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Research Article

TRANSGENIC MOUSE: A VALUABLE TOOL FOR MEDICAL BIOTECHNOLOGY

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ABSTRACT
The mouse has been used as an experimental organism for the study of the effect of various drugs and other factors on the physiology of the organism because its life processes are similar to human. In advanced biotechnological research there is need of observing the effect of different factors affecting the gene expression as well as expression of trans gene in vivo. For all of these studies mouse played an important role in all areas of gene technology. Transgenic mice are having a foreign gene integrated into their genome, which is showing its expression also. These are produced to study the role of various regulatory sequences in gene expression as well as the effect of expression of a foreign gene on overall physiology. Transgenic mice are also helpful in the study of genetic diseases like, various forms of cancer, diabetes. A suitable gene therapy may be designed by mutation and gene knockout studies in mice. Transgenic mice are produced by attempting gene transfer in the early embryonic stage or in fertilized eggs. Genetically transformed zygote or early embryos are then transplanted into the uterus of recipient female. Production of several offspring per pregnancy and short gestation period helps in easy screening of transgenic mice. All of the latest development in medical biotechnology has been possible only due to the convenience in handling and experimentation with transgenic mice.

Keywords: *Transgenic Mice, Medical Biotechnology, Gene Expression*

INTRODUCTION
Medical Biotechnology is the only branch of biotechnology, which directly benefits the mankind by having a direct impact on human health. A large number of medicines are being produced through biotechnology like insulin, growth hormone, etc. Where mice are used as experimental organism. Another important aspect of Medical Biotechnology is gene therapy, in which various strategies are used to treat a genetic disease, that are; 1. Gene augmentation, 2. Gene inhibition, 3. Gene targeting, etc. Any one of these strategies is designed on the basis of the studies of gene expression in a transgenic mouse model of the disease. Mouse models of various diseases are produced by either transgenes is, knock in or knockout strategies.

The mouse serves as the most appropriate model for medical science because it belongs to the class Mammalia and has same basic anatomy and physiology as that of humans. It suffers from a number of diseases that affect human also, like cancer, diabetes, etc. Moreover, by manipulating genes other human diseases can also be developed in mouse that normally not affect it, which can help in understanding of cause of disease and change in physiology as a result of disease.

Use of mouse as a model organism is very convenient due to its short oestrous cycle and gestation period. It produces several progenies at a time. It's in vitro fertilization and culture of embryo for some duration is easy. In 2002, the Mouse Genome Sequencing Consortium announced initial sequence of the mouse genome with its analysis in Nature. The sequencing of the mouse genome has been hailed as providing the experimental key to the human genome since 99 percent of human genes have equivalents in the mouse. Work on mice has already greatly advanced our understanding of gene function and the genetic causes of disease. It has greatly accelerated understanding of general physiology allowing the development of treatments of genetic disorders and improved better targeted treatments for another illness.

The first transgenic mouse was created by Jaenisch and Mintz (1974). They microinjected DNA of Simian Virus into the uteri of a healthy pseudo pregnant female mouse. The viral genes persisted in 40%

of the adult survivors. But they were not able to transfer the transgene to the next generation. Gordon *et al.* 1980, microinjected a plasmid pBR322 containing DNA sequence of Herpes Simplex and Simian Virus into pronuclei of oocytes of mice just after fertilization. This was the first report of getting transgenic mice by direct insertion of purified DNA into nuclei. Later on stable transgenic mouse germline was produced by microinjecting purified rabbit b- globin gene into the single cell embryo of mouse (Costantini and Lacy, 1981; Gordon and Ruddle, 1981). Practical application of transgenic mouse was first explained by Stewart *et al.*, (1984) by creating a genetically engineered mouse model of breast cancer. Which was followed by a number of mouse models of various diseases, like; tumour, hyper cholesteric, atherosclerosis, etc. Capecchi *et al.,* (1987) at the University of Utah, independently described a method for making knockout mice.

