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RESEARCH ARTICLE

KNOCKOUT MOUSE MODELS FOR HUMAN DISEASES

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ABSTRACT

Knockout mouse models are the mice used as a model to study human diseases by knowing the function of genes causing disease or study the function of unknown genes It can be done using transgenic mice with introducing a foreign gene or deleting an existing gene in them. Knocking out a gene that is corresponding to disease will cause the disease in mice. Knockout mice resemble the human disease in similar gene which is used to find new drug targets and try different treatments on mice to see the effect. To introduce a knockout gene in mice, either homologous recombination or Cre-lox system must be used. Knockout mice are produced to study and compare different dysfunction genes with human diseases to come up with a treatment for different cases.

INTRODUCTION

Gene knockout is a technology by using gene targeting in embryonic stem cells to produce mice that contain a deletion of the desired gene. This technique is very useful tool used to identify the function of the gene and has been successfully used in different fields of biology and medicine (Tsien *et al*., 1996). Gene knockout has been very useful in the field of neuroscience in discovering the basis of cellular and molecular behavior (Silva *et al*., 1992), for example if a mouse is deficient in α-calcium-calmodulin-dependant kinase II (αcaMkII), it shows impairments both in the induction of long term potentiation at hippocampal synapses and the acquiring spatial memory. The result supported the notion that synaptic strengthening the formation of certain types of memory (Morris *et al*., 1986). These conventional gene knockout techniques provided animals to inherit gene deletion in all cell type. This kind of genome deletion can lead to severe defect and premature death which provide with the analysis on post development gene functions. If the mutated mice completed its development, the reading of the result often runs on two difficulties. First is doing global gene knock out to attribute different phenotype to a specific type of cells or tissue. Second is hard to exclude the possibility that an abnormal phenotype that is observed in adult animals arise indirectly from the development defect (Tsien *et al*., 1996). Transgenic mouse is made using gene transfer technology which needs to be transfer into early embryos; knockout mice are used as animal model to study the development of humans, disease and disorder. However to create animal models for human diseases, gene knockout is required which generate animals that had their genome altered (Cho *et al*., 2009).

The history of gene knockout began with the help of two instrumental discoveries that lead to creating a knockout mice, the isolation of stem cells and homologous recombination. The importance of these techniques were made clear when Mario Capecchi, Oliver smithies and martin Evans demonstrated the effectiveness and were rewarded Nobel prize for establishing a model of the knock out mouse (Vogel, 2007). One of the major steps in making knockout mice was the isolation of embryonic stem cells (ES cells). The ES cells were isolated from the inner cell mass of a 3.5 days mouse blastocyst (Hall *et al*., 2009). These stem cells could progressively grow in tissue culture and were pluripotent. By injecting into a mouse blastocyst, were able successfully to generate chimeric mice. By producing chimeric mice the stem cells were stably able to differentiate multiple lineages of cell and contributed in the development of the embryo of the mouse. Transmission of the germ line was achieved with the help of pluripotent ES cells. The result was observed in the offsprings and the transmission of the coat color indicated that these cells were derived from ES cells. Thus, stem cells can fuse into the germ line of chimeric mice.
The second major important step of knockout mice was discovering the homologous recombination in mammalian cells. Lin *et al*., (1985) demonstrated the possibility of a

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homologues recombination to reconstruct a functional thymidine kinase gene in mouse L cells. Smithies *et al*., (1985) performed modification in the β-globin locus with inserting a neomycin resistance gene. This work had provided the necessary means to accurately target DNA inserted to the genetic locus of choice, opposite to the random integration that is used in transgenic technology. The combination of these technologies made it easier to create knockout mice. The first genetic locus to test gene targeting was enzyme hypoxane-guanosine phosphoribosyl transferase (hprt) which was tested by using6-thioguanine, for restoring the gene, hprt null cells with hypxanthine, aminopterin, thymidine (HAT) medium can be selected. hprt gene is x-linked so that means one allele is needed to be targeted for selecting a drug. Doetschman *et al*. (1987) proved the possibility to correct mutant hprt gene with homologous recombination in ES cells, although, Thomas and Capecchi (1987) demonstrated distribution in the targeted gene. These experiments cleared the way to find and target non-selectable genes like int-2 and c-abl knockout mice. Since the rise of the first knockout showed a massive growth in animal models derived using this techniquemouse stocks with spontaneous or radiation-induced mutation is considered a thing of the past, the mutated alleles have not correspond with the most cloned genes with using homologous recombination , we can directly utilize to disturb targeted allele (Mak, 1998). For creating a strategy it is usually planned shortly after mouse gene is cloned. Hence, targeted recombination became a well-known tool used to inactivate a gene to be study its function in vivo.

