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Review article

ANIMALS BIOTECHNOLOGY: BIOMEDICAL RESEARCH USING TRANSGENIC ANIMALS AS MODELS

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Abstract Animal model which closely mimic human biology are instrumental for the understanding disease mechanisms as well as for the development of new preventive or therapeutic treatment. Classically, such animal's models have been selected from spontaneous mutants occurring in breeding colonies of laboratory animals or livestock. Today, transgenic techniques offer possibilities to change the physiology of animals and therefore to experimentally generate precise models for biomedical research such as human genetic, metabolic, nutrition or infectious disease. **Chiang Mai Veterinary Journal 2005;3:71-79.**

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Introduction

Transgenic animals carry experimentally introduced cloned DNA stably integrated in their genome. The foreign DNA sequences can be functional genes, called transgenes or sequences designed to interrupt the correct expression of an endogenous gene. Structural genes can be expressed in transgenic animal either under the control of their own regulatory sequences, or under the control of heterologous regulatory sequence. The regulatory sequences present, enhancers and promoters, will determine the stage and cell type specific expression of particular structural gene. Thus, the expression of any defined gene product can be targeted to a tissue of choice.[1-2] The aim of using animal models in biomedical research is to reconcile biologic phenomena between species. Traditional mammalian models in biomedical research include laboratory animal; such as mouse, rat, mice, guinea pig or rabbits also farm animals; for example, swine, cattle, sheep or goats.

The present of short review focus on approaches used in generate mammalian animal models for biomedical research such as basic research, model of diseases, pharmaceutical production and

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xenogenic cells tissues and organs. It will not attempt to give a complete list of all proposed particular models, but emphasis will be placed on principles and recent developments.

Method for introducing genes into animals

In recent year, several techniques have been report to produce transgenic animals. Those include: 1) pronuclear microinjection, the direct microinjection of recombinant DNA into one of the pronuclei of the fertilized mammalian egg;[3-4] 2) the transduction of foreign DNA by retroviruses or retroviral vectors into embryos at various stages of development;[5] 3) the use of genetically transformed embryonic stem (ES) cells as vehicles;[6] and 4) sperm mediated gene transfer.[7]

1. Pronuclear Microinjection

The pronuclear microinjection is method of producing a transgenic animal result in the introduction of few hundred copies of the recombinant transgenes into one of the pronuclei of the fertilized mammalian egg. This technique is currently the most widely applied method for introducing genes into animal. The main advantage of this method is reproducible efficiency and its applicability to many mammalian species. With mice, the overall efficiency (transgenic animals/oocyte injections) is in the range of a few percent.[3] The overall efficiency of microinjection of larger farm animal embryos is somewhat lower, probably mainly due to difficulties in visualizing the pronuclei and due to suboptimal *in vitro* culture condition. Nevertheless, several investigators have successfully produced transgenic rabbits[8-10], sheeps[9,11], pigs,[8-9] goats[12-13] and cows.[14-15]

After microinjection of cloned DNA into one of the pronuclei usually multiple DNA copies integrate in a head-to-tail arrangement at one random chromosomal site into the host genome. If integration takes place into the genome of the zygote, the result animal will carry copies of the transgenes in all its cells, including the germ cells. The stably integrated copies of newly acquired DNA are presented in a hemizygous condition and therefore transmitted to fifty percent of the offspring of a transgenic founder animal. By interbreeding hemizygous first generation carrier siblings, homozygous transgenic animals may be obtained in the second generation. Occasionally, homozygous animals cannot be obtained, because the integration of the transgenes has interrupted an endogenous gene essential for the normal embryonic or fetal development.[16] If the integrated transgenes comprise functional regulatory sequences, they will be expressed. Phenotypical effects of the transgenes expression are inherited as dominant traits.