MATERIALS AND METHODS

Methodology

Transgenic mice are produced by two different techniques: I, Microinjection of DNA into the pronuclei of zygotes, II, Microinjection of DNA in embryonic stem cells into the blastocysts. Each process consists of several steps being the first step common, which is; Construction and purification of transgenic DNA. Transgene gene is constructed with all regulatory sequences for efficient transcription, translation and screening. To avoid any interference with the rest of the genome, gene targeting is preferred by homologous recombination. It requires a long run of similar composition of a particular locus of mouse genome where transgene is directed to be incorporated. Required transgenic construct is designed, cloned in bacterial or viral vector and then purified carefully as traces of impurities can lead to the death of a zygote or an embryo.

Then for proceeding to first method single celled zygotes are harvested from female mouse. To get the maximum number of zygotes donor females are superovulated by hormonal treatment. Always freshly fertilized zygotes are collected before the first cell division (~12 h post conception). Before microinjection to perform DNA is diluted to an optimum concentration of about 2ng/µg. Syringe pressure is also optimized to 50 hec to Pascal. Microinjection is carried out by using automated micromanipulators gently and swiftly without giving any shock to zygotes. Microinjected zygotes are incubated at 37^{o}C in 5 % CO_2 incubator until their transplantation. For implantation pseudo pregnant females are taken and ~30 zygotes are implanted per female by surgical process. After 10 days of birth small tissue sample is taken from pups, their DNA is isolated and checked for the presence of the transgene by southern hybridization or PCR (Cho *et al*., 2009).

In the second method embryonic stem cells are harvested from early embryo. These are cultured in vitro, transfected with foreign DNA and then injected into another embryo. These cells colonise the new host and contribute to germline cells also, leading to the formation of transgenic sperms. Upon mating with normal mice, transgenic offspring are produced which may be screened as in first method.

By using any of the two approaches two types of transgenic mice are created: Knock in and Knockout. Knock in mouse is created to study the expression of a foreign protein. It is generated in a way that expression of the insert is under the control of the researcher and it does not interfere with the rest of the genes. In knockout mouse information about a protein is generated by the elimination of the gene or elimination of functional domains of the protein and then its effect on the organism is studied.

Great care should be taken when deciding how to use genetically modified mice in research. Indeed primary affairs such as selecting the appropriate "wild-type" control mouse used for comparison are frequently overlooked (Crusio *et al*., 2009; Bourdi *et al*., 2011).

Applications

Transgenic mice are frequently used for various purposes, like study of an exogenous gene function, expression and regulation and study of human diseases by producing mouse model of various diseases.

After Stewart's genetically engineered mouse model of breast cancer, Palmiter *et al*., (1985) developed a type of transgenic mice that are prone to tumors through two different approaches. 1. The serendipitous approach, 2. The directed approach. In the serendipitous approach they wanted to increase the levels of

the transgene expression in the mice with elements from the DNA of tumor virus SV40; and they found that the inheritance of an SV40 antigen gene caused the mice to be susceptible to developing brain tumors. In the directed approach cellular oncogenes were expressed in transgenic mice. In spite of the failure of attempt it was found that I-ISV-TK enzyme activity was induced in the liver of the transgenic mice by metals known to activate the endogenous Mt1 gene, and the transgenes could be transmitted to progeny and retained expression.

Then a transgenic mouse as a model for the study of hypertension was designed in which the genetic basis for the disease was known. The mouse Ren-2 rennin gene was introduced into the genome of the rat and found that the expression of this gene caused severe hypertension. Further, as the transgenic mouse didn't over express active rennin in the kidney and had low levels of active rennin in their plasma, they also provided a new model for low rennin hypertension (Mullins *et al*., 1990).

