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Biomedical Application and Future Prospects of Transgenic Animal: Review

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Abstract
Today, transgenic animals represent one of the most potent and exciting research tools in the biological sciences. A transgenic animal is an animal into whose genome foreign Deoxyribose Nucleic Acid (DNA) has been transferred for the purpose of studying and manipulating that DNA. The establishment of stable transgenic animals implies that the foreign DNA is present in gametes or one cell embryos to allow its transmission to progeny. To reach this goal, the foreign gene can be transferred using different methods according to animal species. Several methods have been used for the production of transgenic animals like Microinjection of fertilized ovum, Embryonic stem (ES) cells mediated gene transfer and Retrovirus-mediated gene transfer. The study of human diseases is greatly facilitated by the generation of transgenic animals mimicking health disorders or allowing the evaluation of new pharmaceuticals.
Keywords: Biomedicine, Gene Transferring, Prospects, Transgenic animal

1. Introduction

Over the last three decades biotechnology has advanced to a level where it is generally feasible to make particular changes to the genome, and therefore to the expressed characteristics of living organisms. The product of such a change is called a transgenic or a genetically modified organism. A transgenic animal is an animal whose genome has been altered by the inclusion of foreign genetic material. The purpose of adding a new gene to an organism's genome is to have the organism produce a protein or set of proteins that it did not produce before the gene was added or manipulated (Aine and Meghan, 2005).

Improvement of animal production by transgenesis is still in infancy and despite its intensive use, animal transgenesis is still suffering from technical limitations. The generation of transgenesis has recently become easier or possible for different species thanks to the use of transposons or retrovirus, to incubation of sperm which DNA followed by fertilization by intracellular sperm injection or not and to the use of the cloning technique using somatic cells in which genes has been added or in activated. All these techniques are expected to offer experimenters new and more precise models to study gene function even in large animals. Improvement of breeding by transgenesis has become more reasonable including through the precise allele replacement in farm animals (Louis, 2002).

Transgenic animals represent unique models that are custom tailored to address specific biological questions. Hence, the ability to introduce functional genes into animals provides a very powerful tool for dissecting complex biological processes and systems. This has made it possible to explore the regulation of gene expression as well as the regulation of cellular and physiological processes. Significant uses of live transgenic mammals are in the arenas of agricultural, biological, biotechnological from transgenic animals to humans and biomedical sciences including production of pharmaceuticals and in the field of organ transfer (Wall and Seidel, 1992). Therefore the objectives of this review paper are:

- ✓ To highlight the application of transgenic animals in biomedicine
- ✓ To review available literatures on the methods of producing transgenic animal
- ✓ To assess and describe the feature prospect regarding transgenic animals.

2. Methods of Transgenesis

The establishment of stable transgenic animals implies that the foreign DNA is present in gametes or one cell embryos to allow its transmission to progeny. To reach this goal, the foreign gene can be transferred using different methods according to animal species. Currently, many techniques are used to create these useful animals, and the five most popular techniques; DNA microinjection into the oocyte pronucleus, DNA transfer using ES cells, DNA transfer using retro viral vector, the use of transposon and DNA transfer by nuclear transfer (Louis, 2002).

2.1. DNA microinjection

DNA microinjection has become the most commonly applied method for gene transfer in animals. Using DNA microinjection, mouse was the first animal to undergo successful gene transfer. Microinjection of embryos with DNA has been the traditional approach for generating transgenic livestock. For processes such as cellular or pronuclear injection the target cell is positioned under the microscope and two micromanipulators one holding the pipette and other one, holding a micro capillary needle usually between 0.5 to 5 µm in diameter (larger if injecting

stem cells into an embryo) are used to penetrate the cell membrane and/or the nuclear envelope (David and Matthew, 2013).

2.2. The use of transposons

Transposons are short genomic DNA regions (About 1-3 kb) that autoreplicate and are randomly integrated as multiple copies within the same genome. Integration is achieved by the integrase enzyme encoded by the transposon itself. Foreign DNA can be introduced into the transposon to replace the integrase gene, and the recombinant transposon can be microinjected into one-cell embryos along with the transposon integrase enzyme. Using this method, the foreign gene can be integrated into the embryo genome insects, silk worms, chicken, fish and mammals (Sumiyama et al., 2010).

2.3. DNA transfer into gametes

One logical approach to generate transgenic animals may theoretically consist of introducing foreign DNA in gametes before fertilization. The incubation of spermatozoa in the presence of DNA followed by invivo or invitro fertilization led to the generation of transgenic mice, fish, chicken, rabbits, pigs, sheep and cows (Sato et al., 2002). However, the results obtained with this method are inconsistent. The yield of transgenic animals is usually low and largely unpredictable. Moreover, the integrated DNA is most of the times profoundly rearranged and no more functional. This phenomenon can seemingly be greatly attenuated by selecting the most appropriate ejaculates and by removing DNAse by repeated washing and addition of DNAse inhibitors (Baccetti and Spadafora, 2000).

