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ADVANCES IN TRANSGENIC ANIMAL PRODUCTION AND APPLICATIONS

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Abstract: Transgenic animals are animals which have been genetically transformed by splicing and inserting foreign animal or human genes into their chromosomes. Transgenic animals with changes in the germ line are heritable from generation to generation within the herd, and this heritability has potential to facilitate long-term productivity gains. Transgenic animals with alterations to the germ line are commonly produced through microinjection and three more other methods discussed in this review article. Production of transgenic animal is expense and difficult. Several farms of cloned transgenic animals have emerged throughout the world. 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. Concerns regarding transgenic animals have developed along with the new technologies. 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 disease model, drug research, xenotransplantation and other applications we discussed in this review.
Keywords: Transgenic animals, production, genetic models applications.

Introduction

Trangenesis is the process of introducing a foreign gene called a transgene into an organism which will exhibit and transmit a new property to its offspring. With the advent of transgenic technology and its application in many laboratories around the world.Thereis an increase in the generation and use of genetically modified animals in biomedical, pharmaceutical research and safety testing. The generation of transgenic animals is essential for the in vivo study of gene function during development, organogenesis and aging. It also permits the evaluation of therapeutic strategies in models of human disease, as well as the investigation of disease progression in a manner not possible in human subjects. Commercial applications include the preparation of recombinant proteins, protection of animals against disease, and introduction of new genetic traits into herds.

The first transgenic experiments in mammals were performed in mice (Gordon *et al*., 1980; Gordon and Ruddle, 1981) afterward rabbits, pigs, sheep and cattle (Hammer *et al*.,

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1985, Pursel*et al*., 1987, Rexroad*et al*., 1989, Roschlau*et al*., 1989). 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. Thus transgenic animals have the ability to fulfil the needs of the pharmaceutical industry and in coming years they are looked as potential contributors to the drugs and research in medicine. The transgenic efficiency and precise control of gene expression are the key limiting factors in the production of transgenic animals. Advance studies will allow transgenic technology to explore gene function, animal genetic improvement, bioreactors, animal disease models and organ transplantation (McLaughlin *et al.,* 2001).

Methods for the production of transgenic animals:

The development of transgenic animals having the following sequences. 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. Following recent animal gene transfer techniques used in the production of transgenic animal production:

1. Microinjection Method:

DNA microinjection has become the most commonly applied andfirst predominant method for gene transfer in animals. DNA microinjection is the technique was successfully used for the first time in 1980 (Gordon *et al*., 1980), mouse was the first animal to undergo successful gene transfer.DNA microinjection method is accomplished by the transfer of a desired gene construct from another member of the same species or from a different species into the pronucleus of a fertilized ovum, which is subsequently implanted into the oviduct of recipient animals. This results in the recipient animal giving birth to genetically modified offspring. This method has many advantages like, exogenous genes are expressed in an efficient manner, the size of the inserted DNA molecule has no clear limit and moreover this technique is simple, inexpensive and can be applied to a wide variety of species. However, this technique cannot be used into the cell at later development stage, The injected transgene may cause a change in the normal physiological processes of the animal, time consuming and requires extensive intellectual, financial and material assets (Seidel, 1993) and the success rate of producing transgenic animals by these methods is very low (Wall, 1996).

2. Somatic cell nuclear transfer:

In genetics and developmental biology, somatic cell nuclear transfer (SCNT) is a laboratory technique for creating a viable embryo from a body cell and an egg cell. It is a technique in which the nucleus (DNA) of a somatic cell is transferred into an enucleated metaphase-II oocyte for the generation of a new individual, genetically identical to the somatic cell donor. Somatic cell transfer may be used to generate multiple copies of genetically elite farm animals, to produce transgenic animals for pharmaceutical protein production or xeno-transplantation (Wilmut*et al*., 1997; Polejaeva*et al.,* 2000) or to preserve endangered species. From current advancement of SCNT and molecular techniques, production of a transgenic animal becomes easier. Domestic animals can be cloned using techniques such as embryo splitting and nuclear transfer to produce genetically identical individuals. Although embryo splitting is limited to the production of only a few identical individuals, nuclear transfer of donor nuclei into recipient oocytes, whose own nuclear DNA has been removed, can result in large numbers of identical individuals. somatic cell nuclear transfer (SCNT) research has contributed knowledge that has led to the direct reprogrammingof cells (e.g., to induce pluripotent stem cells) and a better understanding of epigenetic regulation during embryonic development.It is used in both therapeutic and reproductive cloning. Dolly the Sheep, famous for being the first successfully cloned mammal was created using this process (Li *et al*., 2009).