Homologous recombination in embryonic stem cells was employed to produce mice lacking functional LDL receptor genes. It was concluded that the LDL receptor is responsible in part for the low levels of VLDL, IDL and LDL in wild-type mice and that adenovirus encoded LDL receptors can severely back the hypercholesterolemia effect of LDL receptor deficiency (Ishibashi *et al.,* 1993). It was demonstrated that the morphometric method provide valid and complementary information on the degree and distribution of atherosclerosis and suggested that under acute atherogenic situations lesion formation all through the aorta is determined by the similar pathological aspects, in each model. Comparison of the extent of atherosclerosis in the entire aorta within genders also indicated that male LDL receptor deficient mice had considerably more lesions than females (Tangirala *et al*., 1995).

The transgenic mouse models have been proved to be verypotent tool to analyze the physiological importance of modified quota or properties of distinct gene products, such as cardiac ion passages. A system was developed to document and evaluate variations in the electrocardiogram of mouse by using an implantable telemetry system (Mitchell *et al*., 1998). In most cases the addition of foreign gene to the genome resulted in a gain of function, such as the production of a new protein or the expression of an existing protein at a higher level or in a different range of cells. This is a generally useful approach for studying gene function or regulation, but can also be used to model human diseases caused by dominantly acting mutant proteins such as Alzheimer's disease.

In 1996, gene knockout technology combined in the same approach with tumor suppressor genetics in the development of mice that acquired cancers due to the lack of tumor suppressor genes by Jacks. Among one of the prominent application is the expression of insulin like growth factor I in differentiated muscle fibre of mouse by using viral vector. It resulted in increase in muscle mass and strength in young adult mice and prevented changes in muscle fibres due to aging in old mice (Davis *et al*., 1998). In 1999 the mouse model was tested by pharmaceuticals with an antibody vaccine that removes B-amyloid and the mouse showed improved cognitive function by Shenk *et al.,* Subsequently human clinical trials began with this vaccine. Eischen *et al*., (1999) used the Ep-Myc oncomice to demonstrate the functional importance of Arf-p53 circuit as a tumor suppressor, whose failure of action also extenuated apoptosis in Myc-expressing lymphoid cells, promoting hyper proliferation and tumorigenesis. Factor *et al.,* (2000), determined that elevated level of reactive oxygen species (ROS) may be accountable for the vast chromosomal damage and acceleration of hepatocarcinogenesis characteristic for TGF-alpha /C-myc mice. In this study they showed that vitamin E can effectively protect liver tissue from oxidative stress and can stop tumorigenic action of C-myc oncogene. In their experiment they concluded that the vitamin E lessens chromosomal damage and prevents tumor formation in liver of transgenic mouse model. Calvisi *et al.,* (2001), introduced the revival of beta catenin during liver carcinogenesis in transgenic mouse models, in relation to phenotype and tumor grade. They suggested that in liver carcinogenesis, nuclear translocation of beta-catenin and activation of wingless signaling represent an early event, providing a growth advantage in a subset of hepatic tumors with a more differentiated phenotype.

The co- expression of transforming growth factor [TGFJ alpha and C-mycproto-oncogenes has been frequently observed in human hepatocellular carcinoma [HCCI, suggesting an important action of these genes in the malignancy development of the liver (Thorgeirsson and Grisharn, 2002). Attenuation of

DNA damage response in TGF alpha /C-myc mice creates a situation that may favor in progression of hepatic cancer in this model (Hironaka *et al*., 2003). The stimulation of beta catenin gives generative and obvious asset in C-myc/TGF alpha hepatocarcinogenesis supported by Phenobarbital. Beta-catenin activation confers additional growth and invasive advantages in a model of liver cancer already accelerated by synergistic activity of the C-myc and TGF-alpha transgenes (Calvisi *et al*., 2004).

