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Review article

TRANSGENIC ANIMALS AND THEIR APPLICATION IN MEDICINE

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ABSTRACT

Transgenic animals are animals that are genetically altered to have traits that mimic symptoms of specific human pathologies. They provide genetic models of various human diseases which are important in understanding disease and developing new targets. In early 1980 Gordon and co-workers described the first gene addition experiment using the microinjection technology and since then the impact of transgenic technology on basic research has been significant. Within 20 years of its inception, ATryn the first drug approved by USFDA from transgenic animals was developed and it has opened door to drugs from transgenic animals. In addition, they are looked upon as potential future donors for xenotransplantation. With increasing knowledge about the genetics and improvements in the transgenetic technology numerous useful applications like biologically safe new-generation drugs based on human regulatory proteins are being developed.Various aspects of concern in the coming years are the regulatory guidelines, ethical issues and patents related to the use of transgenic animals. This modern medicine is on the threshold of a pharmacological revolution. Use of transgenic animals will provide solutions for drug research, xenotransplantation, clinical trials and will prove to be a new insight in drug development.

Keywords: Transgenic animals, Xenotransplantation, Ethics, Patent.

INTRODUCTION

With the advent of transgenic technology and its application in many laboratories around the world, there is an increase in the generation and use of genetically modified animals in biomedical, pharmaceutical research and safety testing. This development has been additionally accelerated by the decoding the genome of man, mouse and rat.[1] Pharmaceutical companies are faced with the challenge that about 10% of compounds tested in clinical trials make to the market and, out of these, a minority will generate significant profit. The cost

of identifying new drug is immense, about United States (US) $ 800 million and 80% of this cost is spent in clinical trials and development. Transgenic technology has potential to influence the attrition rate in pharmaceutical research by increasing the quality of both targets and compounds. [2]

To date most of the medicines are synthetically produced and will continue in future. However, the challenge for the pharmaceutical industry is the development of 'Biotech medicines' which include therapeutic proteins such as enzymes and antibodies. [3] The global market for recombinant proteins from domestic animals is expected to exceed US$1 billion in 2008 and reach US$18. 6 billion in 2013.[4] The two major animal systems of production are pharmaceutical proteins in milk and egg white from transgenic animals. [1]

Since the first transgenic mice were generated in 1982, transgenic animal models have been used extensively to investigate biomedical important mechanisms underlying a variety of diseases, to develop and evaluate new therapies. [5] Thus transgenic animals have the ability to fulfill the needs of the pharmaceutical industry and in coming years they are looked as potential contributors to the drugs and research in medicine.

Making of Transgenic Animals

There are three types of laboratory animal models mentioned in the literature. They are spontaneous, induced and transgenic. Spontaneous models shape up as a result of naturally occurring mutations. Induced models are produced by a laboratory procedure like administration of a drug or chemicals, feeding of special diets or surgical procedure. The third category includes transgenic models.[6]Transgenic refers to insertion of cDNA (complimentary deoxyribonucleic acid) made from specific mRNA (messenger ribonucleic acid) into cells.[7] A transgenic animal is defined as an animal which is altered by the introduction of recombinant DNA through human intervention. [8]

Following sequence is generally adopted for the development of transgenic animals irrespective of species: [1, 9]

- Identification and construction of the foreign gene and any promoter sequences
- Introduction of DNA directly into the pronucleus of a single fertilized egg by various methods
- Implantation of these engineered cells into surrogate mothers
- Bringing the developing embryo to term, proving that the foreign DNA has been stably and heritably incorporated into the DNA of at least some of the newborn offspring.
- Demonstrating that the gene is regulated well enough to function in its new environment.

The foreign DNA can be inserted into the pronucleus or cytoplasm of the embryo using microinjections or transposon. Other methods of DNA transfer are by lentivirus, sperms, pluripotent cells and cloning. The last three methods allow random gene addition and targeted gene integration via homologous recombination or gene replacement thus causing mutation. [1,6] Targeted mutation refers to a process whereby a specific gene (removal of a gene or part of a gene) is made nonfunctional (knocked-out) or less frequently made functional (knocked-in). [4, 7] A transgenic organism carrying more than one transgenes is known as multiple transgenic. [4]These methods do not create new species, but only offer tools for producing new strains of animals that carry novel genetic information. [10]

Transgenic Animal models of various diseases

An animal model is a living, non-human animal used for research and investigation of human disease, for the purpose of better understanding the disease without the added risk of causing harm to a human being during the entire drug discovery and development process. Transgenic animal models are created by the insertion of a particular human DNA into fertilized oocytes which are then allowed to develop to term by implantation into the oviducts of pseudo pregnant females.[6] There are

different models of transgenic animals for various diseases.

