Gene-Engineered Models for Genetic Manipulation and Functional Analysis of the Cardiovascular System in Mice

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Cardiovascular disease remains a key issue in healthcare. During the last decade, transgenic and gene-targeted mouse technology has provided invaluable insights into cardiovascular molecular biology. Given the similarities between the mouse and human genomes, this study proposes that information experimentally derived using genetically manipulated mice can contribute significantly to the understanding of human cardiovascular pathophysiology. We first introduced the basic principles and methods of genetic manipulation, such as the breeding background in mice and the factors of construct design. Secondly, we reviewed the analyses related to genetic manipulation of the cardiovascular system from embryonic to adult mice. In conclusion, the gene-engineered mouse model is one of the most important tools developed in recent basic and clinical research. *(Chang Gung Med J 2003;26: 868-78)*

Key words: **cardiovascular system, gene-engineered mouse model, gene manipulation, transgenic mouse, knock-out mice.**

Cardiovascular disease continues to be the leading cause of death, and cardiovascular biology remains one of the last frontiers of molecular study. The development of genetically manipulated mouse models of cardiovascular diseases has increased the feasibility of dissecting complex cardiovascular phenotypes using animal genetic models.[1-4] For technical and economic reasons, mice are ideal experimental animals for investigating cardiovascular molecular pathways. Accordingly, a wealth of miniaturized technology has been developed to assess cardiovascular phenotypes.

The two main reasons for manipulating mouse genomes are (1) to elucidate gene regulation *in vivo*, and (2) to define gene function *in vivo* via the resultant phenotype. Transgenic and gene-targeted mice can be used to analyze gene regulation. The phenotype is defined as the observable properties of an organism produced by the genotype. The phenotype includes the molecular (mRNA and protein), biochemical, physiological (single cell, organ, and system), and morphological phenotypes. Given the complexity of the mouse as an organism, the observed phenotype may or may not be the direct result of a single gene mutation. Interaction with other modifier genes and environmental factors may participate in the development of the apparent phenotype.[5]

A. TECHNIQUES INVOLVED IN GENETIC MANIPULATION OF MICE

Two general methods can be applied to modify the mouse genome, namely transgenic and gene-targeted modifications. Both techniques involve the microinjection of foreign DNA into the nucleus of a fertilized oocyte. The main difference between the two techniques lies in the location of the gene integration as detailed below.

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Received: May 14, 2003; Accepted: Sep. 22, 2003
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A.1. Transgenic mouse by microinjection

A "transgene" designates the random insertion of foreign genetic material into chromosomal DNA. This process is non-homologous, and therefore integration can occur anywhere within the genome. Moreover, several copies of the genetic material may be inserted in tandem. The foreign genetic material usually is microinjected into a mammalian genome using a technique developed by Jaenisch and Mintz in 1974.[6] Retroviral infection has been proven to be another way to introduce the extra or exogenous gene into the original genome.[7] In the first attempt to introduce a cloned eukaryotic gene into the germ line researchers used the nuclei of cultured fibroblast.[8] Other researchers used embryonic stem (ES) cells.[9, 10]

Approximately 60 to 80% of microinjected zygotes survive and subsequently can be reimplanted into pseudopregnant foster mothers. The mouse that develops from each microinjected egg is called a "founder" of the particular mutant line. As stated above, each founder may carry one, or more copies of the trans-gene at an unpredictable locus. Ideally, the transgenic mice should be maintained in pathogen-free colonies to ensure good health and ease of transportation.

A1.1. Applying transgenic technology and breeding strategies

The most common applications of transgenic mice are for elucidating the tissue-specific and developmental stage specific regulation of a given gene, and for elucidating the phenotypic effects of transgene expression. In the latter application, many parameters can influence the observed phenotype; for example, stable vs. transient expression, incomplete penetration, and variable expression. If a transgene is integrated into the chromosomal DNA, its expression remains stable over the long term. A gene exhibits incomplete penetration when a phenotypic manifestation fails to appear in all of the individuals with that gene. Moreover, variable expression occurs when different phenotypes result from a specific gene.

