Transgenic and Knockout Mice in Human Disease Research: Novel Insights into Pathophysiology and Perspectives.

Dra. Fatchiyah, M.Kes. Ph.D.
Brawijaya University, Jl. Veteran, Malang 65145
Email: fatchiya@brawijaya.ac.id

ABSTRACT

The ability to engineer the mouse genome has proven useful for a variety of applications in research, medicine and biotechnology. The development of transgenic and knockout technologies has driven an explosion in new animal models of disease. These engineered diseases are a departure from previous animal models in that pathological syndromes are created from *a priori* assumptions about how disease pathogenesis could develop. Both of mice models have become powerful reagents for modeling genetic disorders, understanding embryonic development and evaluating therapeutics. These mice and the cell lines derived from them have also accelerated basic research by allowing scientists to assign functions to genes, dissect genetic pathways, and manipulate the cellular or biochemical properties of proteins. Such models have been useful in providing new information on the functions of receptor of the insulin or insulin-like growth factor family have been implicated in the regulation of pancreatic β-cells and insulin secretion. However, the contrived nature of the systems might generate false information, unless validated by careful reference to human disease and spontaneous disease in other animal models.

Keywords: transgenic, knockout mouse model, *pathological function*

The completion of a high-quality, comprehensive sequence of the human genome, in this fiftieth anniversary year of the discovery of the double-helical structure of DNA, is a landmark event. The genomic era is now a reality. One of the key and distinctive objectives of the Human Genome Project (HGP) has been the generation of large, publicly available, comprehensive sets of reagents and data that, along with other new, powerful technologies, comprise a toolkit for genomics-based research. Genomic maps and sequences are the most obvious examples. Others include databases of sequence variation, clone libraries and collections of anonymous cell lines. The continued generation of such resources is critical, in particular: Genome sequences of key mammals, vertebrates, chordates, and invertebrates. Comprehensive reference sets of coding sequences from key species in various formats, for example, full-length cDNA sequences and corresponding clones, oligonucleotide primers, and microarrays Comprehensive collections of knockouts and knock-downs of all genes are selected animals to accelerate the development of models of disease.

Mice as Model Disease

Classic genetic analyses are performed by observing a phenotype, designing the necessary cross-pollinations or mating, and using the resulting population to perform statistically significant

This paper is presented at the International Seminar, "**Management Strategy on Animal Health and Production Control in the Anticipation of Global Warming for the Achievement of Millennium Developmental Goals.**" This seminar is hold on June, 3-4th 2008 at the ELMI Hotel Surabaya, East Java, Indonesia

experiments to find the mutation and to understand the function of the altered gene. Inherited human diseases provide researchers with many phenotypes, and although the human is the mammal we are generally most interested in learning more about, it is also the one animal we cannot use for genetic experiments for obvious ethical reasons. Mice naturally develop conditions that mimic human disease, such as cardiovascular disease, cancer and diabetes, and the inbred laboratory mouse has therefore been used as a model organism to study inherited human diseases for nearly a century.

Developments in molecular biology and stem cell biology over the last 20 years have allowed researchers to create custom-made mice through gene targeting in mouse embryonic stem (ES) cells. Site-directed mutagenesis in embryonic stem cells and the phenotypic characterization of the corresponding knockout mouse, allows researchers to study gene function as it relates to the entire organism.

Transgenic Mice

One of the simplest ways to study gene function in a mouse is exogenous expression of a protein in some or all tissues. For this type of genetic modification, a new piece of DNA is introduced into the mouse genome. This piece of DNA includes the structural gene of interest, a strong mouse gene promoter and enhancer to allow the gene to be expressed and vector DNA to enable the transgene to be inserted into the mouse genome. Successful integration of this DNA results in the expression of the transgene in addition to the wild type, basal (endogenous) protein levels in the mouse. Depending on the goal of the experiment, the transgenic mouse will exhibit over-expression of a non-mutated protein, expression of a dominant-negative form of a protein, or expression of a fluorescent-tagged protein. By definition, transgenesis is the introduction of DNA from one species into the genome of another species. Many of the first transgenic mice fit this description well as they were generated to study the overexpression of a human protein, often an oncogene. Currently, the phrase "transgenic mouse" generally refers to any mouse whose genome contains an inserted piece of DNA, originating from the mouse genome or from the genome of another species, and the term includes the standard transgenic mouse as well as a knockout mouse (see below).

