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Review

Animal models for human genetic diseases

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The study of human genetic diseases can be greatly aided by animal models because of their similarity to humans in terms of genetics. In addition to understand diverse aspects of basic biology, model organisms are extensively used in applied research in agriculture, industry, and also in medicine, where they are used to model and understand disease and to test new systems of treating disease. The range of model organisms is large, extending from microbes to primates. Mice are widely considered to be the prime model of genetic human disease. In this review, we will mainly focus on the mouse, which is the mammalian species with the best studied genetics.

Key words: Animal models, mice, genetic diseases.

INTRODUCTION

Many drugs, treatments and cures for human genetic diseases have been developed with the use of animal models (Chakraborty et al., 2009; Kari et al., 2007). When animal models are employed in the study of human disease, they are frequently selected because of their similarity to humans in terms of genetics, anatomy, and physiology. Mapping the human genome was not the only scientific focus of the Human Genome Project; at its outset, the value of sequencing genomes of model organisms was recognized. Such organisms include a variety of species, some of which have been particularly amenable to genetic analysis. In part, the sequencing of smaller genomes was also considered as a pilot for large-scale sequencing of the human genome. Also, animal models are frequently having advantage for experimental disease research because of their infinite supply and ease of handling (Simmons, 2008).

Rodents are the most common type of mammal employed in experimental studies. Among these rodents, the majority of genetic studies, especially those involving disease, have employed mice, because their genomes are so similar to that of humans. Mouse as an animal model provides a novel way to study a signaling pathway in genetic disorder that is critical for embryonic development (Barrott et al., 2011). Mouse models can also be used in medicine and diagnostic purposes such as antibody production. Then these antibodies after conjugation with specific enzyme can be used for the diagnosis of various infections, using enzyme linked immunosorbent assay (ELISA) (Sharif and Hashmi, 2010). Other common experimental organisms include fruit flies, zebra fish, and chicks. The most versatile organism to study mammalian gene function is the mouse as there is an extensive tool kit for modifying the genome and specific genes encompassing gene-driven and phenotype-driven approaches (Rosenthal and Brown, 2007).

Mice are popular as an animal model because of their availability, low cost, size, fast reproduction rate and ease of handling (Simmons, 2008). They are widely considered to be the prime model of inherited human disease and share 99% of their genes with humans (Sanger Institute Press, 2002). These diseases include several types of cancer, heart disease, glaucoma, hypertension, metabolic and hormonal disorders, obesity, diabetes, osteoporosis, skin pigmentation diseases, deafness, blindness, neurodegenerative disorders (such as Huntington's or Alzheimer's disease), birth defects (such as cleft palate and anencephaly) and psychiatric disturbances (including anxiety and depression) (Rosenthal and Brown, 2007).

Mouse models for a rare genetic disorder of the blood platelets, May-Hegglin anomaly (MHA) showed same symptoms as occur in humans (American Institute of Physics, 2011). Also in genetic prion disease, histopathological examination of transgenic mice

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brain samples served as an ideal platform for the investigation of this disease similarly to human (Levi et al., 2011).

INVERTEBRATE MODELS

Invertebrate models are often easy and inexpensive to maintain, and can offer very large numbers of offspring and rapid generation times. These characteristics make them ideally suited to high-throughput genetic screening. The roundworm *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster* are the two most widely studied invertebrates (Segalat, 2007). *D. melanogaster* is employed in a wide variety of studies ranging from early gene mapping, via linkage and recombination studies to large scale mutant screens to identify genes related to specific biological functions. Some biological malfunctioning in human can also be observed in drosophila. Myo VIIa protein defect which causes usher syndrome in human (Irshad et al., 2005) also lead to deafness in *drosophila* (Todi et al., 2005). *Caenorhabditis elegans* is valuable for studying the development of simple nervous systems and the aging process (Spradling et al., 2006).

VERTEBRATE MODELS

Zebrafish

There has been a very significant increase in the use of zebrafish for the study of disease processes in humans. Zebrafish reproduce easily and quickly and have morphological and physiological similarities to mammals. Those who study zebrafish hope that use of the species will lead to progress in several aspects of the drug development process, including target identification, disease modelling, lead discovery and toxicology (Zon and Peterson, 2005). Zebrafish models have been developed for several human diseases, including blood disorders, diabetes, neurodegenerative diseases and muscular dystrophy (Rubinstein, 2003).