Other important issues regarding breeding strategy are optimizing of the mutation expression, maximizing the number of viable offspring, and minimizing the potential confounding effect of background genes from the breeder parents. The effects of background genes can be determined by repeated backcrossing into inbred strains to prevent the expression of the polymorphic modifier genes in different mouse strains. However, no "best" strain is ideal for all phenotypes. Random segregation, and continuous inbreeding of +/+ (wild type), +/- (heterozygous) and -/- (homozygous) littermates, may generate unusual alleles that yield false positives or negatives of the mutation phenotype. Both inbreeding and segregating backgrounds should be considered carefully. The most commonly used strains are C57BL/6 (hardy blastocysts, long-surviving microinjection, and high response to superovulation), FVB/N (large pronuclei, long-surviving microinjection, and high response to superovulation), and SV129 (ES cell line).

Generally, a transgenic gene overexpresses the gene of interest. However, the presence of a gene does not necessarily indicate expression of the gene, and if the gene is expressed, then it is not necessarily expressed in a functional protein. The two limits of the transgenic expression depend on the number of copies of the transgene and also on the integration sites. First, several copies may be inserted at a single site in a chromosome, causing various dosage effects. Second, the random insertion of a transgene may not be led by its own regulatory elements or promoter. Transgenes expressed in the wrong tissues at the wrong time or the wrong stage of development can severely and negatively influence the proper function of the cell, tissue, or organ.

The expression of antisense RNA or dominant-negative cDNA can down-regulate the expression of a gene or ablate the gene function in a transgenic mouse. Neither the antisense nor the dominant negative approach generally completely suppresses gene expression/function.

A.1.2 Factors of construct design

The most critical aspect of the transgenic mouse is the construct design. Relevant issues to be considered include (1) the promoter to drive the gene of interest, (2) the length of the transgene, (3) the effect of prokaryotic vector sequences, and (4) the method for distinguishing the transgene from endogenous genes.[11]

Promoter: The transgene is likely to be integrated into a different chromosomal location in the genome in each founder mouse. Current technology aims to direct transgene expression using tissue-spe-

cific promoters. For example, the α-MCH and MLC2v promoter limits the expression of a targeted gene mutation to the heart.

Length: Relatively small promoter sequences may suffice for directing cell-specific expression in transgenic mice.[12] However even when a long promoter is included, it may fail to express the endogenous gene, owing to the distal enhancer element or over-riding control regions, including locus control regions.[13] The use of yeast artificial chromosome (YAC) constructs has advanced from the use of a few plasmid-compatible kilobases of a transgenic sequence throughout the megabase.[14] However, YAC is no longer used for microinjections due to its high frequency of self recombination. Recently, to obtain a large quality of bacterial artificial chromosome (BAC) DNA, the construct was amplified in *Escherichia coli* to normal plasmid DNA and prepared using a palsmid purification kit suitable for the isolation of large DNA constructs. BAC DNA is circular. It may be preferable to linearize the construct before injection, otherwise breakage within the gene of interest may occur.

Vector sequences and distinguishing exogenous vs. endogenous genes: Ideally, the vector sequence should be removed before being introduced into the mouse gene. The vector sequence may inhibit the transgenic expression. A transgene-specific marker or a reporter gene should be used to distinguish the transgenic and endogenous genes.

Reporter gene: Using a reporter gene driven by a promoter of interest allows investigators to determine easily the temporal and tissue-specific pattern of expression of a given gene. The most commonly used reporter gene is β-galactosidase *(LacZ)*; *LacZ* is frequently used with a nuclear location signal. More recently, green fluorescent protein (GFP), which enables the localization of expression in live cells has come to be widely used. However, the expression of a reporter gene is known to produce unintended phenotypes. For example, over-expression of GFP in cardiac tissue can lead to cardiomyopathy in mice.