To generate a standard transgenic mouse, a bacterial or viral vector containing the transgene and any desired markers are injected into a fertilized mouse egg. The DNA usually integrates into one or more loci during the first few cell divisions of preimplantation development. The number of copies of the transgenic fragment can vary from one to several hundred, arranged primarily in head-to-tail arrays, and the transgenic founder mice are mosaic for the presence of the transgene. Founders are very likely to have germ cells with the integrated transgene, and therefore will be able to vertically transmit the integrated gene, and all cells of the progeny transgenic mouse contain the transgene. This method is relatively quick, but includes the risk that the DNA may insert itself into a critical locus, causing an unexpected, detrimental genetic mutation. Alternatively, the transgene may insert into a locus that is subject to gene silencing. If the protein being expressed from the transgene causes toxicity, excessive overexpression from

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multiple insertions can be lethal to some tissues or even to the entire mouse. For these reasons, several independent lines mice containing the same transgene must be created and studied to ensure that any resulting phenotype is not due to toxic at the site of transgene insertion.

ES Cells & KO Mice

Embryonic stem (ES) cells are pluripotent cell lines with the capacity of self-renewal and a broad differentiation plasticity. They are derived from pre-implantation embryos and can be propagated as a homogeneous, uncommitted cell population for an almost unlimited period of time without losing their pluripotency and their stable karyotype. Even after extensive genetic manipulation, mouse ES cells are able to reintegrate fully into viable embryos when injected into a host blastocyst or aggregated with a host morula. After these pre-implantation embryos are implanted into a surrogate mother, they develop into mosaic offspring known as chimeras. The tissues of chimeric mice are comprised of a mixture of cells that originated from both the host embryo and the ES cells. The contribution of each originating cell population is seen most visibly in the fur, which is generally striped black (from host cells) and brown (from ES cells). Healthy ES cells can give rise to descendants in all cell types, including functional gametes to produce more and more mice containing the desired genetic modification. If the proportion of ES cell descendents in the coat of the animal is high, the probability that ES cells are represented in gametes is also high, since ES cells mix thoroughly with host cells early in embryogenesis. ES cells give rise to brown coat color because they are Aw/Aw dominant White-bellied Agouti), and the host cells give rise to black coat color because they are a/a (recessive non-agouti). The ES cells used most commonly are from the 129 strain of mice, while the host embryos are from the C57BL6 strain of mice. If the chimeras are bred to a/a non-agouti mice (for example C57BL6 or Black Swiss), then any brown offspring (Aw/a) must have arisen from ES cell-derived gametes, and 50% of the brown offspring are expected to carry the genetic modification.

A knockout mouse has had both alleles of a particular gene replaced with an inactive allele. This is usually accomplished by using homologous recombination to replace one allele followed by two or more generations of selective breeding until a breeding pair are isolated that have both alleles of the targeted gene inactivated or knocked out. Knock out mice allow investigators determine the role of a particular gene by observing the phenotype of individuals that lack the gene completely. When an investigator wants to replace one allele with an engineered construct but not affect any other locus in the genome, then the method of choice is homologous recombination. To perform homologous recombination, you must know the DNA sequence of the gene you want to replace. With this information, it is possible to replace any gene with a DNA construct of your choosing. The method has a few more details than will be illustrated on this page, but the essential information is retained. Many genes that participate in interesting genetic pathways are essential for mouse development, viability or fertility. Therefore, a traditional knockout of the gene can never lead to the establishment of a knockout mouse strain for analysis. Conditional gene modification using

Cre-lox technology allows the gene of interest to be knocked-out in only a subset of tissues or only at a particular time, circumventing lethality. Because gene targeting can be controlled both spatially and temporally, the function of a given gene can be studied in the desired cell types and at a specific time point. This genetic dissection allows researchers to define gene function in development, physiology or behavior.

The first knockout models of insulin resistance aimed at the disruption of major molecules in IR signaling. The successful generation of viable, heterozygous IR knockout mice showed that 50% of IR expression is sufficient for the maintenance of physiological blood glucose concentrations. In contrast, homozygous IR-deficient mice rapidly develop diabetic ketoacidosis and die within 3–7 days after birth, showing the indispensability of IR for the control of glucose metabolism.

Mice with a specific disruption of the IR gene in βcells show a selective loss of insulin secretion in response to glucose and a progressive impairment of glucose tolerance, indicating that insulin stimulates its own secretion via IRs on βcells, thus playing an important functional role in glucose sensing by the pancreatic βcell. Therefore, defects in insulin signaling at the level of the βcell may contribute to the observed alterations in insulin secretion in type 2 diabetes. Interestingly, a βcell specific IGF-1R knockout model also underscored a role for this receptor in the control of glucose stimulated insulin secretion and glucose tolerance.

Conclusion

Mouse models with single genetic defects can yield important information about the action of insulin in glucose metabolism but may not always match the human disease states. Thus it is critical that new, relevant animal models employing more sophisticated strategies for gene inactivation be developed to extend our understanding of the mechanisms underlying type 2 diabetes.

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