For doing experiments scientist commonly used small animals model like mouse for example to test different treatment and effects, mainly because it's relatively easy to acquire, breed, transport and to house. The mouse genome is a well-studied and there are a high number of different strains that are well suited with almost all experiment. They reproduce fast, short period to get the next generation, they can easily be maintained in small area with little cost needed. But on the other hand, their small size can cause some problems because of the low quantity tissue obtained in some experiments but the high volume lessens this impact. As all animal models, using mice helped scientists to progress into new advancement but some people debate whether it is humane to use animals as they are subjected to danger and ultimately death (Streba *et al*., 2012). Experiments on animal are dated to 500 BC; animal experimenting first started with observing the structure and functions of organ and was recorded in Greece. In 1800 the main tool that use passive voice sentence had to study physiological process that occurs in organism was by animal experimenting. Louis Pasteur used dogs to test anti-rabies vaccine in the 1885, whereas diphtheria anti-toxin was synthesize by injecting toxin into guinea pigs. In the 19th century a law has passed down where this law passed and who passed it concerning experimenting with live animal, as Jeremy Bentham proclaimed, the ethic principle that tried to correlate the benefit obtained with the level of action required to obtain it. But also encourages the sense of morality in using animals for experiment, due to experimenting with animal and willingly subjecting them to pain. In 1824, in England the society for the preservation of cruelty to animals was founded, set to be the first organization to have given animals right (Streba *et al*., 2012).

Knockout means to remove a certain gene completely from a genome or inactivate the gene forever by different methods. Knockout mouse models are the mice used as a model to study human diseases by knowing the function of disease causing genes. It can be used also to study the unknown genes functions (Kitada *et al*., 2007). Mice were used because of the similarities between mice and humans in biochemical, genetic, and physiological aspects. In particular, to make use of mice must undergo genetic manipulation to make them have the human disease gene. Mice are also used because their reproduction took less than three months and have a big litter size (Doyle *et al*, 2012). Transgenic mice are the mice that are genetically modified by inserting a foreign gene into them to modify or add new traits in the mouse. These organisms with the transgenes to study certain gene function like cancer genes, and try different treatment studies on such organism to find cure for it. One of the best ways of studying gene function is to inactivate the gene and see the effects of inactivation. Inactivation means the deletion or disruption of the gene sequence, and that would lead in non- expression of disease genes. The method could be used is homologous recombination. The production of knockout mouse should go in the order of producing a vector to target the identified gene, inserting the sequence, selection of cell with insertion, take embryonic stem cells with knockout gene, injecting the embryonic stem cells in fresh mouse embryo, and crossing mice to get homozygous mice (Nature, 2015).

To cause a deletion in the genome, the deleted sequence must be identified and known even after deletion. Then, the vector is constructed, and it should contain the gene of interest which is a marker gene like GFP or the common marker Neo^R gene. It should also, contain two DNA sequences fragments that would match with the targeted gene. Also, it must have a second negative selection marker gene which is thymidine kinase (TK) as in figure 1. The Neo^R gene is a neomycin resistance gene that makes the targeted or transformed cell resist neomycin antibiotic in the media, so the grown cells contain the sequence. TK is ganciclovir-sensitive gene that will help to know if the gene is replaced with Neo^R because random insertions may occur, so to know whether Neo^R and TK are inserted or Neo^R only. The dead cell has the TK and Neo^R, while growing cells will have Neo^R only (Nature, 2015).