A.2. Gene targeted (knock-out/knock-in) mouse

Total gene ablation has been enabled by the recent development of gene-targeted knock-out technology in mice. Two types of targeting constructs have been used to modify genes in ES cells, namely replacement-type and insertion-type vectors. Each construct can be used to create a point mutation model and a knock-out model using a "hit and run" vector with selection marker cassettes. Two important requirements must be met for successful gene-targeted ablation. These requirements are the permanent culturing of totipotent ES cells and the targeted disruption of a gene by homologous recombination (HR).[15]

A.2.1. ES cell line and HR technique

Mouse embryonic stem cells are derived from the inner cell mass of a 129/Sv mouse blastocyst, which is pluripotent and differentiates into all types of tissue. Gene-targeted ablation has been impossible to perform in rats due to an absence of appropriate ES cells.[16]

Gene-targeting constructs must be precisely engineered to prevent anomalous gene integration and unintended mutations. The precise molecular and biochemical mechanism of homologous recombination has been determined. HR occurs approximately 1000-times less frequently than non-HR integration. Either ES cells can be co-cultured with neo-resistant fibroblasts or LIF should be provided in the culture media. Linearized targeting construct generally is introduced into the ES cells by electroporation. Linearized construct is integrated by homologous recombination at a very low frequency of approximately one in a million cells. Including selectable markers (G418-neomycin resistance cassette) in the targeting vectors is standard practice in gene-targeting to enrich gene-targeted ES cells. Other positive/negative selectable markers, such as hygromycin and hypoxanthine:guanine phosphoribosyl transferase (HPRT), also have been successfully used to enrich cells that have undergone a desired gene targeting event. Selected ES cell colonies are expanded and screened by Southern blot analysis. Positive ES cells are injected into blastocysts derived from superovulated female mice, frequently of the C57BL/6 inbred strain. Collection is usually performed on 3-day-old embryos. The injected blastulas are transferred to a pseudo-pregnant mother. The success rate is around 1/126.

A.2.2. Chimera and breeding strategies for gene-targeted mice

The pup is called a chimera (F0, *agouti*) because

it contains cells from two independent sources, ES cells from 129/Sv with light grayish-brown fur and blastulas from C57BL/6 with black fur. Accordingly, the color of the coat of the chimeric pups is a useful early marker of successful mutation, at around 3 weeks after birth. To detect germ-line transmission, a test cross is performed to breed chimeras (+/-) with an inbred strain (+/+), including black C57BL/6J, to generate F1 offspring with agouti dominant and black color of the heterozygous or black wild type. Heterozygous F1 identified using Southern blot analysis or PCR are mated to generate F2 with one quarter homozygous mutants (Fig. 1).