3. Retrovirus-mediated gene transfer:

Retro virus-mediated gene transfer method is is mediated by means of a carrier or vector; generally a virus or a plasmid. Retroviruses are commonly used as vectors to transfer genetic material into the cell because of their ability to infect host cells .A retrovirus is a virus that carries its genetic material in the form of RNA rather than DNA. The code in the viral RNA is reverse transcribed to produce DNA, which is then incorporated into the host cell. The offspring derived from this method are chimeric, i.e., not all cells carry the retrovirus or an organism consisting of tissues or parts of diverse genetic constitution. This method was successfully used in 1974 when a simian virus was inserted into mice embryos, resulting in mice carrying this DNA. The most important features of retrovirus as vectors are the practically ease and effectiveness of gene transfer and target cells specificity.Retrovirus-mediated gene transfer is a powerful tool that can be used to understand gene functions. There are varieties of retrovirus vectors and efficient packaging cell lines are developed for the efficient functional expression cloning methods.These techniques can provide a better

platform to develop transgenicanimals for breeding new animal varieties and promote the development of medical sciences, livestock production and other fields. This method is advantageous because this system is technically simple, readily integrates and passes through the germ lines allowing for their propagation into subsequent generations and causes minimal disruption to host DNA. However,there is some risk of interfering of viral sequences may with transgene expression.

4. Embryonic stem (ES) cells mediated gene transfer:

Embryonic stem (ES) cells mediated gene transfer method based on findings of Gossler*et al*., (1986). The presence of transgenes can be tested at the embryonic state in this method. ES cells are pluripotent cells, found in the inner cell mass (ICM) of embryos at the blastocyst stage of development which not yet differentiated and maintain the ability to develop into any type of tissue during the embryonic and foetal development. DNA can be introduced into the ES cells in vitro (Capecchi, 1994). The desired DNA containing embryonic stem cells grown at blastocyst stage, are incorporated into the host's embryo and then embryo inserted in the uterus of a surrogate mother, resulting in a chimeric animal. The method uses homologous recombination of DNA to permit precise targeting of DNA in embryonic stem cells. If the homologous sequence to be introduced into the cell carries a mutation or a gene from another species, the new sequence will replace the specific targeted gene. This is the method of choice for gene inactivation therefore called as "knock-out" method, mainly used in the study of the genetic control of developmental processes

Application of transgenic animals:

1. Used for developing disease model

Mice have been used to model for diseases of human beimngs, because of their physiological, anatomical and genomic similarities to humans. Transgenic animals are produced as disease models) such as Alzheimers, cancer, AIDS. Transgenic animals enable scientists to understand the role of genes in specific diseases. The benefits of using transgenic animals include the possibility of the replacement of higher species by lower species- through development of disease models in mice rather than in dogs or non-human primates, Pigs could be used as an effective model for the study of growth hormone releasing hormone (GHRH) defects (Draghia – Akli*et al*., 1999). Transgenic animals such as mice have been found to be valuable in investigations into gene function and for analysis of different hereditary diseases (Moore, 1995; Masood, 1997).

2. Application in Drug and Industrial production:

Transgenic animals are used for production of proteins such as alpha-1-antitrypsin, produced in liver, used in treatment of emphysema or cystic fibrosis. This process is less expensive than production of protein through culture of human cells (Boyd Group, 1999). The human lungs are constantly get affected by foreign particles such as dust, spores and bacteria. To prevent these, neutrophils releasing the elastase enzyme but this enzyme harmed the elastin in the lungs which maintains the elasticity of lungs. So, human body releases a protein α1 proteinase inhibitor which has been successfully expressed in sheep (Khatib, 2005). Recombinant human proteins produced in the mammary glands of transgenic animals (Meade *et al.,* 1999; Rudolph,1999). Pharmaceutical proteins are now used for commercial purpose (Ziomek, 1998). Two scientists at Nexia Biotechnologies in Canada spliced spider genes into the cells of lactating goats. The goats are used to manufacture silk, milk and secrete tiny strands from their body by the bucketful. By extracting polymer strands from the milk and weaving them into thread which is light and tough material that could be used to prepare military uniforms, medical micro sutures and tennis racket strings. Americans are more supportive (60%) for above use of transgenic animals. The mammary gland of transgenic goats is used to produce Monoclonal Antibodies. A recombinant bispecific antibody is produced by using transgenic cattles with in their blood (Grosse-Hovest*et al.,* 2004).Another application includes newly generation of trans-chromosomal animals in which a human artificial chromosome containing the complete sequences of the human immunoglobulin heavy and light chain loci was introduced into bovine fibroblasts, which were then used in nuclear transfer. Trans-chromosomal bovine offspring were obtained that expressed human immunoglobulin in their blood. This could be a significant step forward in the generation of human therapeutic polyclonal antibodies (Kuroiwa*et al.,* 2002).