Casanovas *et al.,* (2005) have suspected a new process of "evasive resistance" to angiogenesis reducing medicines pointing the VEGF signaling pathway, comprising activation of other proangiogenic inducers that can evidently substitute for VFGF signaling so as to sustain tumor angiogenesis. Diseases having a genetic basis can be modeled with knockout, knock-in, and condition mutant gene targeted mice (Lepage and Conlon, 2006). The transgenic mouse technology is a powerful tool that can be used for creating animal models for cardiovascular disease to identify molecular pathogenic mechanisms and for identifying the physiological actions of a novel gene (Tian and Wang, 2006). Sometimes, simply the excessive activation of a specific gene can produce a phenotype that proved to be significant for the study of normal development or of any disease. For example, more production of the LDL receptor and Apo E proteins, which influence cholesterol depositions, can provide knowledge pertaining to the occurrence of atherosclerosis. The mouse has become the preferred species manipulation aimed at creating disease. Although mice are not sensitive to dietary stimulation yet, more transgenic mice have been widely produced that alter the susceptibility to atherosclerosis (Baglione and Smith, 2006). Humanized liver containing mice have functionally differentiated human hepatocytes and are less susceptible to APAP toxicity, compared to ICR mice. Transgenic mice designed to express cloned oncogenes and knockout mice with defective tumor suppressing genes have proved to be excellent models for the study of human cancer. A wide variety of these oncomice have been created to cover a broad spectrum of cancers affecting all organs of the human body and they are being improved to become more representative of human cancer. The disease symptoms and potential drugs or treatments can be tested against these mouse models (Douglas *et al*., 2007). In another study, the MMT V-Ras + Myc oncomice were used to document the functional importance of another cell cycle regulator, the cell cycle- stimulating phosphatase CDC25 A: Genetically increased expression of CDC25A in double transgenic mice has been found to accelerate mammary gland tumorigenesis, supporting the significance of up regulated expression of CDC25A in human cancers (Ray *et al*., 2007). A mouse has also been created with altered glucose metabolism by over expression of Phosphoenolpyruvatecarboxykinase in skeletal muscles, which yielded more physically fit, sexually active mice with longer life (Hakimi *et al*., 2007). *In vivo* MRS [magnetic resonance spectroscopy] analysis of modified fatty acyl unsaturation in hepatic tumor production of a TGI alpha/C-myc transgenic mouse model was performed. In correlation with the IPLC, mass spectrometry, Western blot, and microarray analyses, they were able to confirm the ability of *in vivo* MRS to detect precancerous abrasions in the mouse liver before visual neoplastic developments were distinguishable by MRI (Griffitts *et al*., 2009). Ectonucleoside triphosphate diphosphohydrolase type 5 (ENTPD5) deficient mice suffer advancing hepatopathy, hepatocellular tumors and spermatogenic arrest because ENTPD5is a soluble enzyme that hydrolyses purine nucleoside diphosphates. In humans, ENTPD5 is similar to the PCPH proto oncogene and lack of regulation of this gene is key factor in some human cancers. Such mouse models are valuable for the understanding of the influence of ENTPD5/ PCPH on cellular proliferation and neoplasia (Read *et al*., 2009).

Latest advancements in mouse models have made it possible to design mice with genetic modifications that result in hepatocyte destruction, over time, in the loss of native hepatocytes (Strom *et al*., 2010). Factor *et al.,* (2011) worked on the genomic modeling of tumor onset and progression in a mouse model of aggressive human liver cancer. In conclusion their study provides a comprehensive characterization of sequential molecular changes during a stepwise progression of preneoplastic lesions towards HCC (hepatocellular carcinoma) and highlights a critical role of metabolic disorders and innate immunity al early stages of liver cancer. It was concluded that antitumor functioning of MLN2238 in different types of mouse models of B-cell lymphoma and PCM, supports clinical development. MLN9708 is being evaluated in multiple phase I and 1 / 2 trials (Lee *et al*., 2011).

Conclusion

It has been clear from the above mentioned literature that transgenic mice played a vital role in the advancement of medical biotechnology. Not only they bear all clinical trials but provide a valuable tool during the development of any drug therapy and understanding of human physiology in response to any drug or transgene.

REFERENCES

Baglione J and Smith JD (2006). Cardiovascular Disease: Methods and Protocols. *Molecular Medicine* **129** 83-95.