This method in its improved version might become attractive in future for some species. The spermatozoa were incubated with the antibody-DNA complex and further used to fertilize oocyte by in vitro fertilization in mouse, by artificial insemination in chicken and by injection into uterine horn in pig. In all cases, up to 30% of born animals were transgenic and the trans-genes were expressed and transmitted to progeny without any rearrangement. Interestingly the same antibody recognized the same antigen in several mammals including human and in lower vertebrates. This method might greatly simplify gene addition in animals (Qian et al., 2001).

2.4. The use of retro viral vectors

A retrovirus is a virus that carries its genetic material in the form of RNA rather than DNA. Retroviruses are used as vectors to transfer genetic material into the host cell. The result is a chimera, an organism consisting of tissues or parts of diverse genetic constitution. A chimera is an animal that consists of two or more tissues that have different genetic compositions produced by genetic engineering. This means that some of the cells are transgenic and some of the cells in the organism are not (Margawit and Endag, 2003).

The chimeric mouse was constructed by introducing cells from one strain of mouse into the embryos of another strain of mouse by microinjection into the blastocyst stage embryo which is a day-5 embryo. The embryo was then implanted into a surrogate mother, and allowed to grow into a chimeric adult mouse that exhibited characteristics of each strain of mouse (Aine and Meghan, 2005).

Retroviral vectors are under intensive study to tentatively transfer gene to human somatic cells and proceed to gene therapy. Similar vectors have been designed to transfer foreign genes into mammalian embryonic cells. A family of vectors capable of infecting chicken primordial germ cells and of generating transgenic animals has been described. This approach appears much simpler than microinjection and might become used extensively in future (Lois et al., 2002).

2.5. Gene transfer using embryonic cells

Gene replacement by homologous recombination is performed in routine in bacteria and yeast. It can be achieved in somatic mammalian cells although with a relatively poor efficiency. This approach is very attractive since it can lead to specific gene inactivation, to targeted point mutation in an animal genome or to the replacement of a given gene by a non-related one. Cells in which it occurred must be selected and further used to generate a living embryo. This proved to be feasible on condition to use embryonic stem cells capable of forming chimeric embryos after microinjection into blastocysts or morula (Collodi, 2001).

Although laborious this protocol has become popular and genes are frequently inactivated in mouse. Despite repeated efforts, the extension of this method to species other than mouse is failed. This is clearly due to the fact that the recombined ESCs have more or less the capacity to participate to the development of chimeric embryos but that transmission of the mutation to progeny has been observed so far only in two mouse lines (Smithies, 2001).Two recent studies indicate that chicken cultured primordial germ cells retransferred to embryos can participate to their development and transmit their genes to progeny (Petitte et al., 2002).

2.6. Gene transfer by nuclear transfer

This method was extended to sheep about 15 years ago but the success was restricted to experiments in which cells used as nuclear donors were taken from early embryos and not previously cultured. These observations gave

credence to the idea that animal cloning by nuclear transfer was possible only with nuclei from totipotent or pluripotent cells. A systematic study carried out in sheep revealed that fully differentiated cells as well as embryonic or foetal cells could successfully give their genes to generate normal animals The conditions defined for cloning animals starting from differentiated cells was retained soon after to generate transgenic sheep by gene addition (Schnieke et al., 1997). On the other hand, it has been repeatedly observed that cells from adults and cells cultured for long periods of time is a less efficient material for cloning by nuclear transfer. The reasons why these phenomena occur are unknown. Mutation of essential genes for embryo development may occur progressively in living animals and in cultured cells (Kang et al., 2004).

Gene addition by the cloning technique has been extended to goat, cow (Arat et al., 2001). This suggests that gene replacement will become a reality in several species other than mouse in the coming years (Dai et al., 2002).

3. Biomedical Application of Transgenic animal

Biomedicine is the application of the principles of the natural sciences, especially biochemistry, molecular biology, and microbiology, to human and veterinary clinical medicine. The field of biomedicine offers tremendous potential to treat pain and suffering in humans and animals worldwide. Biomedical research using genetically engineered and cloned animals is being conducted to produce therapeutic drugs for the treatment of human diseases, for the production of organs for human transplantation, and to study the effects that individual genes on body function (Allison and Davis, 2008)

3.1. Pharmaceutical production

Gene 'pharming' involves the production of recombinant biologically active human proteins in the mammary glands of transgenic animals. This technology overcomes the limitations of conventional and recombinant production systems for pharmaceutical proteins (Rudolph et al., 1999) and has advanced to the stage of commercial application (Dyck et al., 2003). The mammary gland is the preferred production site mainly because of the quantities of protein that can be produced in this organ using mammary gland-specific promoter elements and established methods for extraction and purification of that protein (Rudolph et al., 1999).

