

Genetically Manipulated Animals and Their Use in Experimental Research

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Abstract

Genetically manipulated animals generated by transgenic and gene-targeting (knockout) technology have contributed tremendously to our understanding of gene function and regulation at the molecular level in the context of the whole organism. They are also becoming powerful tools for human disease studies and therapeutic drug development. An overview of the current development of these animals and their application in biological and medical researches is provided in this article.

Ann Acad Med Singapore 1999; 28:560-4

Key words: Animal models, Gene targeting, Genetic engineering, Knockout, Transgenic

Introduction

Animals have long served as experimental models for studying human biology and diseases. With the biomolecular revolution in the past three decades, genetically manipulated animals are playing increasingly important roles in our efforts to create and expand the various animal models for experimental research. The term “genetically manipulated animals” here refers to those animals whose genetic material (DNA) have been permanently altered either by inserting new gene(s) or by deleting certain endogenous gene(s) through genetic engineering methods to create a genotype of experimental choice. These genetically altered animals include transgenic animals and gene-targeted animals (knockout animals). They are already having a profound impact on biological and medical research. They do not include the different animal strains that are obtained through many generations of inbreeding or forced cross breeding. In this short review, the author will first introduce how transgenic animals and knockout mice are created. Then, a few examples are given on how these genetically manipulated animals can serve as animal models to help us understand the basic human biology and diseases as well as in developing therapeutic tools to treat human diseases.

Transgenic Animals

Transgenic animals are animals that have had their genomes altered by injecting or infecting the embryos with extra copies of a gene. To date, transgenic animals

from many different species have been created including transgenic mouse, rat, pig, cattle, sheep, chicken, *Xenopus* (frog), fish, *Caenorhabditis elegans* (nematode worm), and *Drosophila melanogaster* (fruit fly). Amongst these, transgenic mice make up more than 90% of all transgenic animals. This is mainly because the house mouse has been an established experimental model mammal, with well-established genetics and many isolated genes. The first transgenic mouse strain was generated in 1976 by infecting the mouse embryos with infectious Moloney leukemia retrovirus (M-MuLV).¹ Infecting mouse embryos with retroviruses constitutes one of the methods to generate transgenic mice. Currently, the most widely and successfully used method for generating transgenic mice is to directly inject recombinant DNA into the pronucleus of the fertilized egg.^{2,3} The injected DNA then stably integrates into the host chromosome by a mechanism that is not very well understood. The mouse that has integrated the foreign gene is called the “founder” mouse. Subsequent breeding from the founder mice can generate a homozygous transgenic mouse line with every single cell of the mouse carries the transgene (Fig. 1). Traditionally, DNA with a size of up to 50kb can be readily introduced into transgenic mice. With the recent technology development in genome research, yeast artificial chromosomes (YACs) carrying huge human genes (a few hundred kb) have been successfully introduced into transgenic mice.⁴ These YAC transgenic mice hold great promises for human disease studies as a lot of human genes are very large.

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Gene-targeted (Knockout) Animals

Animals with their genomes altered by replacing a designated normal gene with a defective copy of the gene are called targeted mutations, null mutations or knockouts. This is accomplished by homologous recombination of chromosomal and extrachromosomal DNA sequences at a specific chromosomal site. Currently, only knockout mice can be efficiently generated in higher vertebrates. To introduce a planned alteration of the mouse genome, mouse embryonic stem cells (ES cells) are first manipulated by *in vitro* cell culture. Cells containing the desired alteration are injected into recipient blastocysts. These blastocysts are reimplanted into pseudo-pregnant foster mothers. Chimeric pups generated in this way are then crossed to generate a pure heterozygote. Homozygotic gene-targeted mice will then be generated by crossings between the heterozygotic mice (Fig. 2).^{5,6}

Applications of Genetically Manipulated Animals

Gene Function Studies

Genetically manipulated animals have contributed greatly to our understanding of gene function and regulation in the context of the whole organism. By analysing the pathophysiology of transgenic animals over-expressing a particular gene product or knockout mice missing a gene product, the function of the gene can be deduced. Furthermore, by following the temporal and spatial expression of the transgene products, we can obtain the knowledge of how the particular gene is being regulated *in vivo*. This has greatly enriched our knowledge of genes at the molecular level and provided the fundamentals for elucidating complex biological processes such as human diseases.

