The Strengths and Limitations of the Use of Transgenic and Knock-out Animal Models

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Over the past 15 years, experiments utilizing transgenic and knockout mice have significantly advanced our understanding of the roles of individual genes and proteins in normal development and function and in disease. Due largely to the availability of thousands of different types of genetically engineered animals, mice are now the most commonly used species in biomedical research. Here, we discuss the strengths and limitations of genetically engineered mice, and provide an example MRI study demonstrating their use.

As of the year 2003, both the human and mouse genome have been fully sequenced. The genome is the full set of genes, hereditary information encoded in the DNA, of an organism. A comparison of the mouse and human genomes reveals that approximately 99% of all mouse genes are similar (homologous) to a human gene (6). Whereas the genome or genotype describes the genetic constitution of an organism, the phenotype is an observable trait of an organism. Genetically engineered mice, such as mice where a specific gene has been deleted or knocked out, or where extra genetic material has been added to the genome (transgenic), enable us to study the roles of specific genes by observing or measuring the resulting phenotype. However, care must be taken to properly design and interpret studies using genetically engineered animals (1,2), as potential pitfalls exist and should be avoided. Genetically-engineered mice are usually created on a well-defined and uniform background genotype, which is generally one of a few common inbred strains. For studies of the roles of specific genes, it is critical to select the appropriate control strain with the same genetic background. The choice of background strain is also important, since the function of a gene of interest may be influenced by other genes present in a particular background strain. One example of a poor choice of background strain would be using the common C57Bl/6 mouse for research on the roles of different genes in hearing, since C57Bl/6 mice are susceptible to noise-induced hearing loss. In general, it is important to be aware that traits expressed in a mouse may arise due to the gene of interest, due to gene expression that may be unique to the particular background strain, or due to an interaction of the genomic "environment" of the background strain with the gene of interest. Also, there may be compensatory pathways that are modulated in a genetically engineered mouse, and these may also affect the phenotype. Given these potential confounding effects, temporal and tissue-specific regulation of gene expression are important approaches that may circumvent phenotypes such as embryonic lethality and developmental defects, which may occur in certain transgenic and knockout mice. Also, the use of pharmacological agents can often serve to complement and validate experimental results obtained using genetically engineered animals.

When considering the use of transgenic and knock-out mice in biomedical research, it is also important to distinguish between models of genetic disease and models of acquired disease. For example, there are over 100 genetically-altered models of cardiomyopathy

that demonstrate various degrees of hypertrophy and heart failure (7). And while these murine models have been highly valuable in defining the natural function of these genes, they serve as models of human disease only to the extent that a corresponding mutation is known to cause a similar genetic disease in humans. In order to study the role of an individual gene in the pathophysiology of diseases acquired by humans, it is often more productive to first create an accurate animal model of that acquired disease (such as by coronary occlusion or aortic constriction) and then to define the function of the gene of interest in the progression of that particular acquired disease.

To give a concrete example of an MRI study using genetically engineered mice, we review our study of the role of inducible nitric oxide synthase (iNOS) in post-infarct left ventricular (LV) remodeling of the heart following myocardial infarction (MI) (3,4). Specifically, we tested the hypothesis that iNOS plays a negative role in post-MI LV remodeling. To test this hypothesis, 12 untreated wild-type (WT) C57Bl/6 mice, 12 iNOS^{-/-} mice on the same genetic background, and 5 WT C57Bl/6 mice treated with 1400W (Alexis Biochemicals, San Diego, CA), a highly selective iNOS inhibitor, were studied by MRI before and at 1, 7, and 28 days after experimental MI. Previously, we showed that C57Bl/6 mice exhibit a post-MI LV remodeling phenotype similar to that seen in humans (5). MI was induced by a 1 hour occlusion of the left anterior descending coronary aftery followed by reperfusion. 1400W was administered (1.25 mg/kg-hr) starting 1 hour post-reperfusion until day 14 post-MI using implanted Alzet micro osmotic pumps. Imaging studies included (a) localizer scanning, (b) cine MRI, and, (c) at day 1, gadolinium-enhanced MRI. Gadolinium enhanced MRI demonstrated similar day 1 infarct sizes in treated, knockout, and untreated WT mice (38.7±9.4%, 35.2±4.2%, and 36.8±4.5%, p=NS). Example mid-ventricular end-systolic images at day 28 are shown in Figure 1, illustrating the phenotype of iNOS^{-/-} mice and the affect of iNOS inhibition in this disease model. Specifically, severe LV cavity dilatation and circumferential wall thinning are seen in the untreated WT mouse (Figure 1A). In contrast, preservation of cavity size and confinement of wall thinning to the anterior segment are seen in the knockout and 1400W-treated mice (Figure 1B,C). Figure 2 shows ESV and EF data summarizing cardiac size and function over 28 days. Specifically, deletion of the gene encoding for iNOS and iNOS inhibition led to reduced ESV at days 7 and 28 vs. untreated WT mice (p<0.05) and increased EF at day 28 vs. untreated WT mice (p<0.05). This study, using MRI as a phenotyping tool, showed that iNOS^{-/-} mice and WT mice treated with a a selective inhibitor of iNOS have similarly reduced post-infarct LV remodeling. Using both a genetically engineered mouse and pharmacolgical inhibition in a WT mouse strengthened the study, as this combination helped alleviate potential concerns related to possible compensatory pathways in genetically engineered mice, or possible lack of specificity using only a pharmacological approach.