Products derived from the mammary gland of transgenic goats and sheep, such as antithrombin III (ATIII), α-antitrypsin or tissue plasminogen activator (tPA), have progressed to advanced clinical trials (Kues et al., 2004). The enzyme α-glucosidase from the milk of transgenic rabbits has drug status and has been successfully used for the treatment of Pompe's disease (Van den et al., 2001). With the advent of transgenic crops that produce pharmacologically active proteins, there is now an array of recombinant technologies that will allow selection of the most appropriate production system for each required protein. The production of edible vaccines in transgenic crop plants against, for example, foot and mouth disease, might become an important application for animal health (Wigdorovitz et al., 2004).

The production of therapeutic proteins in chickens offers a number of advantages. Firstly, their generation interval is short, which means that there is less of a time lag between the development of lines of genetically engineered poultry and the production of therapeutic proteins. Secondly, chickens produce a lot of protein in the eggs they lay, and those proteins can be purified from eggs using well-established protocols (Allison and Davis, 2008).

3.2. Antibody production in transgenic animals

Numerous monoclonal antibodies are being produced in the mammary gland of transgenic goats (Meade et al., 1999). Cloned transgenic cattle produce a recombinant specific antibody in their blood (Grosse et al., 2004). Purified from serum, the antibody is stable and mediates target cell- restricted T cell stimulation and tumour cell killing. An interesting new development is the generation of trans-chromosomal animals. Trans-chromosomal bovine offspring were obtained that expressed human immunoglobulin in their blood. This system could be a significant step forward in the production of human therapeutic polyclonal antibodies (Kuroiwa et al., 200).

Currently, the source of human polyclonal antibodies is from human volunteers who donate plasma, but the current supply cannot keep up with the demand. Production of human polyclonal antibodies in genetically engineered cattle would allow for the large scale production of antibodies. Antibodies are collected from the blood of transgenic cows through plasmapheresis, in much the same way as they are currently collected from human donors. Following purification, antibodies have the potential to be used to fight infections, assist humans with compromised immune systems, or to treat autoimmune diseases such as rheumatoid arthritis (Allison and Davis, 2008).

3.3. Blood replacement

Functional human hemoglobin has been produced from intransgenic swine. The transgenic protein could be purified from the porcine blood and showed oxygen-binding characteristics similar to natural human hemoglobin. The main obstacle was that only a small proportion of porcine red blood cells contained the human form of

hemoglobin (Swanson et al., 1992).

3.4. Xenotransplantation

Today more than 250,000 people in the world 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 in Western societies. To close the growing gap between demand and availability of appropriate human organs, porcine xeno-grafts from domesticated pigs are considered to be the best alternative (Kues et al., 2004). Essential prerequisites for successful xenotransplantation are; overcoming the immunological hurdles, preventing the transmission of pathogens from the donor animal to the human recipient, ensuring the compatibility of the donor organs with human anatomy and physiology are the major considerations, but immunological graft rejection was obstacles in porcine-to-human organ transplantation (Yamada et al., 2005). Hyperacute rejection (HAR) occurs within seconds or minutes, when, in the case of a discordant organ (e.g. in transplanting from pig to human), pre-existing antibodies react with antigenic structures on the surface of the porcine cells and activate the complement cascade; in other words, the antigen-antibody complex triggers formation of the membrane attack complex (Kuwaki et al., 2005).

When using a discordant donor species such as the pig, overcoming HAR and acute vascular rejection are the pre-eminent goals. Two main strategies have been successfully explored for long- term suppression of HAR: synthesis of human regulators of complement activity in transgenic pigs and the knockout of α-gal epitopes, the antigenic structures on the surface of the porcine cells that cause HAR. The successful xenotransplantation of porcine organs with a knockout of the 1,3-α-galactosyltransferase gene, eliminating the 1,3-α-gal- epitopes produced by the1,3-α-galactosyltransferase enzyme, has recently been demonstrated (Kuwaki et al., 2005). A particularly promising strategy to enhance long-term graft tolerance is the induction of permanent chimerism via intra portal injection of embryonic stem (ES) cells to the organ receiver (Frandrich et al., 2002).