2. Retrovirus mediated gene transfer

The next step in the evolution of transgenic technology was accomplished by the infection of preimplantation mouse

embryo with retrovirus[2] but, the use of retroviruses and retroviral vectors has not found widespread application. Retroviruses are nature's natural gene delivery system. In a single protein package comes nucleic acid that can redirect a cell's synthetic machinery to express the viral genes as well incorporate the viral genome into the host cell genome. This is mainly due to the size limitations for transuded DNA as well as to the unresolved problems of the reproducible expressing a transuded eukaryotic gene. The viral information was successfully transferred into the genome of the recipient animal, and the technique of utilizing retroviruses as vectors for specific foreign DNA sequences was soon developed.[17-18] Recently lentivirus constructs have been made and used to infect embryonic tissue resulting in the generation of transgenic rat and mice.[19] The uses of retroviruses for gene transferring are concerns about the reactivation of retrovirus causing a viral infection and the activation of oncogenes making the transgenic animal more susceptible to the development of tumors.

3. Embryo Stem Cell (EScell)

In recent years, several techniques have been reported to produce transgenic animals. These include ES cell mediated techniques.[6] A rapidly evolving method to produce transgenic mice makes use of ES cells as carriers of recombinant DNA into the early embryo. ES cells lines are derived from explanted blastocysts. They retain their normal karyotype and their pluripotent embryonic character even after DNA transfection (viral transduction, calcium phosphate precipitation, electroporation or microinjection). When such cells are placed back into a carrier blastocyst, they can colonize the developing embryo and contribute to the germ-line of a resulting chimeric organism.[6] Functional ES cell-derived germ cells will transmit the *in vitro* mutated genome to the next generation. So far, ES cells have been obtained from mice and hamster only. Therefore, this approach is not yet applicable to other mammalian species

An advantage of this methodology is the possible selection of appropriately transformed cells prior to the introduction into the host embryo. In addition, the use of ES cells is attracting increasing attention because they allow the application of methods to target mutations to selected genes by homologous recombination. Recently developed ingenious selection system[20] and powerful screening method,[21] make it possible to rescue and identify the few rare cells which have undergone homologous recombination of an endogenous target gene with a transfected mutant copy.

4. Sperm Mediated Gene Transfer

The use of sperm to deliver the transgenic material to the oocyte during fertilization, initial experiments have involved incubating the sperm with the DNA then using in vitro fertilization techniques to transfer the DNA into the oocyte during fertilization.[7] To increase the efficiency

of sperm uptake of DNA various approaches are being taken. One is to attach the recombinant DNA to the sperm head via an antibody fused to the DNA.[22] Another approach has been to place the DNA inside the sperm head by electroporation[23-24] or lipofection.[25-26] This method has the advantage that there is no manipulation of sperm and fertilization occurs via natural means.[27]

Trangenic animals for basic research

The generation and analyses of transgenic animals carrying different constructs that lead to different phenotypes will be among the initial steps to the understanding of the relationship between different genes and the role of each one in the development of the organisms. The more recent approach consists of systematically knocking out laboratory animal's genes without any particular hypothesis on their function using RNA interference.[28-29]

Transgenic animals as model of diseases

Genetically modified laboratory animals provide a powerful approach for studying gene expression and regulation, and allow the direct examination of structure-function and cause-effect relationships in pathophysiological processes.[30] Various approaches have been used to experimentally generate potential disease models. The most straightforward approach is the expression of a transgenes in order to cause an elevated level of the gene product either in the circulating plasma or in a particular cell type. It is most widely used application consists in the tissue specific expression of oncogene encoded regulatory proteins, which has led to a variety of transgenic mouse lines exhibiting the constitutive development of particular tumor.[1-2] Similarly, by introducing the human Cu/Zn-superoxide dismutase gene into the genome of mice, the level of functional enzyme could be increased up to six fold in the brain and other tissues.[15] Such transgenic mice may therefore provide insight into the consequences of increased dosage of the Cu/Zn-superoxide dismutase gene in Down syndrome.

The severe, inherited human disease perinatal lethal osteogenesis imperfects has been reproduced in mice by inserting and expressing a mutated pro-alpha1 type I collagen gene.[31] The expression of as little as 10% mutant collagen in transgenic fetuses resulted in a dominant lethal phenotype due to a reduced type I collagen content. This observation suggests that the presence of nonfunctional subunits may severely inhibit the correct assembly of multimeric structures. This should allow the study of the consequences of inappropriate assembly of other collagens or of cytoskeleton proteins, such as actins or intermediate filaments. Although antisense RNA (RNA strand which complementary to messenger RNA) has been shown to effectively repress the expression of specific gene of *in vitro* cultured cells at the translational level, with transgenic mice only one successful attempt has been reported: by co-express-