The applied concepts and techniques are the

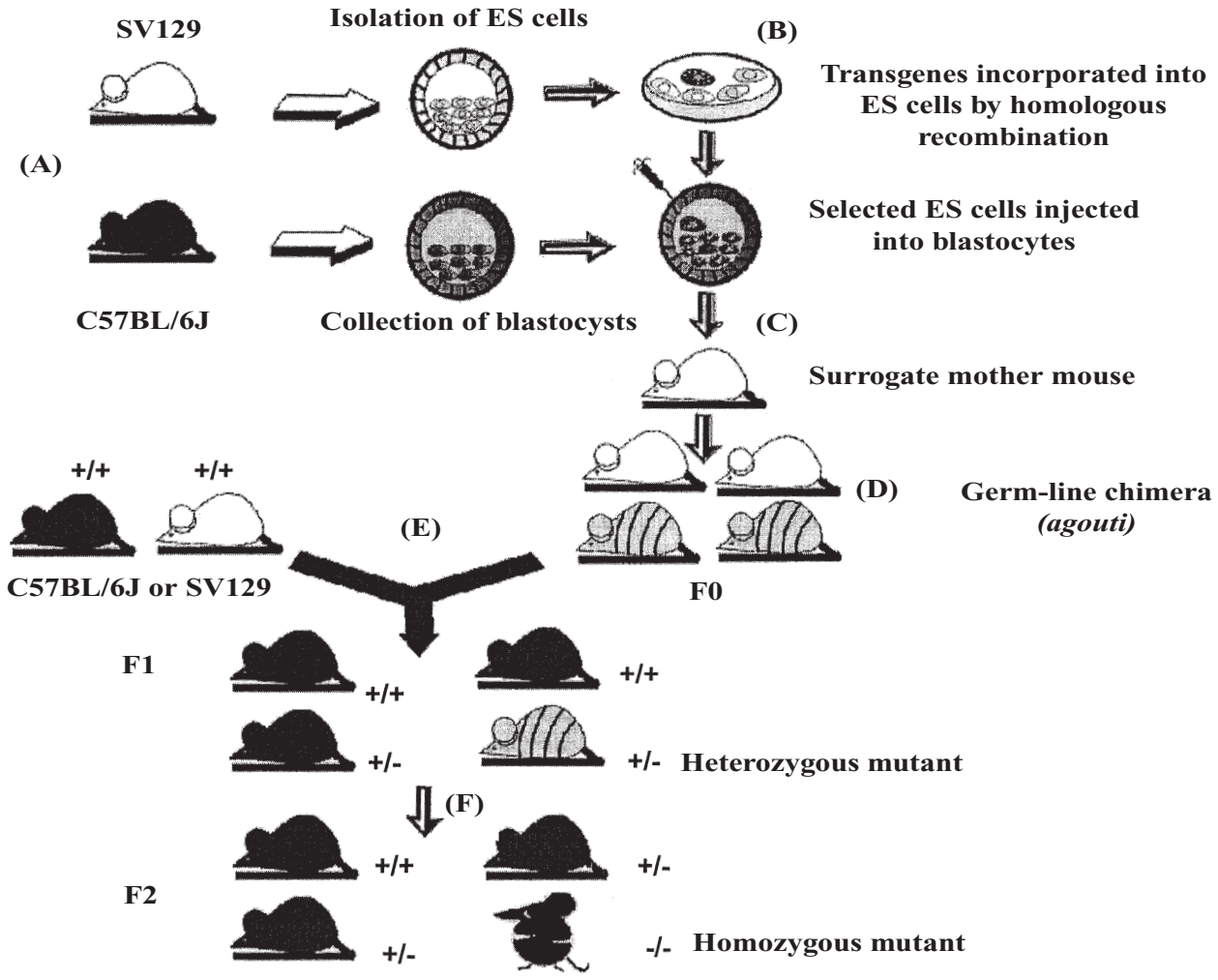

Fig. 1 The summary bellow shows how to make the knock-out or knock-in mice. (A) First, mouse embryonic stem cells are derived from the inner cell mass of a 129/Sv mouse blastocyst. (B) Linearized targeting construct integrated by homologous recombination is generally introduced into the ES cells by electroporation. (C) Selected ES cell colonies are expanded and screened by Southern blot analysis, then are injected into a blastocyst derived from superovulated female mice, frequently of the C57BL/6 inbred strain. (D) The pup is called a chimera (F0) because it contains cells from two independent sources, ES cells from 129/Sv with agouti, light grayish-brown fur, and blastulas from C57BL/6 with black fur. (E) Breeding chimeras (+/-) with an inbred strain (+/+), black C57BL/6J or white 129/Sv, to generate F1 offspring with agouti dominant and black color of the heterozygous or black wild type. (F) Heterozygous F1 identified by Southern blot analysis or PCR are mated to generate F2 with one quarter homozygous mutants.

same in both the knock-out and knock-in mice. Furthermore, the generation of knock-in mutants has been suggested to "clean-up" the inserted selection cassettes, thus preventing reduced expression of the mutant protein.[17] A common solution to inserted cassettes is to use three *LoxP* sites, positioned such that both the *neo* gene and the gene of interest are floxed.[18] Notably, no side effects have been noted for the cre and *LacZ* genes.[19, 20]

The knock-in technique has been applied specifically to study the point mutation gene or promoter efficiency. Mixed genetic background knock-out mice frequently have a wider range of phenotypes.[21] The analysis of knock-out mice has revealed that the mutation of the phenotype is often less severe than expected, perhaps because of the functional redundancy of the genes and the compensatory mechanism among gene family members. Consequently, double or multiple knock-out mice may be required. The major disadvantage of gene-targeted mice is the very early expressed genes that result in embryonic lethality. However, this problem can be solved using conditional gene manipulation.