3. Application in Disease control:

Scientist developed the mice by altering the genes of the mousepox virus in Australia 50. Some scientist also thought to develop genetically modify mosquitoes so they cannot produce malaria but other scientist worry about these mosquitoes that they could have unforeseen possibly risk if, they are released into the environment (Pew Initiative on Food and Biotechnology, 2003).

4. Xenotransplantation:

Now a day approximately about 250000 people are alive due to the successful transplantation of an appropriate allo-transplantation. Sometimes there is limitation of

appropriate organs or rejection of live organ donation. So, to rectify this problem porcine xenografts from domesticated pigs are considered to be the best choice. Pigs which are genetically modified can be used as a source animal for tissues and organs in human beings for transplantation purpose by delete the gene responsible for the human rapid immune rejection response (Hagelin, 2004). In Canada, a National survey on xenotransplantation showed that only 48% found acceptable for 'the use of animals as a source of living cells, tissues or organs to prolong human life (Canadian Public Health Association, 2001). To overcome the Hyperacute rejection & acute vascular rejection, synthesis of human regulators of complement activity are produced in transgenic pigs. Survival rates, after the transplantation of porcine hearts or kidneys expressing transgenic regulators of complement activity proteins to immunosuppressed nonhuman primates, reached near about 23 to 135 days. So, the Hyperacute rejection can be overcome in a clinically acceptable manner (Bach, 1998). For long term graft tolerance induction of permanent chimerism via intraportal injection of embryonic stem (ES) cells or the co-transplantation of vascularised thymic tissue (Yamada *et al.,* 2005).

5. Blood replacement

Transgenic swine are used to produce human haemoglobin. The protein obtained from transgenesis could be purified by using procine blood which is similar to human haemoglobin (Swabson*et al.,* 1992).

6. Agriculture

Transgenic pigs containing a human metallothionein promoter or porcine growth-hormone gene construct referred significant improvements in economically traits including growth rate, body fat/muscle ratio (Nottle*et al.*, 1999). Transgenic pigs are used to produce pork by using spinach desaturase gene which produce large amount of non-saturated fatty acids, used for diet purpose and was advantageous to reduce the risk of stroke and coronary disease (Niemann, 2004). Transgenic animals are used for milk production. Generally, there is an improvement in milk composition. For this purpose transgenic mice have been developed, at the same time some unwanted side effects can occur (Kumar *et al.*, 1994). Transgenic pigs are use to increase milk production by altering the composition of lactose (Wheeler *et al.*, 2001). In the pig, transgenic expression of a bovine lactalbumin construct in sow milk has been resulting in higher lactose contents and greater milk yields, correlated with improved survival and development of piglets (Wheeler *et al.,* 2001). Transgenic sheep are used for wool production in which transgenic sheep carrying a keratin-

IGF-I construct showed that expression in the skin and the amount of clear fleece was about 6.2% greater in transgenic as compared to non-transgenic animals (Damak, 1996). Scientists are attempting to produce disease-resistant animals, such as influenza-resistant pigs, but a very limited number of genes are currently known to be responsible for resistance to diseases in farm animals (Muller, 1992).

7. Transgenic animals are used in toxicity and vaccine testing.

Transgenic technology could provide a means of transferring or increasing nutritionally beneficial traits. For example, enhancing the omega-3 fatty acid in fish consumed by humans may contribute to a decreased occurrence of coronary heart disease. In fact, transgenic pigs that contain elevated levels of omega-3 fatty acids have been produced (Lai *et al*., 2006).Recent progress has produced prion-free suppressed prion livestock with diseases likebovine spongiform encephalopathy (BSE) in cattle (Richt *et al*.,2007). 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.

Conclusion

In coming years genetically modified animals will play a significant role in the field of biomedicine especially in animal disease model, drug development, animal disease control, xenotransplantation, antibody production, animal pharming and blood replacement. The regulatory aspects and ethics should be given due consideration while using transgenic animals. As transgenic animals become more mainstream, a small yet growing portion of the animal production industry. It will shift its operations from farming livestock for food production, to transgenic animals for pharmaceutical production. The world market is growing for human pharmaceutical products. Producing transgenic animals is still relatively expensive. However, costs are trending down and transgenic animals have certain advantages over traditional laboratory methods for producing human proteins. More commercial use of transgenic animals in food production is also likely. Regulators will need to review existing policies and guidelines regarding transgenic animals. New policies regarding transgenic and cloned animals may be necessary to ensure the safety and health of transgenic animal. Transgenictechnologies will ensure that further research and analyses will be demanded by animal producers, regulators, environmentalists, and the general public.

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