A. Human Immunodeficiency Virus/ Acquired Immunodeficiency Syndrome (HIV/AIDS):

Tg26 HIVAN Mouse Model was the first transgenic model developed in 1991 for HIV. Since than many models have developed, Rosenstiel et al gives summary of 32 transgenic murine HIVAN models developed. [11] These transgenic animals can express HIV-1 proteins; develop symptoms and immune deficiencies similar to the manifestations of AIDS in humans. [12] Other models are AIDS Mouse and Smart Mouse. [6]

B. Alzheimer's disease: No animal models existed for the disease before transgenic technology was employed. Immunization of Amyloid precursor protein A42 in transgenic mice showed that vaccination against Alzheimer's disease could have potential as a therapeutic approach.[2, 6] Different animal models like Alzheimer's mouse, amyloid pathology mouse models like PDAPP mice, Tg2576 mice, [13, 14] TAU transgenic mouse models like ALZ7 mice, 7TauTg mice and presenilin transgenic models like ApoE knockout are developed to study Alzheimer's disease. [13, 15]

C. Cardiovascular disease: Various transgenic animal models for gain and or loss of function of angiotensin, endothelin, natriuretic peptides, catechoalmines, Calcium binding-signaling, sodium channel transporters, and nitric oxide synthesis involved in cardiovascular regulation are used to study cardiovascular diseases.[16] Transgenic models of heart failure and hypertrophy like Gene overexpression of Calmodulin, Gene mutation of alpha cardiac myosin heavy chain and Knockout gene model of transforming growth factor are developed.[17] Mutation of the ApoE gene that is critical for uptake of chylomicrons and very low-density lipoprotein particles, results in a model that develops atherosclerotic lesions histologically similar to those found in humans.[2]

D. Diabetes Mellitus: Transgenic models are developed for studying the genes, and their role in peripheral insulin action. Models of insulin secretion such as glucokinase, islet amyloid polypeptide, and hepatic glucose production in type 2 diabetes are developed. [18]A transgenic mouse model that expressed Insulin Dependent Diabetes Mellitus by inserting a viral gene in the animal egg stage is also developed.[19] There are other models like beta receptor knockout mouse, uncoupling protein (UCP1) knockout mouse,acute and chronic models for the evaluation of antidiabetic agents. [18, 20, 21, 22]

E. Angiogenesis: Mouse models of angiogenesis, arterial stenosis, atherosclerosis, thrombosis, thrombolysis and bleeding addresses techniques to evaluate vascular development.[23] Inhibition of angiogenesis is currently one of the biggest opportunities for new cancer therapies. With the help of angiogenesis transgenic animal models inhibitors are identified which act on specific mechanisms of angiogenesis. [2]

F. Cancer diseases: Oncomouse was first transgenic animal to be patented. Its germ cells and somatic cells contain an activated human oncogene sequence introduced into the animal at an early embryonic stage to ensure that the oncogene is present in all the animal cells.[6] Mechanisms for tumor progression and metastasis via E-cadherin, and other adhesion molecules is possible by various transgenic knockout models. [7]

Transgenic animal models are used in the assessment of mutagenicity, carcinogenicity and tools for understanding metabolic enzymes and receptors. [24] There are transgenic animal models for mutagenicity assays approved by the World Health organization like LACItransgenic model (Big Blue® construct) and LACZ transgenic model (Muta™Mouse construct). [25]Variety of transgenic animals have been generated by different strategies in experimental immunotherapy of cancer, each aims to activate different immune system components. Some of them are transgenic rodent models expressing tumor associated antigens like MUC1 transgenic mice, Oncogene transgenic mice to study immunotherapeutic strategies, transgenic mice expressing immune effector cell molecules like Fc-receptor transgenic

mice [5] The preclinical transgenic model of Matrix Metalloproteinase (MMP) inhibitors studies the bioactive products of MMP and their possible effects on cell physiology. [26] Animal models for Huntington's disease, skeletal muscle disease and other diseases are also developed.[27, 28]

Disease models are needed in medicine so that one can discover the targets for drug development. Adding and deleting genes in these animals provide them new properties that make them useful for better understanding of disease or manufacturing a cure. It is not ethical or safe to perform the initial tests in humans, so transgenic animals are used. As the testing of new vaccines and drugs must first be performed on animals, these disease models are indispensable. [6]

Products from Transgenic Animals

Most transgenic species are studied for research applications as well as potential commercial pharmaceutical productively. Here are some of the transgenic animals and their products in development.