A.3. Conditional and inducible transgene expression and knock-out genes

Some knock-out genes can lead to a lethal embryonic phenotype, which is useless for studying adult biology. Consequently, the ideal "genetic switch" should be reversible and specific for the targeted gene, and should not interfere with other cellular components or with general metabolism.[17] The development of tissue-specific conditional mutations could allow the mutation to be inserted into specific cell types, and moreover allow inducible mutations to be turned on/off at the desired developmental stage.

A.3.1. Conditional (spatial and temporal) gene targeting

A common method for tissue-specific conditional mutation involves designing a Cre/lox system.[22] *Cre*, as a regulated transgene usually driven by a tissue-specific promoter, is a 38-KDa protein that recombines DNA between two *LoxP* target sites. *LoxP* sequences are 34-bp, and include two 13-bp inverted repeats, flanking an asymmetric 8-bp core sequence. A promoter that selects the tissue in which the gene is to be expressed controls the *Cre* recombinase linked to the targeted gene and thus controls the targeted gene.

A.3.2. Inducible mutations

Conventional mutations are determined from mouse models of human hereditary diseases in which the mutation is present continuously from the embryonic stages. A temporary mutation would be an ideal tool for examining functional questions about gene function and avoiding compensation or redundancy by other genes or mechanisms over the course of development. Many inducible systems have been developed at either the transcriptional or the post-transcriptional levels.[23]

Attempts to develop the "switch" involve heavy metals, heat shock, ecdysomes and hormones to control endogenous eukaryotic promoters. However, the methods mentioned above are associated with serious problems, including pleiotopic effects (when a single gene is responsible for distinct and seemingly unrelated phenotypic effects), leakiness, inducibility, and toxicity.[24] Accordingly, the tetracycline (TA) regulatory system has been developed.[25] In the original system, the TA-controlled transactivator (tTA) could not bind DNA when TA was present ('turn-off'), whereas the reverse tTA (rtTA) element could bind DNA only when TA was present ('turn-on'). To date, none of the systems for on/off-control have been effective in promoting contractile gene expression.

Websites exist that provide specialized information on mouse genomics and biology (Tables 1 and 2). The increase in the body of knowledge has made coordinating and integrating all information to support the biomedical research community critical.

B. FUNCTIONAL ANALYSIS OF CARDIOVASCULAR SYSTEM IN MICE

The most important issue of the post-HGP era is assigning functions and information to each gene. The simplest way to understand how a gene works is to determine the effects of interference with gene function by mutational analysis. This genotype-based "reverse genetics" approach is advantageous in that it facilitates the molecular analysis of mutations. Small animals, such as mice, are ideal in vivo whole animal model systems.

The mouse is ideal model organism for analysis

Table 1. Transgenic Mice: Internet Resources

Website	Comment
1. http://biomednet.com/mkmd	Knock-out mice
2. http://gdbwww.gdb.org	Mouse Genome Database
3. http://jcsmr.anu.edu.au/group_pages/mgc/MedGenCen.html	Medical genome Center, Australia
4. http://mammary.nih.gov	Mammary tumors
5. http://tbase.jax.org	Jackson's Laboratory
6. http://tikus.gsf.de	GSF German genome project
7. http://mips.gsf.de/cgi-bin/proj/medgen/homemouse	
8. http://www.avs.com	
9. http://www.celera.com	Commercial site for mouse genome
10. http://www.bioscience.org/knockout/knochome.htm	Knock-out mice
11. http://www.emma.rm.cnr.it	European mouse mutant archive
12. http://www.genetrap.org	Gene trap resources
13. http://www.informatics.jax.org	Mouse Genome Databases
14. http://www.mgu.har.mrc.ac.uk	MRC mammalian genetics unit
15. http://www.mouse-genome.bcm.tmc.edu/	Baylor College of Medicine
16. http://www.nih.gov/science/mouse/	NIH mouse initiative
17. http://www.rodentia.com/wmc/	Whole mouse catalog
18. http://www3.ncbi.nlm.nih.gov/omim	On-line Mednelian Inheritance in Man