ing an antisense basic myelin protein minigene in myelin producing cells, the myelination in the central nervous system of the transgenic mice was significantly reduced, resulting in shiverer phenotypes.[32] Using mutant ES cells, two groups have reported the successful introduction into mice of deficiencies for the X-linked gene encoding hypoxathin-phosphoribosyltransferase (HPRT).[33-34] A deficiency of this enzyme in humans causes severe neurological disorders, described as Lesh-Nyhan syndrome. Although the syndrome is well understood in terms of molecular genetics, little is know about how the disorder develops; no preventive treatment is available. Surprisingly, the HPRT-deficient mice do not represent a model for the human disease, because mice seem to use different pathways of purine metabolism and do not exhibit the typical neuronal damage observed in human patients. Nevertheless, these experiments clearly demonstrate the potential of the use of the ES cells for the introduction of specific mutations into mice.[35-36]

Transgenic animals for pharmaceutical production

Another use for transgenic animals involves the biological production of valuable human proteins, enzymes, hormones and growth factors. Transgenic animals such as cattle, sheep, goats and pig have several significant advantages for the production of recombinant protein over other systems, including their potential for large-scale production, correct glycosylation patterns and post-translational modifications, low running costs, rapid propagation of the transgenic founders and high expression stability.[37-38] The most promising site for production of recombinant proteins is the mammary gland because of the quantities of protein that can be produced and the ease of extraction or purification of the respective protein, but other body fluids including blood, urine and seminal fluid have also been explored.[39] Large amounts of numerous heterologous recombinant proteins, hormones, growth factors, enzymes, blood factors, vaccines, antibody or structural proteins have been produced by targeting expression to the mammary gland via mammary gland-specific promoter elements and the biological activity of the recombinant proteins was assessed and therapeutic effect have been characterized.[37] The enzyme α-glucosidase from the milk of transgenic rabbits has orphan drug registration and has been successfully used for treatment of Pompe's disease. This is a rare glycogen storage disorder, which is fetal in children under 2 years and currently application with recombinant α-glucosidase is the only way to treat this metabolic defect.[40] Biologically active human lactoferrin has been produced in large amounts in the mammary glands of transgenic cows and will probably be developed as a biopharmaceutical for prophylaxis and treatment of infectious disease.[41]

Xenogenic cells tissues and organs

Another promising area of application

for transgenic animals will be the supply of xenogenic cells, tissues and organs. Xenogenic cell therapy has been advanced to preclinical studies. Porcine islet cells have been transplanted to diabetic patients and were shown to be at least partially functional over a limited period of time.[42] Bovine neuronal cells were collected from transgenic fetuses, and when transplanted into the brain of rats resulted in significant improvements in symptoms of Parkinson's disease.[43] Furthermore, porcine or bovine fetal cardiomyocytes or myoblasts might provide a therapeutic approach for the treatment of ischemic heart disease. Similarly, xenogenic porcine cells might be valuable for the repair of skin or cartilage damage.[44] Porcine xenografts are considered the solution of choice in transplantation of an appropriate human organ because porcine anato-my and physiology are not different from that human, the organs are similar in size to human organs, pigs grow rapidly and have short reproduction cycles with large litters and transgenic techniques for modifying the immunogenicity of porcine cells and organs are well established.[45-46] Further improvements in the success of xenotransplantation will arise from the possibility of inducing a permanent tolerance across xenogenic barriers.[47] A particularly promising strategy for long term graft acceptance is the induction of a permanent chimerism via intraportal injection of ES cells.[48]

Conclusions

Transgenic animal systems combine the virtues of cell culture and congenic breeding strategies while avoiding the negative aspects of each system. Using transgenic techniques, a characterized genetic sequence may be evaluated within the specific geomic background of the whole animal. Therefore, transgenic animals may be utilized to study the regulation of specific genetic sequence in a realistic fashion. However, researchers continually identify or develop new animal models to evaluate genetic disorder, metabolic and nutrition metabolic disease, diagnostic and therapeutic procedures, pathogenic mechanism and the efficacy of novel drug development. There are also contributing data to studies of aging and disease processes of humans and animals.

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