The chick

The chick *(Gallus gallus)* embryo is easily obtained and also has the advantage of being very large and relatively translucent, making delicate microsurgical manipulations easy. It thus offers an excellent system in which molecular studies can be combined with classical embryology. The chick is a good model largely because its embryo is easily obtained. Popular experimental manipulations of chick embryos include surgical manipulations and tissue grafting, retrovirus mediated gene transfer, electroporation of developing embryos and embryo culture. RE1-silencing transcription factor (REST) region in Human phenotype DFNB55 for hearing impairment is also expressed in the chicks. The REST gene was found to be expressed in supporting ear cells of chick auditory epithelium (Irshad et al., 2005; Roberson et al., 2002). Rapid advances are being made in chicken transgenics, embryonic stem (ES) cell technology, and cryopreservation of sperm, blastodisc cells, primordial germ cells and ES cells (Stern, 2005).

The frog

Frogs of the genus *Xenopus* (African clawed frog) have been particularly important models for investigating both embryonic development and cell biology. There has also been seminal work on chromosome replication, chromatin and nuclear assembly, cell cycle components, cytoskeletal elements and signaling pathways (Beck and Slack, 2001).

The rat

The rat, being considerably larger than the mouse, has for many years been the mammal of choice for physiological, neurological, pharmacological, and biochemical analyses. The bigger size of rat is more advantageous than mouse for collecting tissues (more tissue) and for surgeries. A complete genome sequence has been published (Gibbs et al., 2004). Rat models are also used for Human deafness diseases. For example a hearing disorder due to mutation in Myosin XVA gene causes DFNB3 phenotype in human (Irshad et al., 2012). The mouse and rat models used for this disease are shaker 2 mouse and LEW/Ztm-*ci2* rat respectively (Held et al., 2011). Genetic analysis in laboratory rats, however, is much less advanced than in mice. It is partly because of the relatively high cost of rat breeding programs and because until recently it has been much more difficult to modify the rat germ line by gene targeting (Herrera and Ruiz, 2005).

The mouse

The mouse *(Mus musculus)* is particularly well suited to genetic studies and is an extensively used model of mammalian development. Its short generation time like rat has allowed large-scale mutagenesis programs and extensive genetic crosses and various features aid in mapping genes and phenotypes. Mouse is more amenable to experimental research than rat. The ability to construct mice with predetermined genetic modifications to the germ line (by transgenic technology and gene targeting in embryonic stem cells) has been a powerful tool in studying gene function and in creating

models of human disease (Davidson and Christiaen, 2006).

One of the significant health problems in the world population is hearing disorders or deafness (Irshad et al., 2005). Mouse models for deafness have revealed a variety of defective structures and functions found in humans. These models were also found helpful in the elucidation of some patho-physiological processes in the human ear (Leibovici et al, 2008). Usher syndrome is a deafness-blindness disorder. Many of the mouse models for hearing impairment disorders such as Usher syndrome have been identified due to their characteristic circling and head-tossing behavior that results from vestibular dysfunction (Williams, 2008). Another hearing disorder due to mutation in Transmembrane cochlear expressed gene 1 (Tmc1) were identified in both recessive and dominant forms of hearing loss in humans (Santos et al., 2005). The tmc1 protein might have an important function in K+ channels of inner hair cells. The mouse models which have been selected for mutation in tmc1 gene are Tecta knockout deafness (dn) and Beethoven (Bth) mouse (Kurima et al., 2002; Vreugde et al., 2002). In recent years, it has become essential to use mouse models as a tool for studying genetic diseases, especially in cases of monogenic disorders (Ganeshan et al., 2010).

COMPARISON OF MOUSE WITH LARGE ANIMAL'S MODELS

Large animal models such as cat, cow, sheep, pig and dog may be used as genetic model for human disease. Rodents however, have relatively short generation times and large numbers of offspring and so are more suited to genetic studies. Large animal models are disadvantaged by practical limitations and ethical concerns. The history of the use and development of large animal models goes back decades when researchers first identified and characterized a canine model of a genetic disease now known as a lysosomal storage disease (Krabbe disease) more than 45 years ago (Fankhauser et al., 1963; Haskins, 2009). A distinct advantage of the large animal models is that they manifest clinical signs, which originally brought them to the attention of the veterinary and research communities. In contrast, murine targeted genetic disease models may manifest biochemical characteristics but not the clinical characteristics of the human disorder, as in the mouse model of Tay-Sachs disease (Yamanaka et al., 1994). But it also depends on the disease that is modeled. Several symptoms of psychiatric disorders can be modeled very well in rats and mice.

In addition, large animal model species are, relative to murine models, outbred, better reflecting human patients, and they mount robust immune responses, as mentioned earlier. However, these qualities do not always ensure an evaluation predictive of the potential response in humans. For example, preliminary work in both canines and nonhuman primates on the use of adeno-associated viral vectors to treat hemophilia B in humans failed to reveal immune-mediated responses that limited the effectiveness of the gene therapy in humans (Manno et al., 2006; Nichols et al., 2009). In terms of comparison, large animal models are more expensive experiments to maintain and they require a longer time scale (Ellinwood and Colin, 2009).