Table 2. Mouse Genomic Information: Internet Resources

1. http://cardiogenomics.org	Cardiovascular phenotypes
2. http://genex.hgu.mrc.ac.uk	Mouse 3D atlas
3. http://www.ncifcrf.gov/vetpath/nexropsy.html	Virtual mouse necropsy
4. http://www.nhlbi.nih.gov/resources/pga	Programs for genomic applications
5. http://www.st-and.ac.uk	3D

of the gene function. Mice resemble humans closely enough to be a satisfactory model organism, yet are relatively easy to manipulate genetically. For example, ES cell techniques and the mouse genome are both mapped for genetic studies. The advantages of the mouse model include low cost (of generation, breeding, and maintenance), short bleeding time, and relatively well characterized genetic markers. However, the small size (approximately 0.05% of that of a human) and rapid heart rate (328 - 780 beats per minute) of the mouse present challenges for the functional analysis of the cardiac phenotype of genetically engineered mice.[3] The analytical techniques include assessing the embryonic, neonatal and adult mouse heart and vessels under both basal and stressed conditions (Table 3).

The most critical issues related to physical analysis include miniaturization and refinement of laboratory protocol, difficulties in anesthesia and

Table 3. Timing of the Appearance of Principal Cardiac Features in Mouse and Human Embryos

	Mouse (day)	Human (day)
Implantation	4.5	5-6
Vasculogenesis		19
Endocardial tubes forming	7.5	20
Tubular heart	8.0	21
Heart beats	8.5	22
Looping	8.5-9.0	23
Septum primum, and endocardial cushing begin	9.0-9.5	23-28
Cavity formation	10.5	28
Interventricular separation beginning	10.5	30
Ostium secumdum		38
Intra-atrial separation complete	12.0	43
Separation completely	14.5	56.5
Superior vena cava and aortic branches formed		
Multiple layer spiral system		

surgery, and differences in background, gender, and age, and cost.[26] Before the mouse model can be used to study a cardiovascular phenotype, the basic conserved and changed features of mouse cardiovascular physiology must be known[27] (Table 4).

Table 4. Characteristics of Mouse Cardiovascular System

Anatomy and basic physiology	1. 40 chromosomes with 2.5 years life span. 2. Small size and heart rate 5-10 times. 3. Left superior vena cava drains to right atrium. 4. One pulmonary vein opens to left atrium. 5. No second atiral septum formation 6. Large septal coronary artery. 7. Larger epicardial coronary artery than left main coronary artery (3.7 vs. 0.16 mm). 8. Higher location of SA node and not apparent Purkinje cells. 9. No obvious ST segment.
Cardiommyocytes ($1.5\ \mu m)^3$	1. α-MHC V1 form α-MHC and V1 form 5. 2. First peak of DNA synthesis on embryonic day 12.5; lowest around birth; and the 2nd peak on day 7 after birth.
Metabolism	Higher energetic requirement and metabolism with increased in oxidative capacity (ATPase activity, SERCA2a activity, density of mitochondria).
Blood distribution	1. Brain (1/4 vs. human). 2. Renal and splanchnic arteries (1/2 vs. human).
Genome and gene organization	1. Two β-globin genes in mouse. 2. Sequence divergence between human and mouse at splice sites. 3. Differences in imprinting and promoter usage. 4. Specifies differences in gene expression during early development.