Human Disease Studies

It is now possible to design animal models for a certain disease through the introduction of a disease-related gene *in vivo* by molecular biology techniques, making it unnecessary to depend on spontaneous mutants. Many useful transgenic and gene-targeted animals have been created to mimic human disease conditions and serve as models for human disease studies. These engineered diseases are a departure from previous animal models in that pathological syndromes are created from an assumption about how a disease pathogenesis could develop. For an animal model of a human disease to be truly useful, the physiological and pathologic phenotype which resembles that in human must have a genetic cause identical to that in human.⁷ For example, transgenic mice carrying the human poliovirus receptor gene (PVR) was produced in 1991.⁸ These mice were found to be susceptible to poliovirus infection, which essentially develops only in humans and monkeys. The various lines of the transgenic PVR mice were then accessed for

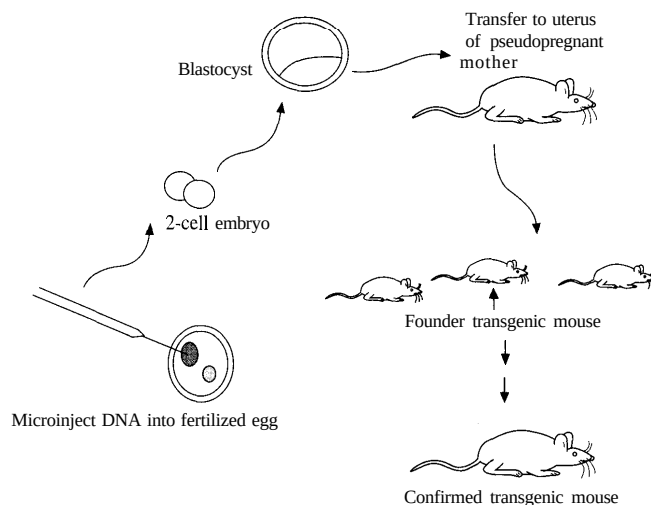


Fig. 1. An outline of a common procedure of generating transgenic mice.

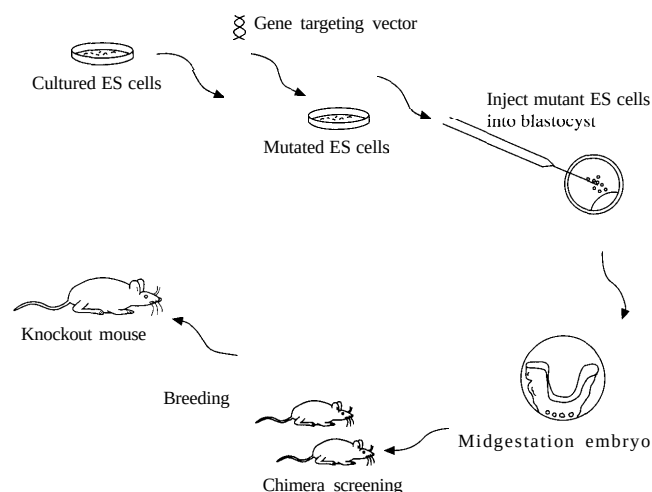


Fig. 2. An outline of the method to generate knockout (gene-targeted) mice.

their usefulness as an experimental system for the neurovirulence test (NV test) of oral polio vaccine to replace monkeys.⁹ Two of the transgenic mice lines were found to be useful in oral polio vaccine NV test. To date, more than 12,000 transgenic PVR mice (line TgPVR21) have been used in comparative studies of polio oral vaccines to evaluate NV.⁷