Goats: Monoclonal Antibodies (MAbs) , Ig fusion proteins, tPA (tissue Plasminogen Activator), ATryn (recombinant human antithrombin III) is the first transgenic recombinant protein from transgenic animal approved by the United States Food and Drug Administration (USFDA) in January 2009. [9,29]

Chickens and Eggs: vaccines; interferons, cytokines; Human Serum Albumin (HSA), insulin, MAbs. [9]

Pigs: organs for xenotransplantation, human hemoglobin, human protein C. [9,30,31]

Cows: Factors VIII and IX, protein C, recombinant antithrombin III (rATIII), recombinant HSA, and human milk protein. [9]

Mice: expression of malaria protein for vaccine development; MAbs, ATIII, beta interferon; cystic fibrosis transmembrane regulator; Factor X, HSA, tPA, myelin basic protein; prolactin; fibrinogen and antineoplastic urinary protein. [9]

Rabbits: recombinant human C1 inhibitor, human erythropoietin, human alpha antitrypsin, human interleukin 2, tPA, alpha glucosidase, and human growth hormone. [9]

Sheep: sheep milk includes fibrinogen (major constituent, with thrombin and Factor XIII) human Factor VII, Factor IX, activated protein C and alpha-1-antitrypsin. [9,31]

Other Species: Frogs, nematodes, and marine invertebrates have been used to study various promoter elements and gene transfer technology. [9]

Currently in most pharmaceutical companies, relatively large numbers of targets are validated to varying extents and progressed to the high throughput screening stage. The drugs acting on these targets are then used in clinical trials where the attrition rate is generally high, this makes it extremely costly. It is believed that the greater use of transgenic models could reduce the required throughput for achieving success and thereby significant impact on costs.[10]

Drugs from Transgenic Animals and Other Applications:

Proteins started being used as pharmaceuticals in the 1920s with insulin extracted from pig pancreas. In the early 1980s, human insulin was prepared in recombinant bacteria and is now used by most of the diabetic patients. This success was limited as bacteria cannot synthesize complex proteins such as monoclonal antibodies or coagulation blood factors that must be matured by post-translational modifications to be active or stable in vivo. These can be fully achieved only in mammalian cells which can be cultured in fermenters or used in living animals. Several transgenic animals can produce recombinant proteins but presently two systems started being implemented. The first is milk of transgenic mammals and the second system are chicken egg white. A variety of recombinant proteins which includes antibodies, vaccines, blood factors, hormones, growth factors, cytokines, enzymes, milk proteins, collagen, fibrinogen and others are being developed in transgenic animals. [1,3,31]

The mammary gland is the preferred production site, because of the quantities of protein that can

be produced in this organ and established methods for extraction and purification of these proteins.Products derived from the mammary gland of transgenic goats and sheep are ATIII, α-antitrypsin and tPA. ATIII is employed for the treatment of heparin-resistant patients undergoing cardiopulmonary bypass. The enzyme α-glucosidase from the milk of transgenic rabbits has been successfully used for Pompe's disease. [4, 31]

Blood, seminal plasma, urine, silk gland and insect larvae haemolymph are other theoretically possible systems. Blood most of the time cannot store high levels of recombinant proteins which are naturally unstable also biologically active proteins in blood may alter the health of the animals. Milk avoids essentially these problems and is presently the most mature systems to produce recombinant proteins from transgenic organisms. Now experiments validate egg white as a source of foreign proteins including recombinant vaccines. [1, 31]

Blood replacement

The current production system for blood products is donated human blood, and this is limiting because of disease concerns, lack of qualified donors, and regulatory issues. Genetically engineered animals, such as cattle carrying human antibody genes which are able to produce human polyclonal antibodies, have the potential to provide a steady supply of polyclonal antibodies for treatment of various infectious and medical conditions like organ transplant rejection, cancer, and autoimmune diseases and other diseases. [32]

There are currently at least 33 different drugs in clinical testing including several in pivotal trials that contain variable regions from transgenic mice encoded by human sequences. Also there are 17 therapeutic MAbs approved by the USFDA which are in different phases of drug development. [33]

Functional human haemoglobin has been produced in transgenic swine. The transgenic protein purified from the porcine blood showed oxygen-binding characteristics similar to natural human haemoglobin but only a small proportion of porcine red blood cells contained human form of haemoglobin. [4]

Xenotransplantation of porcine organs to human patients

Today more than 250,000 people are alive only because of the successful transplantation of an appropriate human organ (allotransplantation). However, progress in organ transplantation technology has led to an acute shortage of appropriate organs, and cadaveric or live organ donation does not meet the demand. To close the growing gap between demand and availability of appropriate human organs, porcine xenografts from domesticated pigs are considered to be the best alternative.[4]

Essential prerequisites for a successful xenotransplantation are: [4, 34]

1. Prevention of transmission of zoonoses
2. Compatibility of the donor organs in anatomy and physiology
3. Overcoming the immunological rejection of the transplanted organ.