B.1. Developmental analysis of cardiovascular system in mice

As well as modifying *in vivo* genetic functions, transgenesis provides a new way of visualizing the cell fate map for cardiovascular development. The process of embryonic development in humans remains remarkably ineffective. At least 50 to 60% of embryos die before birth[28] and 25% of human abortions remain unexplained.[29]

Owing to ethical concerns, the subject of development has been approached through the creation of mutant mouse strains, especially in which a reporter gene is inserted.[30] Such mice are useful not only in characterizing a newly made *Cre*-expression line, but also for analyzing cell lineages. However, one of the most important concerns is to ensure that the reporter molecule is neutral, and should not interfere with normal gene functions. Accordingly, the development of neural crests and conduction systems has been carefully elucidated.[31]

Table 5. Methods for Analysis of Cardiovascular Phenotypes

Basic Functions	1. Echocardiography Magnetic resonance imaging, computer tomogram 2. Micromanometers Tail cuff plethysmography 3. Aortic flow probe Doppler echocardiography 4. Electrocardiography 5. Telemetry recorder and electrophysiological study
Stress Functions	1. Exercise test Treadmill and swimming 2. Drug/nutrition supply by intake, intravascular, intra-peritoneal, subcutaneous and intra-muscular areas Western diet, and osmotic infusion pump 3. Surgical artificial techniques i. Pressure overload: transverse aortic/pulmonary aortic constriction ii. Volume overload: A-V fistula iii. Myocardial infarction and ischemia 4. Hypoxia 5. Vascular injury
Pathologic Studies	1. In situ hybridization 2. Immunohistochemistry 3. Confocal microscopy, electronic microscopy, 3D reconstruction 4. Cell replication and apoptosis 5. Sacrifice at different stages i. Embryonic stage ii. Neonatal and perinatal mortality iii. Young adults iv. Elder adults 6. Clinical chemistry parameters

B.2. Phenotypic analysis of cardiovascular system in mice

Mouse models of cardiovascular diseases have also provided new and important insights into the pathophysiology of human disease. The cardiovascular phenotype can be precisely assessed at the cellular and organ level using well-known and novel sophisticated *in vitro*, *ex vivo* and *in vivo* methods. Cardiovascular phenotypes are usually expressed from the time of early gestation or early life.

B.2.1. Representative cardiovascular phenotypes

The phenotypes selected by gene manipulation can be divided into those with congenital heart disease, coronary artery disease, cardiomyopathy, arrhythmias, atherosclerosis, thrombosis, and hypertension (Table 5). All these models can improve the understanding of the related molecular networks.

B.2.2. Dissecting the molecular pathways

Genetic engineering of mouse models can be used to dissect the molecular pathway in complex cardiovascular diseases. The phenotype is analyzed first. (Table 6) For example, atherosclerosis is modified by inflammation, metabolism, hypertension, and coagulation. The mouse model can be applied to elucidate the interaction between different variables.[32] Alternatively, the complex part of the pathway can be dissected from the specific and known pathway. For example, the rennin-angiotensin system for cardiovascular regulation can be studied in detail using different receptors or vasoactive peptide gene-targeted mice.[33] The interaction between the adrenergic axis and heart disease is clarified using the genetically engineered mouse model.[34,35] There are additional models for the valvulogenesis, conduction, congenital heart disease and heart failure using the knock-out animal mice.[36,41]