Many efforts have also been made to develop a small animal model for HIV infection. The absence of a suitable animal model is one of the major barriers to the development of an effective therapy for AIDS. Although no transgenic mouse model has been generated to date that will provide an avenue for vaccine development, current transgenic HIV models have already provided tremendous insights into the pathogenesis of HIV as well as for the development of molecular-based thera-

pies.¹⁰ In a few instances, AIDS-related complications have been modelled successfully in transgenic mice that resemble aspects of HIV-induced disease in humans. For example, transgenic mice expressing the HIV-1 *nef* gene in T cells using T-cell specific promoter resulted in significant reduction of CD4+ T cells but not CD8+ T cells.¹¹ In another study, transgenic mice expressing *nef* in lymphoid cells resulted in severe immunodeficiency syndrome associated with thymic atrophy, splenomegaly, lymphadenopathy and increased mortality.¹² These results indicate that the HIV *nef* gene is most likely involved in causing immunodeficiency of the host. Although transgenic technology has not yet proven its utility for vaccine development, it holds the promise of being an active participant in the development of both safe and effective molecular therapy approaches for the treatment of AIDS.¹⁰

Transgenic mice have also been generated to mimic human Alzheimer's disease (AD). Two major histological characteristics of AD are amyloid plaques and neurofibrillary tangles (NFTs). Mice which are programmed for neuronal expression of the human 751-KDa β -amyloid precursor protein (β -APP) developed β -amyloid deposits in their brains as well as Alz50 immunoreactive neurons specific for AD patients.¹³⁻¹⁵ In addition, they also show age-dependent learning and memory impairment in the relative absence of general behavioural or other neurological deficits.¹⁶ These characteristics resemble the presenile structures observed in the early AD pathology. Recently, transgenic mice carrying a 650kb of YAC containing the human APP gene on chromosome 21 were generated.^{17,18} APP is an extremely large gene (about 400kb) that are expressed in a tissue and developmental stage specific manner. Thus, YAC transgenic animals which contain the complete genomic sequence of APP will allow the stringent testing of the roles of the AD mutations on amyloidogenesis in an animal model.⁴

Gene targeting by homologous recombination has also been extensively used to generate mouse models for human diseases. Currently, mouse is the only species in which gene-targeting (knockout) mutants can be created. Two mouse models of cystic fibrosis (CF) have been generated by targeted mutation of the CF gene *Cfr* in mouse.^{19,20} The two *Cfr*⁻/*Cfr*⁻ mice exhibit symptoms that are roughly similar to the symptoms observed in affected children. In most cases, these mice die shortly after birth. The strategy used for the production of the mouse CF models is applicable to all cases where a genetic disease is identified at the molecular level and the mouse homologous gene is cloned. For example, mice with sickle cell anaemia or various thalassaemias; mice with null mutation at the PPCA gene involved in the lysosomal storage disorder galactosialidosis have been produced.^{6,21} It is likely that many other similar models will be produced in the years to come for the

most prevalent human disorders.

Gene targeting also has its limitations. For embryonic-lethal phenotypes and systemic indirect dysfunction, knockout mice cannot be used to analyse adult pathophysiology. To circumvent these pitfalls, methods to achieve gene knockout in a regulatable or tissue-restricted manner are desired. The bacteriophage Cre recombinase has been used to generate site-specific recombination in a tissue-specific or inducible manner in transgenic mice.^{22,23} It is hoped that this new technology will eventually generate targeted null mutations at a specific developmental stage or a specific tissue of the mouse. More mouse models of human disease can be expected when this technology matures in the future.