The immunological hurdles are:

(a) Hyperacute rejection response (HAR) occurs within seconds or minutes.

(b) Acute vascular rejection occurs within days.

(c) Cellular rejection occurs within weeks after transplantation.

(d) Chronic rejection is a complex immunological process resulting in the rejection of the transplanted organ after several years.

Due to demand and unavailability of appropriate organs, xenotransplantation is considered as the solution of choice. The pig seems to be the optimal donor animal because their organs have a similar size as human organs, porcine anatomy and physiology is same and maintenance is possible at high hygienic standards at relatively low costs. [34, 35]

Two main strategies have been successfully explored for long-term suppression of HAR, the knockout of α-gal epitopes which are the antigenic structures on the surface of the porcine cells that cause HAR and synthesis of human complement regulatory proteins in transgenic pigs. [31, 34, 36]

Problems with drugs from transgenic animals:

Erythropoietin could not be expressed in the mammary gland of transgenic cattle. The recovery rates of Factor VIII protein were low. [34] Another concern is leakage of a target protein into the circulation by way of the mammary epithelial cells and as measured by increased plasma levels of the protein designed to be expressed only in the animal's milk. [9] There is also a risk of transmission of infection from animal to man. [31] There are some unique concerns such as premature lactational shut down observed in some animals expressing recombinant proteins in their mammary gland. [37]

While there are problems associated with transgenic animals, the benefit derived from them is far superior and with the increase in technology this could be solved.

Drugs from Transgenic Animals in Clinical Trials

The approval of ATryn (rATIII) by USFDA has opened gates for other drugs from transgenic animals. [29] Recombinant C1 inhibitor produced in the milk of transgenic rabbits has completed phase III trials and is expected to receive registration. [4, 31]

A topical antibiotic against Streptococcus mutans, for prevention and treatment of dental caries, has completed phase III trials. [34] Vaccine used in Alzheimers disease has restored neurological performance in the mice, and is currently in phase II human clinical trials. [6]α-Glucosidase from the rabbit is in clinical trial phase II/III for Pompe's disease. [31, 34] Products such as α-anti-trypsinused for cystic fibrosis, α-AT deficiency and tPA used for coronary clots are currently in phase II/III clinical trials and are expected to be on the market within the next few years. [34]

Ethics in Transgenic Animals

Genetic modification of micro-organisms and plants has least concern with regards to ethics but when it comes to genetic modification of animals and particularly humans, more objections are registered. There remains concern that with advances in transgenic animal technologies the number of animals used for research may actually increase rather than reduced because of a wider range of diseases and conditions. [38]

Ethical concern with oncomouse is that it usually suffers in order to collect relevant information, which contradict the principles of animal rights.[6]Adenopolyposis coli knock-out mutant mice are clinically normal until the intestinal polyps develop, after which mice become anemic and lose weight. Each newly created transgenic strain has the potential to cause poor health and suffering in the animals hence measures need to be undertaken to minimize animal suffering. [39]

Other ethical concerns are breaching species barriers and animal life should not be regarded as a chemical product subject to genetic alteration and patentable for economic benefit. Also genetic engineering of animals interferes with the integrity or telos of the animal. Telos is defined as the set of needs and interests which are genetically based, environmentally expressed, and which collectively constitute or define the form of life or way of living exhibited by that animal, and whose fulfillment or thwarting matter to that animal. [37]

In India attitude towards animals is tinged with religious and ethical colour which makes religious sentiments and public awareness necessary to be taken in consideration.[40] The 3R (Reduction, Refinement, Replacement) aim to minimize pain experienced by the animals used in experiments.[29]Inspite of the problems listed above, transgenic animals may represent a "refinement" in comparison to some other traditional experimental models of disease where animals bear a heavy load of suffering. A genotype is an excellent model of disease for selected body functions at the molecular or cellular level while the corresponding phenotype is completely healthy. Thus it becomes necessary to consider the moral implications of producing such a species as well as measures of reducing animal suffering. [39]

Patents on Transgenic animals

Patenting of animal models is the need of hour, because it is an indispensable tool for screening of novel molecule to various diseases. A human

pathological condition in animals is most important to determine the therapeutic efficacy of novel molecule. They allow facilitation of the screening process to eliminate inactive moieties and assess the pharmacologist to identify the therapeutic potential and characterize the toxicological profile of novel chemical or biological entities. [6]