Bourdi M, Davies JS and Pohl LR (2011). Mispairing C57BL/6 sub strains of genetically engineered mice and wild-type controls can lead to confounding results as it did in studies of JNK2 in acetaminophen and concanavalin a liver injury. *Chemical Research in Toxicology* **24** 794-796.

Calvisi DF, Factor VM, Loi R and Thorgeirsson SS (2001). Activation of beta-catenin during hepato-carcinogenesis in transgenic mouse models; relationship to phenotype and tumor grade. *Cancer Research* **61** 2085-2091.

Calvisi DF, Ladu S, Factor VM and Thorgeirsson SS (2004). The activation of beta catenin provides proliferative and invasive advantages in C-myc/TGF-alpha hepatocarcinogenesis promoted by Phenobarbital. *Oxford Journals Life Science and Medicine Carcinogenesis* **25** 901- 908.

Capecchi MR and Thomas KR (1987). Site directed mutagenesis by gene targeting in mouse embryo derived stem cells. *Cell* **51** 503-512.

Casanovas Hicklin DJ, Bergers G and Hanahan D (2005). Drug resistance by evasion of antiangiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors. *Cancer Cell* **8** 299-309.

Cho A, Haruyama N and Kulkarni AB (2009). Generation of Transgenic Mice. *Current Protocol Cell Biology* (John Wiley & Sons, Inc.) **42** 19.11.1-19.11.22.

Costantini F and Lacy E (1981). Introduction of a rabbit globin gene into the mouse germ line. *Nature* **294** 5836.

Crusio WE, Goldowjtz D, Holmes A and Wolfer D (2009). Standards for the publication of mouse mutant studies. *Genes, Brain and Behavior* **8** 1-4.

Davis B, Elisabeth R, Shoturm Daria I, Musaro A, Rosenthal N and Lee H (1998). Viral mediated expression of insulin like growth factor-I blocks the aging related loss of skeletal muscle function. *PNAS* **95** 15603-l 5607.

Douglas H, Erwin F, Wagner and Richard DP (2007). The origins of oncomice: a history of the first transgenic mice genetically engineered to develop cancer. *Genes Development* **21** 2258-2270.

Eischen CM, Weber JD, Roussel MF, Sherr CJ and Cleveland JL (1999). Disruption of the ARF-Mdm2-p53 tumor suppressor pathway in Myc- induced lymphomagenesis. *Genes Development* **13** 2658-2669.

Factor VM, Conner EA and Thorgeirsson SS (2011). Genomic modeling of tumor onset and progression in a mouse model of aggressive human liver cancer. *Oxford Journals Life Science and Medicine Carcinogenesis* **32** 1434-1440.

Factor VM, Laskowska D, Jensen MR, Woitach JT, Popescu NC and Thorgeirsson SS (2000). The vitamin E reduces chromosomal damage and inhibits hepatic tumor formation in a transgenic mouse model. *Proceedings of National Academy of Science* **97** 2196-2201.

Gordon J and Ruddle F (1981). Integration and stable germ line transmission of genes injected into mouse pronuclei. *Science* **214**(4526) 1244-6.

Gordon JW, Scangos GA, Plotkin Di, Barbosa A and Ruddic FH (1980). Genetic transformation of mouse embryos by microinjection. *Proceedings of National Academic Science* USA **77** 7380- 7384.

Griffitts J, Tesiram Y, Reid GE, Saunders D, Floyd RA and Towner RA (2009). *In vivo* MRS (magnetic resonance spectroscopy) assessment of altered fatty acyl unsaturation in liver tumor formation of a TGF alpha/C-myc transgenic mouse model. *Journal of Lipid Research* **50** 611-622.

Hakimi P, Yang J, Casadesus G, Massillon D and Tolentino-Silva F *et al.*, (2007). Overexpression of the cytosolic form of phosphoenol pyruvate carboxykinase (GTP) in skeletal muscle repatterns energy metabolism in the mouse. *Journal of Biological Chemistry* **282**(45) 32844-32855.