METHODS OF INDUCING MUTATIONS IN MOUSE

This task is approached in two main ways: one that is directed and disease driven, and the other one is non-directed and mutation driven. The non-directed method uses radiation and chemicals to cause mutations. One common technique associated with this method is the large-scale mutation screen. On the other hand, the directed, disease-driven approach can employ any one of a number of techniques, depending on the exact type of mutation involved in the disease under study. Common directed techniques include transgenesis, conditional gene modifications, single-gene knock-outs and knock-ins and chromosomal rearrangements (Hardouin and Nagy, 2000).

Transgenesis is a directed approach, to make a transgenic mouse, an exogenous DNA sequence is transferred into the germ line of an animal. Genetically modified mice can be produced by a variety of routes. Transgenes can be inserted into the germ line by direct transfer into the zygote, gametes, and embryonic or somatic cells that eventually contribute to the germ line. Of the possible routes described above, the fertilized oocyte and embryonic stem cell routes are the most popular (Strachan, 2010). Mouse models with a single gene or multiple genes mutation are excellent research tools for understanding the role of a human genetic disease (Chadman et al., 2009).

Non-directed (phenotype driven) and large-scale animal mutagenesis

Two of the most effective ways to produce mutations are by exposing organisms to X-rays or to the chemicals such as triethylenemelamine (Whitney and Russell, 1980) and N-ethyl-N-nitrosourea (ENU) (Johnson and Lewis, 1981). ENU can produce mutations with many different types of effects, such as loss and gain of function (Simmons, 2008).

X-rays often cause translocation mutations and large deletions that involve multiple genes (Bedell et al., 1997), whereas ENU treatment is linked to point mutations which mean mutations within single genes (Hardouin and Nagy, 2000).

Transposon-mediated insertional mutagenesis

As an alternative, mutagenesis methods have been developed whereby known DNA sequences are allowed to integrate randomly into animal genomes. Such insertional mutagenesis involves the inactivation of an endogenous gene through the integration of either defective reporter transgenes (gene trapping) or certain transposons. As a major step toward understanding human genes and modeling disease, international consortia have very recently been using a combination of mutagenesis approaches to produce mutant phenotypes for all of the 20,000 or so mouse genes (Strachan, 2010).

Transposons cause random insertional gene inactivation by jumping within a genome. Transposons are mobile elements: they can move around to different positions within the genome of a single cell. As they move to new locations, they sometimes integrate into genes and can inactivate them. DNA transposons have been widely used in germ-line mutagenesis in *D. melanogaster* and C. *elegans.* In addition to applications involving germ-line mutagenesis, vertebrate transposon systems can be used in the mutagenesis of somatic cells for cancer gene discovery and in modeling somatic cancers. They can also be used in standard transgenesis via pronuclear microinjection, offering both high efficiency and single-copy transgenes (Dupuy, 2010).

Directed (genotype driven) mutation

As opposed to the use of X-rays and ENU, transgenesis is a directed approach. Transgenic animals are produced by adding foreign genetic information to the nucleus of embryonic cells, thereby inhibiting gene expression. This can be achieved by either injecting the foreign DNA directly into the embryo or by using a retroviral vector to insert the transgene into an organism's DNA. The first mouse gene transfers were performed in 1980 (Hardouin and Nagy, 2000). Both knock-out and knock-in models are ways to target a mutation to a specific gene locus. These methods are particularly useful if a single gene is shown to be the primary cause of a disease. Knock-out mice carry a gene that has been inactivated, which creates less expression and loss of function; knock-in mice are generated by inserting a transgene into an exact location where it is overexpressed (Brown et al., 2006). The mouse genome database (MGD) is the community of laboratory mouse. MGD has obtained all genome data generated by the International Knockout Mouse Consortium (IKMC) to see associations between mouse models and human genetic diseases (Blake et al., 2010).

Both knock-in and knock-out animals are generated in the same way: a specific mutation is incorporated into the endogenous gene, and then it is transferred to the next generation through breeding. The use of embryonic stem (ES) cells is required for this technology. ES cells are totipotent cells that can be cultured from early embryos (Evans and Kaufman, 1981). Using homologous recombination in ES cells create site-specific mutations necessary in genes to produce accurate models of defects caused by human chromosomal rearrangements. In this way, many types of mutations can be introduced into a model's gene including null or point mutations and complex chromosomal rearrangements such as large deletions, translocations or inversions (Rosenthal and Brown, 2007).

Gene targeting by zinc finger nucleases

Two types of recent technological development are now offering the prospect of rapidly extending gene targeting to many other mammals and vertebrates. One breakthrough involves novel pluripotent stem cells and the other breakthrough is described in this section and concerns zinc finger nucleases, artificially constructed endonucleases that are designed to make a doublestrand DNA break at just one location in a genome, within a predetermined target gene. Successful gene targeting with zinc finger nucleases was first conducted in zebrafish in 2008 and in rats in 2009 Zinc finger nucleases are engineered to have separate DNA-binding and DNA-cleaving domains and are produced as fusion proteins by ligating different protein-coding DNA sequences. Like restriction nucleases, zinc finger nucleases work as dimers. To make a double-strand DNA break, the two monomers bind to and cut complementary DNA strands (Mino et al, 2009).