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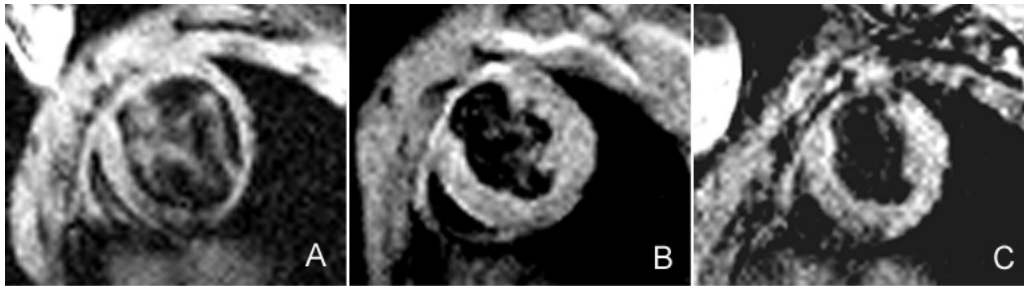
Fig. 1. Mid-ventricular short axis images 28 days post-MI of (A) an untreated WT mouse, (B) a WT mouse treated with 1400W, and (C) an iNOS knockout mouse. Reduced cavity size and circumferential extent of wall thinning are evident in (B) and (C).
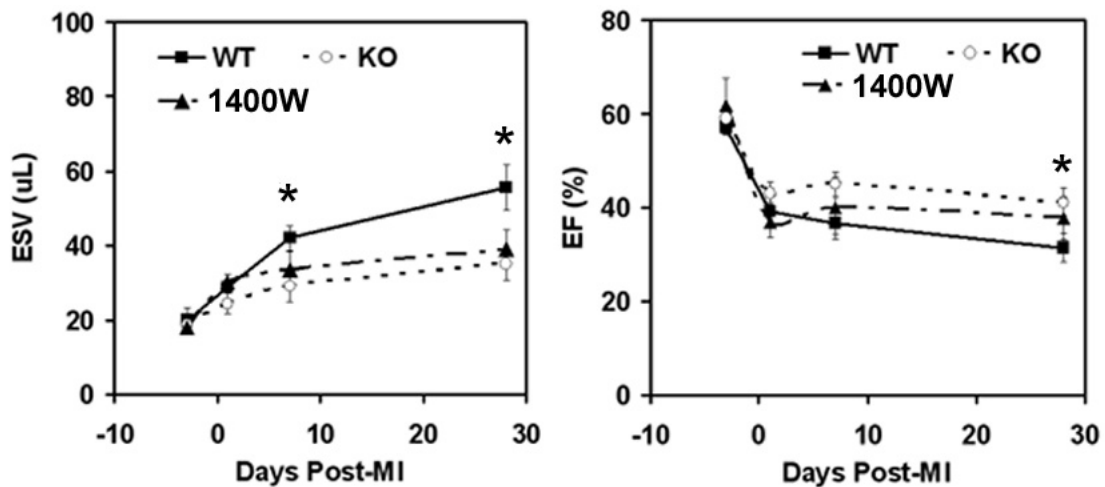

Fig. 2. End systolic volume (ESV) and ejection fraction (EF) at baseline and during the first 28 days following MI. Inhibition of iNOS (1400W) reduces ESV and increases EF to a degree similar to that seen in iNOS knockout (KO) mice compared to untreated wild-type (WT) mice. *$P < 0.05$