Hironaka K, Factor VM, Calvisi DF, Conner EA and Thorgeirsson SS (2003). Dysregulation of DNA repair pathways in a transforming growth factor -alpha/C-rnyc transgenic mouse model of accelerated hepatocarcinogenesis. *Laboratory Investigation* **83** 643-654.

Ishibashi S, Brown MS, Goldstein JE, Gerard R, Hammer RE and Herz J (1993). Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus mediated gene delivery. *Journal of Clinical Investigation* **92** 883-893.

Jacks T (1996). Tumor suppressor gene mutations in mice. *Annual Review of Genetics* **30** 603-636.

Jaenisch R and Mintz B (1974). Simian virus 40 DNA sequences in DNA of healthy adult mice derived from pre implantation blastocysts injected with viral DNA. *Proceedings of National Academic Science* USA **71** 250-1254.

Lee EC, Fitzgerald M, Bannerman B, Donelan J and Janz S (2011). Antitumor activity of the investigational proteasome inhibitor MLN9708 in mouse models of B-cell. *Clinical Cancer Research* **17** 7313-7323.

Lepage DF and Conlon RA (2006). Animal model for disease. *Cardiovascular Disease: Method and Protocols* **2** 41-67.

Mitchell GF, Jeron A and Koren G (1998). Measurement of heart rate and Q -T interval in the conscious mouse. *American Journal of Physiology* **274** 747-751.

Mullins JJ, Peters J and Ganten D (1990). Fulminant hypertension in transgenic rats harboring the mouse Ren -2 gene. *Nature* **344** 541-544.

Palmiter RD, Chen HY, Messing A and Brinster RL (1985). SV4O enhancer and large T Antigen are instrumental in development of choroids plexus tumors in transgenic mice. *Nature* **316** 457-460.

Ray D, Terao Y, Fuhrken PG, Ma ZQ, DeMayo FJ, Christov K, Heerema NA, Franks R, Tsai SY and Papoutsakis ET (2007). Deregulated CDC25A expression promotes mammary tumorigenesis with genomic instability. *Cancer Research* **67** 984-991.

Read R, Hansen G, Kramer J, Finch R, Li L and Vogel P (2009). Ectonucleoside triphosphate diphosphohydrolase type 5 (Entpd5) deficient mice develop progressive hepatopathy, hepatocellular tumors and spermatogenic arrest. *Veterinary Pathology* **46** 491-504.

Sato Y, Yamada H, Iwasaki K, Tateno C, Yokoi T, Yoshizato K and Horii I (2008). Human hepatocytes can repopulate mouse liver: histopathology of the liver in human hepatocytes transplanted chimeric mice and toxicological responses to acetaminophen. *Toxicology Pathology* **36** 581- 591.

Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H and Guido T (1999). Immunization with amyloid Beta attenuates Alzheimer disease like pathology in the PDAPP mouse. *Nature* **400** 173-177.

Stewart TA, Pattengale PK and Leder P (1984). Spontaneous mammary adenocarcinomas in transgenic mice that carry and express MTV/myc fusion gene. *CelI* **38** 627- 637.

Strom SC, Davila J and Grompe M (2010). Chimerjc mice with humanized liver tools for the study of drug metabolism, excretion, and toxicity. *Hepatocytes: Methods and Protocols* **640** 491-509.

Tangirala RK, Rubin EM and Palinski W (1995). Quantization of atherosclerosis in murine models: correlation between lesions in the aortic origin and in the entire aorta, and differences in the extent of lesions between sexes in LDL receptor deficient and Apo lipoprotein E- deficient mice. *Journal of Lipid Research* **36** 2320-2328.

Thorgeirsson SS and Grisharn Joe W (2002). Molecular pathogenesis of human hepatocellular carcinoma. *Net Genet* **31** 339-346.

Tian XL and Wang QK (2006). Cardiovascular Disease: Methods and Protocols. *Molecular Medicine* **129** 69-81.