Gene knockdown by RNA interference

RNA interference (RNAi) is a very rapid, straightforward method that is used extensively to block expression of specific target genes in cultured cells from a variety of different animal species. By cleaving transcripts, RNAi can cause a severe decrease in the expression of a target gene, but not normally the complete abolition of gene expression that is expected in gene knockouts. Despite this restriction, highly efficient *in vivo* RNAi knosckdowns have produced a variety of informative loss-of-function phenotypes. *In vivo* RNAi has been performed on a large scale in *D. melanogaster* and *Caenorhahditis elegans.* RNAi knockdown can result in developmental phenotypes in rodents, and successful RNAi-mecliated gene knockdowns have also recently been performed in monkeys with the aim of testing the efficacy and safety of RNAi therapeutics (Strachan, 2010).

Conditional gene inactivation

Microbial site-specific recombination systems are used naturally in microbes, including bacteria and yeast. In such systems, the recombinase specifically recognizes a

short defined DNA sequence and will induce recombination between two copies of the recognition sequence. Recognition sites for microbial recombinases can be engineered easily into gene-targeting vectors and can be inserted into specific locations in an animal genome. In genetically modified mice, the most widely used site-specific recombination systems are the Cre-loxP system originating from bacteriophage PI, and FLP (flippase) system from the plasmid of *Saccharomyces cerevisiae.*

The Cre-loxP system has been applied in many different ways in genetically modified mice. It can allow the site-specific integration of transgenes, the conditional activation and inactivation of transgenes, and the deletion of unwanted marker genes. The mice containing the Cre recombinase under the control of tissue-specific or inducible regulatory elements are crossed to the mice with the desired loxP sites. When Cre is expressed, recombination occurs at the loxP sites, which delete the intervening sequences, and the resulting mutation is induced in specific regions and times (Simmons, 2008).

CONCLUSIONS

Animal models are essential for our investigation of how genes function in cells, in awareness of human disease, and in testing novel drugs and have proven to be a useful tool for discovering targets for therapeutic drugs (Sleeper et al., 2009). Nonetheless, despite promising results with certain preclinical treatments in animal models, the same treatments do not always translate to human clinical trials. As a result, many diseases are still incurable. Most available animal models are made in mice, and they recreate some aspects of the particular disease. This statement is particularly true for neurodegenerative diseases because mice are the primary models for studies of the roles of specific genes in normal development and physiology of the nervous system. However, these studies can be difficult to extrapolate to human brain function. One reason that mouse models might not completely mimic human disorders is that mice simply might not be capable of expressing some cognitive human disease symptoms that are apparent to the observer (Wolfgang and Golos, 2002). Moreover, the lack of disease expression may be due to evolutionary distance. For example, Huntington's disease patients show dyskinesia (involuntary movements), whereas mice do not, such as R6/2 mice models do not show dyskinesia (McBride et al., 2008; Watson et al., 2012).

Perhaps using nonhuman primates might alleviate some of these discrepancies because their physiology is closer to that of humans. In fact, some researchers have pursued this possibility despite the technical difficulties and additional costs to perform transgenesis in primates. For example, a transgenic model of Huntington's disease was recently developed using rhesus macaques that replicated some of the characteristic pathologies of the disorder as it occurs in humans (Yang, 2008).

Theoretically, nonhuman primates should be the best animal models of disease. The genetic diseases reflect many facets of model development. Scientists identified many of these diseases because of striking and obvious clinical signs, often in the veterinary pediatric period (Haskins, 2009; Sleeper et al., 2009; Wang et al., 2009). Among large animal models, great apes and chimpanzees have rarely been used as disease models because of ethical concerns, the large cost in maintaining them, and various practical difficulties. Various small nonhuman primates are tested for research but are not as well suited to genetic analyses. Instead, rodents (rats and particularly mice) have been widely used in modeling disease and investigating gene function.

Genetic manipulation of animals normally involves introducing foreign DNA into the germ line to create a transgenic animal. New genetic technologies are increasing the range of disease models. Until recently, knocking out gene function in vertebrates was very largely performed in mice (Strachan and Andrew 2010). Being mammals, rodents are quite closely related to us at biochemical, physiological, and genetic levels, and there are many practical advantages in using them as experimenral models. Mice are less expensive to maintain than rats and have been particularly friendly to genetic analyses. In conclusion, mouse is the principal model for understanding gene function and mammalian development, for modeling human disease and testing treatment regimens.

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