Xenotransplantation

Xenotransplantation of pig organs into humans represents an attractive solution to the worldwide organ shortage. However, it is well known that, when transplanted into primates, pig organs are subject to a very aggressive immune reaction which ultimately leads to the hyperacute rejection of the graft.^{24,25} Research has shown that it is the binding of the naturally occurring cytotoxic xenoreactive antibodies (XNAs), mainly to the carbohydrate structure Gal α 1-3Gal epitope on the porcine endothelial cells and the activation of the recipient's complement cascade which lead to the hyperacute rejection.²⁶ At present, these are the primary immunological barrier to transplantation of pig organs into primates. Analysis of the immune response from patients who received porcine foetal islets or liver as a temporary support for hepatic failure confirmed that similar immune reactions occur in human as well.^{27,28} It is obvious that the interaction between XNAs and pig endothelial cells must be reduced if successful xenotransplantation of pig organs into humans is to be achieved. Knockout mice with defective α -1,3-galactosyltransferase have been generated. These mice bound substantial lower amount of human XNAs than normal mice and induced a lesser activation of the human complement.²⁹ However, the knockout technology is not yet available in pig. Future technology development to generate knockout pigs may overcome this barrier of xenotransplantation. An alternative genetic manipulation is to produce transgenic pigs which express the H-transferase which inhibits Gal α 1-3Gal antigen.²⁶ Transgenic pigs expressing human complement regulators decay-accelerating factor (DAF), MCI' or CD59 have been generated.³⁰⁻³² These transgenic pigs express high levels of the human proteins on the surface of their endothelial cells of all their organs.³¹ *In vivo* testing by heterotopically transplanting transgenic pig heart into immunosuppressed non-human primates showed much longer organ survival comparing to control hearts.³² In one case, the DAF transgenic pig hearts continued to beat for a median of 40 days.^{26,30}

Future technology development as well as a more complete understanding the function of genes involved in the human immune response to xenotransplanted pig organs will definitely help in realising the goal to use pig organs to replace a failing human organ. However, this may still take many years.

Bioreactors and Drug Factories

Transgenic animals function as drug factories to produce large quantity of human proteins for therapeutic applications is another exciting aspect of the application of these animals. Many human proteins are in great demand for therapeutic usage. For example, human blood proteins such as Factor VIII, Factor IX for haemophiliacs, tissue plasminogen activator for people suffering from strokes and heart attacks, and protein C for patients undergoing joint replacement surgery. All these proteins are present in donated human blood in tiny amount, and hence their production through purification of the human blood is extremely expensive and difficult. On the other hand, transgenic animals can be created to produce large amount of a particular human protein with only a fraction of the cost. To date, transgenic livestock including transgenic sheep, goat, cow and pig have been produced to produce various human proteins in their milk. For example, protein C transgenic pigs produced one gram of protein C in each litre of milk—200 times the concentration in normal human blood.³³ Transgenic livestock bioreactors by far outperformed the conventional huge stainless steel cell culture bioreactors. We anticipate that there will be great developments in this area in the near future.

Gene Therapy

Gene therapy, the use of gene transfer to treat diseases, excites the imagination and captures the public's attention because it promises at a single step almost magically to cure the previously incurable. The advancement of transgenic and gene-targeting technology has generated an enormous amount of knowledge for gene function and regulation. This in turn has stimulated the advancement of gene therapy technology for human diseases. Many clinical protocols have been developed in recent years and clinical trials have been started in many different human diseases. So far, gene therapy is essentially limited at the "gene complementation therapy" in which an active copy of the mutant gene (the "therapeutic gene") is introduced into the affected cells.⁶ Successful *ex vivo* therapy for severe combined immunodeficiency ADA- SCID patients and *in vivo* therapy of CF mediated by adenoviruses are two of the examples of the recent successes of gene complementation therapy.^{34,35} The other type of gene therapy, "gene correction therapy", in which the endogenous mutant gene is replaced by the "therapeutic gene" is not yet available.⁶ Recent reports

in using binary approaches with modified gene-targeting vectors and controlled expression of the non-mammalian recombinase in animals hold great promise for conditional gene-targeting in animals as well as human gene correction therapy in the future. However, clinical and basic researchers still have much to do before gene therapy can live up to its promise.

Conclusion

Genetically manipulated animals have proven to be powerful tools for basic biological research as well as therapeutic applications in medicine. These animals greatly expanded the variety of animal models available for human disease studies and therapeutic drug development.

Acknowledgements

The author would like to thank Dr Nataraja V Balasubramaniyan for his help in preparing this manuscript.

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