Despite further challenges, appropriate lines of transgenic pigs are likely to be available as organ donors within the next five to ten years. Guidelines for the clinical application of porcine xenotransplants are already available in the United State of America (USA) and are currently being developed in other countries. The general consensus of a worldwide debate is that the technology is ethically acceptable provided that the individual's well-being does not compromise public health and economy, xenotransplantation will be viable, as the enormous costs of maintaining patients suffering from severe kidney disease using dialysis or supporting those suffering from chronic heart disease could be reduced by a functional kidney or heart xenograft (Neiman et al., 2005).

3.5. Farm animals as models for human diseases

Mouse physiology, anatomy and life span differ significantly from those of humans, making the rodent model inappropriate for many human diseases. Farm animals, such as pigs, sheep or even cattle, may be more appropriate models in which to study potential therapies for human diseases that require longer observation periods than those possible in mice, e.g. atherosclerosis, non-insulin- dependent diabetes, cystic fibrosis, cancer and neuro-degenerative disorders (Hansen and Khanna, 2004).

Cardiovascular disease is an increasing health problem in aging Western societies, where coronary artery diseases account for the majority of deaths. Because genetically modified mice do not manifest myocardial infarction or stroke as a result of atherosclerosis, new animal models, such as swine that exhibit these pathologies, are needed to develop effective therapeutic strategies (Grunwald et al., 1999).

The pig could be a useful model for studying defects of growth-hormone releasing hormone (GHRH), which are implicated in a variety of conditions such as Turner syndrome, hypochrondroplasia, Crohn's disease, intrauterine growth retardation or renal insufficiency. Application of recombinant Gonadotropin Hormone Releasing Hormone and its myogenic expression has been shown to alleviate these problems in a porcine model and development of further ovine and porcine models of human diseases is underway (Forsberg et al., 2005).

An important aspect of nuclear-transfer-derived large animal models for human diseases and the development of regenerative therapies is that somatic cloning does not result in shortening of the telomeres and thus does not necessarily lead to premature ageing Telomere shortening is usually correlated with severe limitation of the regenerative capacity of cells, the onset of cancer, ageing and chronic disease with significant impacts on human lifespans (Schazlein and Rudolph et al., 2005). Expression of the enzyme telomerase, which is primarily responsible for the formation and rebuilding of telomeres, is suppressed in most somatic tissues postnatally (Schatzlein et al., 2004).

4. Future prospects of transgenesis

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The techniques for obtaining transgenic animals in species of agricultural interest are still inefficient. Some approaches that may overcome this problem are based on cloning techniques. Using these techniques it is feasible to reduce to less than 50% the number of embryo receptor females, which is one of the most important economic limiting factor in domestic species. It would also facilitate the further proliferation of transgenic animals. Recent results relate these techniques with still low success rates high rates of perinatal mortality and variable transgenic expression that requires to be evaluated before generalizing their application (Edward et al., 2003). Considerable effort and time is required to propagate the transgenic animal genetics into commercial dairy herds.

Rapid dissemination of the genetics of the parental animals by nuclear transfer could result in the generation of mini herds in two to three years. However, the existing inefficiencies of nuclear transfer make this a difficult undertaking. It is noteworthy that the genetic merit of the 'cloned' animals can be fixed, while continuous genetic improvements is introduced in commercial herds by using artificial insemination breeding program. In an alternative scenario of herd expansion, semen homozygous for the transgene may be available in four to five years. Extensive breeding programs will be critical in studying the interaction and co-adaptation of the transgene(s), with the background polygenes controlling milk production and composition. Controlling inbreeding and confirming the absence of deleterious traits so that the immediate genetic variability introduced by transgenesis is transformed (Karatzas, 2003).

Conclusion and Recommendations

Throughout history, animal husbandry has made significant contributions to human health and well- being. The combination of recent advances in reproductive technologies with the tools of molecular biology (gene targeting and array analysis of gene expression) adds a new dimension to animal breeding. 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. As the complete genomic sequences of all farm animals become available, it will be possible to improve targeted genetic modification in animal breeding and to develop strategies to cope with the future challenges in global agricultural production. 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. Based on the above conclusion the following recommendation is forwarded;

- When introducing new, sophisticated techniques like genetic modification of animals, the benefits of new product(s) or techniques should be evident and clearly demonstrated to the general public.
- Veterinarian should acquire basic knowledge about the application and techniques of animal transgenesis and this should be part of the academic curriculum.
- Even though transgenic animals have such like benefit still it was not started in Ethiopia, so this technique should be introduced in Ethiopia and applicable for biomedicine
- The transmission of pathogens from the donor animal to the human recipient should be considered.

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