The two major aspects in granting patents to animal model are morality and reproducibility. [6] Other concerns like restrictive licensing of the patents can hinder transfer of knowledge. [41] Preclinical animal models are important in drug discovery, because they lay the fundamental for human trials. Patents remain one of the important ways of recovery of the investments made by the Pharmaceutical companies in research. The Indian Patent Law section 3i and 3 j states that all the surgical processes and animals are not patentable, hence animal models are not patentable in India. If the suitable amendments are made then animal models can be patentable in India and it would open novel vistas in the research arena in India. [6]

Regulation in Transgenic Animals

Pharmaceutical research on animals in India depends on Department of Biotechnology, National Institute of Immunology and Environment Protection Act. An Animal Welfare Board is constituted, with a Committee for the Control and Supervision of Experiments Animals (CPCSEA) which is in charge of legal and ethical aspects or animal research. General Guidelines on caring for animals in research are in accordance with the International Committee for Laboratory Animal Science (ICLAS) Guidelines. However there is no specific law or guidelines for cloned and transgenic animals. In 2000, Indian Council of Medical Research Report promotes transgenic animal research as long as it would pursue a higher scientific goal. [40, 42]

With changes in the overall process of drug discovery, US patents of animal models encourage scientists in America and Europe to produce animal models which are very close to human disease and hence contribute significantly to the process of drug discovery. [6] The Indian regulatory authorities need to be prepared for such challenges of ethics, regulation and patents in transgenic animals.

Future prospects

Xenogenic cells, in particular from the pig, hold great promise with regard to successful cell therapy for human patients. Porcine islet cells transplanted to diabetic patients has shown to be partially functional over a period of time. Porcine fetal neural cells have been transplanted into the brain of patients suffering from Parkinson's disease, Huntington's disease, stroke and focal epilepsy. The advantages of porcine neural cells over their human counterparts are the abundant availability. [34]The pig could be a useful model for studying defects of growth-hormone releasing hormone, which are implicated in conditions such as Turner syndrome, hypochrondroplasia, and intrauterine growth retardation.[4]

Eggs provide other non-invasive harvesting medium. Significant quantities of two human proteins, interferon beta-1a and a humanized monoclonal antibody (miR24) were expressed in the whites of eggs laid by transgenic hens. miR24 is being developed for malignant melanoma. [3]

There are transgenic animals for the development of unique biological materials like polymers based on spider silks that may be useful as suture or plastic materials in facial and orthopedic restorative surgery. [43,37] It is impractical to obtain sufficient quantities of plasma butylcholinesterase (BChE) to treat humans exposed to organophosphorus agents in agriculture and chemical warfare, the production of recombinant BChE in milk of transgenic animals is under investigation. [44] It is proven that the amount of human antithrombin III obtained per year from transgenic goats is equivalent to this resulting from 90,000 human blood samplings. [1] Thus transgenic animals have the capacity of mass production and an effective alternative to various human byproducts. Improvements in transgenic technology include inducible gene expression,

artificial chromosomes and advancement in nuclear transfer. [34, 45, 36]

Emerging transgenic technologies: [4, 31, 45]

- Lentiviral transfection of oocytes and zygotes,
- Chimera generation via injection of pluripotent cells into blastocysts
- Culture of spermatogonia and transplantation into recipient males
- Ribonucleic acid interference

Researchers are using transgenic animals to develop therapies for a wide range of diseases discussed above and other diseases like Anaemia, Emphysema, Haemophilia, Malaria and Rheumatoid arthritis. [3]

CONCLUSION

Major prerequisites for success and safety of transgenic animals will be a continuous refinement of reproductive biotechnologies. In coming years genetically modified animals will play a significant role in the field of biomedicine especially in drug development, animal disease models, xenotransplantation, antibody production, gene pharming and blood replacement. [4] The regulatory aspects and ethics should be given due consideration while using transgenic animals.

From research, pigs and transgenic animals derived products like milk, eggs seems to be promising in developments of therapeutic strategies. Drugs from transgenic animals can minimize the attrition rate in clinical trials by increasing the quality of the target and compound combinations making the transition from discovery into development. Transgenic technology can impact at many points in the discovery process, including target identification and target validation. It also provides models designed to alert researchers early to the potential problems with drug metabolism and toxicity which will help in providing better models for human diseases. [2]

Transgenic in general is a rapidly advancing field, and within 20 years of its inception it has produced the first USFDA approved drug for transgenic animals. Thus the use of transgenic animals has the capacity to overcome the current and future needs in medicine and is now a necessity rather than a matter of choice.

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