorous immunosuppression, transgenic pig organs have survived in primates for up to 60 days without evidence of rejection (Waterworth et al., 1997). Another approach to alleviate HAR has been to eliminate the galactosyl transferase gene by homologous recombination (Sandrin et al., 1994). Although homologous recombination is not available in the pig, it is possible to insert transgenes into the pig genome. Sandrin et al. (1995) suggested the idea of competing with the -galactosyl transferase in pigs by expressing the -1,3 fucosyl transferase (H-transferase) gene that humans use to form the blood group O antigen. Recently, it has been reported that -galactosyl transferase gene could be knocked-out in pig fibroblast cells (Polejaeva & Campbell, 2000). Thus, the knocked-out pigs will be generated in near future by somatic cell nuclear transfer.

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