In this step which step ES cells would be obtained because they have the pulripotency ability in which they can differentiate in most kind of cells that will lead to have an embryo (Nature, 2015). Then, these cells are under brief electrical pulse which is called electroporation. It causes the cell membrane to be more permeable which will lead to the uptake of vector in the cell. After that, the cells are cultured in a media containing neomycin to get the transformed cells with Neo^R. Then, the selected cells are cultured in media containing ganciclovir to select cells that took Neo^R only. Cells that grow on media will have Neo^R only not Neo^R and TK together (Nature, 2015). Write in the form of story

The heterozygous ES cells l be injected in a normal fresh mouse embryo to make it part of embryo cells. Later this cell placed in a surrogate mother to get an offspring with both normal cells and heterozygous knockout cells (Nature, 2015). The newly produced mouse is called chimeric mouse. The chimeric mouse identified by the coat color in which the knockout cells were originally taken from a black father that mated with a white female. In this condition the chimeric mouse will have both colors in its skin coat (Nature, 2015).

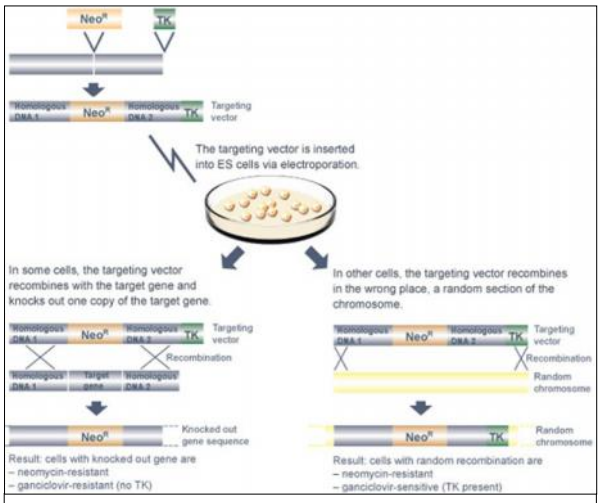

Figure 1: vector construct and selection method

The obtained chimeric mouse is crossed with a normal white mouse to produce a homozygous knockout mouse, so 50% of black offspring are heterozygous. Then, a heterozygous male is crossed with a heterozygous female to have a 25% homozygous offspring, which can be identified using a molecular biology technique like PCR (Nature, 2015).

A phenotypic comparison between the knockout mouse with a wild type normal mouse to see the differences and this might be used to see the unknown function of gene. A study was done to have a knockout mouse with apoE gene disrupted which cause Atherosclerosis (Gupta *et al*., 1997).

DISCUSSION

Knockout mice technology saved a lot of people by which the scientist understood the mechanism of the diseases caused by certain gene having mutations or dysfunction. Using knock out methods, scientists can compare the normal phenotype with mutated phenotype in order to understand the cause of a specific disease. And can cure many diseases. In the atherosclerosis study obtained a mouse with the knockout gene and further studies was done on it, but generally, the last product will be an organism with a disrupted or deleted gene for more researches (Elia, 2009). These mice can be used to test drugs on them in case we deleted a gene that will cause disease if got deleted, instead of testing drugs on human with such diseases, do that on knockout mice. There are many marker genes that can be used but Neo^R because it is the most common together with TK gene. The mechanism used in this process is called homologous recombination, while there are many other ways like Cre-lox system, in which the Cre enzyme can degrade or cleave the sequence between the two loxP sites on the genome. In which the Cre enzyme work in a specific region of the DNA (Ventura, 2004). This technique is more accurate than the homologous recombination technique. This method can be used to study and discover a new treatments for the genetic diseases in which the cause is deletion or disruption of the gene sequence. Knockout mouse technology can be improved in many ways to have accurate results. In my opinion, it can be used or tried on monkeys since they are closely related to Human beings, and they have mostly the same metabolic pathways and mechanisms. Knocking out a monkey or any animal from the Homo Genus will help a lot in understanding of such complicated mechanisms for genetic diseases.

CONCLUSION

Knockout mice technology saved a lot of people through studying different drug effects and efficiency. Human diseases that are related to genes are a common practice in knockout mice to study them and find and test drug on such genes. Knockout nice technology still needs to be practiced on many genes that are still unknown for their function.

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