Table 6. Representative Cardiovascular Phenotypes with Closely Related Genes

Congenital heart disease	
1. Hypoplasia of ventricular wall	1. transcription factor: MEF2C, dHAND, RXR, N-myc, TEF-1, NF-1, WT-1. 2. cytoskeletal proteins: ALP. 3. signal transudation: neuregulin, ervB2/4. 4. others: VEAM-1, α4 integrin, VEGF-4, tie2.
2. Valve defects	NF-Atc, Smad6, TGF-β, FOG-2, HB-EGF and TACE, CHF1/Hey2.
3. Defects in outflow tracts	1. DiGeorge syndrome: VEGF, TBX1, 2. ET-1, ECE-1/ET_A, NT-3, trkCreceptor, dHAND, hox1.5, Sox4, rae28, NFH-1, NF-1, neuropilin, Pax3, RXRα, connexin43, NMHC-B, BMP.
4. Septum defects	NKX2.5, PBSF/SDF-1, ActRIIB, RXR, VCAM-1, NF-Atc, FOG-2, N-myc, ET, Sox4, MFH1, NT-3, trkCreceptor, NF1, Jumonji.
Cardiomyopathy	
1. Hypertrophy cardiomyopathy (HCM)	MLC, MyBP-C, T-cap. GLUT4, fratzxin, kinin B2 receptor, melusin, natriuretic peptide receptor-A gene.
2. Dilated cardiomyopathy (DCM)	dystrophin, beta-sacroglycan, delta-sacroglycan, alpha-dystrovrevin, metavinculin, desmin, lamin A/C, caveolin-1, caveolin-3, lysosomal cysteine peptidase cathepsin L
3. HCM with DCM	MHC, Cardiac actin, Titin, Alpha-TM, Tn-T, MLP
4. HCM with Restrictive Cardiomyopathy	TnI
Coronary artery disease	TnI, MMP, TNF, VEGF.
Hypertrophy related genes	α1B receptor, Gαq, Gq, Ras, RhoA, rac1, PKCβ2, NF-AT3, IL-6, STAT3, IGF-1B, TAK1, TNF-α, βARK1, β1 receptor, β2 receptor, Gαs, Gi, SERCA2a, calwequestrin, FKBP-12, MMP-1.
Arrhythmia	1. Long QT syndrome: KvLQT1, mink, Kv1.1, Kv2.1, Kv4.2, Kv1.4, ankyrin, ERG1 B potassium channel eliminates I, KCNE1. 2. Conduction defect: HF-1b, connexin43/40/45. 3. Others: Tfam, Gi, AT1, NKX2.5, L-type channel, RhoA.

C. OUTLOOK AND THE FUTURE

Using both humans and mice to elucidate the molecular pathways, made possible using targeted mouse models, has been and will remain important in studying cardiovascular development and pathophysiology. The knock-out mice equivalent of recessively inherited conditions in humans due to the loss of gene functions provides invaluable information in genetic studies. However, the knock-out models contribute only a small amount to the understanding of human cardiovascular diseases. The complexity of some cardiovascular diseases in humans is more compatible with susceptibility related to combinations of small, quantitative changes in genetic functions, which is analogous to the situation in mice that are heterozygous for genetic modification.[32,42] Therefore, future studies must be used to examine heterozygotes and single-nucleotide polymorphisms.

Mouse models of disease frequently have important limitations including the phenotypes often diverge considerably from those of the human disorders they were meant to resemble, even where the corresponding phenotypes in humans and mice are known to arise from similar mutations in orthologous genes.[37] However, the scarcity of human study material requires that research strategies be optimized and expression studies in early human development and mouse development be coordinated.

Acknowledgements

We would like to thank I-Chan Tsai, Hsueh-Hua Wu and Li-Ying Chien for their technical assistant. This work was partially supported by grants NHRI-EX91-9108SC and NHRI-EX92-9108SC (to Dr. Chu) from the National Health Research Institute, Taiwan and grants from the National Institute of Health, USA (to Dr. Chen).

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基因工程鼠簡介：小鼠心臟循環系統之基因操作及功能分析

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心臟循環疾病爲人體健康相當重要課題之一。於過去10年中，基因工程鼠技術包括基因轉殖鼠及基因剔除鼠兩項，已確實成功提供一嶄新工具來研究心血管疾病。這是因爲小鼠與人之間，有相當基因類似性。而此篇綜論簡述第一部份，爲簡介基因工程鼠之基礎技術，如基因轉殖鼠類之基因遺傳背景考量，植入基因架構之建立；進而介紹基因剔除鼠之基礎技術，如胚胎幹細胞培養方法，同質基因重組概念，嵌合鼠之培育，以及甚至介紹條件式及誘發式基因工程鼠之培育概念。第二部份爲介紹如何分析鼠類之心臟循環系統功能，例如小鼠類胚胎之循環系統評估方式法，以及如何分析小鼠類循環系統表現型，並介紹代表性之小鼠心臟循環系統表現型，以配合分子生物層次之研究。綜合而言，基因工程鼠已成爲今日基礎與臨床研究不可或缺之利器，吾人應有深入之認識。(長庚醫誌 2003;26:868-78)

關鍵字：心血管循環系統，基因工程鼠模型，基因操控，基因轉殖鼠，基因剔除鼠。

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