Genetically modified animal models to study cardiovascular disorders

Ciro Indolfi, Daniele Torella*, Lorena Belardo*, Massimo Chiariello*

Division of Cardiology, Magna Graecia University, Catanzaro,
**Division of Cardiology, Federico II University, Naples, Italy*

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Address:

Prof. Ciro Indolfi

Laboratorio di Cardiologia Interventistica Università degli Studi "Magna Graecia" Via T. Campanella 88100 Catanzaro

Introduction

The techniques for generating genetically modified animals have had an enormous impact on cardiovascular research. Genetically modified animals have proved to be extremely useful for the understanding of the regulation of many gene products and the role of specific genes in complex biologic processes, such as the embryonic development of the cardiovascular system, lipoprotein metabolism, hypertension, and the development of atherosclerosis. The ability to knock out genes and to express transgenes in animals has produced a paradigm shift for cardiovascular scientists, changing the manner in which they approach their research and create entirely new topics for investigations. The generation and use of genetically modified animals have represented far more than a sophisticated tool for molecular geneticists; few cardiovascular scientists have been unaffected by the revolutionary impact of transgenic and gene-targeted animals. Traditional physiologists and developmental pathologists have increasingly turned to genetically modified animals to address their research.

Of fundamental importance to the development of transgenic technology were the experimental techniques of mammalian embryologists, who devised methods for collecting and manipulating fertilized one-cell mouse eggs and established the *in vitro* nutritional requirements for culturing eggs from the one-cell stage to the blastocyst stage of development. With the development of micromanipulation techniques, it became possible to inject DNA directly into the nucleus of cultured somatic cells. At approximately the same time, developmental biologists demonstrated that it was possible to microinject germ cells with DNA and RNA and observe the expression of the encoded protein product. Animals in which foreign DNA has been integrated into the host chromosomes are called transgenic . In recent years, the term has also occasionally been used to describe gene-targeted animals generated by homologous recombination in embryonic stem cells.

Traditional transgenic techniques permit the insertion of a cloned fragment of DNA into a random location in the mouse genome, thereby adding a new gene to the normal complement of genes in the chromosomal DNA. At the same time that transgenic techniques were being established, largely independent scientific advances were making it possible to introduce specific, predetermined mutations into the endogenous genes of the mouse. These gene-targeting techniques have typically been used to disrupt a gene of interest to generate mice that are deficient in that gene product. Thus, gene-targeting techniques make it possible to subtract, or knock out , specific genes in the genome.

These techniques had been used to knock out hundreds of different genes, including many relevant to the development of the heart and blood vessels, hypertension, lipoprotein metabolism, and atherosclerosis[1].

Vascular biology in genetically altered mice

The majority of studies of vascular function in genetically altered mice have been related to the role of endothelium. For this reason, studies related to endothelium and nitric oxide (NO) will be highlighted as ex-

amples of the types of studies that are being done in mice and of new insight into the regulation of blood vessels that is emerging.

A major focus of research in vascular biology relates to the role of endothelium in health and disease. Thus, it is perhaps not surprising that the majority of studies on vascular function in genetically altered mice are related to endothelial function. Studies to date suggest that endothelial function is generally similar in blood vessels of normal mice compared to blood vessels of other species.

In homozygous endothelial nitric oxide synthase-deficient mice (eNOS-/-), relaxation of the aorta and carotid and pulmonary arteries in response to acetylcholine is absent. Relaxation of the aorta in response to A23187 (a calcium ionophore) is also absent in eNOS-/-, providing direct evidence that endothelium-dependent relaxation of the aorta and several major arteries is mediated by eNOS. These results obtained in eNOS-/- mice are consistent with studies in vessels of control mice in which pharmacological inhibitors provided indirect evidence that endothelium-dependent relaxation was mediated by NO[2].

Vascular remodeling after arterial legation is impaired, and vascular proliferation is increased in eNOS-deficient mice. Vascular remodeling during chronic hypoxia is also altered in these mice. Finally, eNOS is an important regulator of ischemia-induced angiogenesis. Thus, together, these results have provided direct evidence that endogenous NO produced by eNOS is an important regulator of vascular tone, growth, and remodeling as well as of the migration of vascular muscle[2].

The role of inducible nitric oxide synthase (iNOS) in modulating neointimal formation after arterial wall injury is not clear. To determine whether the induction of iNOS gene expression promotes or attenuates the neointimal response to injury, Chyu et al.[3] used a murine model of perivascular injury induced by placing a periadventitial collar around the carotid arteries in both wild-type and iNOS knockout mice. Twenty-one days after cuff placement, a 40% reduction of the neointimal area occurred in iNOS knockout mice compared to the wild-type mice.

iNOS produced a sustained increase in NO compared to eNOS, and it was expressed in the arterial walls of vascular smooth muscle cells after mechanical injury. Since NO inhibits vascular smooth muscle cell proliferation *in vitro*, the inhibition of NO output by iNOS was expected to increase neointima formation after injury. However, N^G-nitro-L-arginine methyl ester treatment in a rat carotid injury experiment failed to demonstrate such an increase. This chemical may possess effects other than the inhibition of NO production, which may confound the interpretation of the data. This study in iNOS gene knockout mice avoided such problems. Twenty-one days after injury, less neointima formation existed in iNOS knockout mice than in wild-type mice. These findings support the hypothesis that iNOS expression in injured vascular smooth muscle cells promotes proliferation and neointima formation[3].

These findings highlight the divergent functional roles of eNOS and iNOS genes, which is likely to be of biological significance. Molecular mechanisms responsible for this diversity remain to be defined[3].

Advantages of genetically altered mice. Mice that overexpress or are lacking in expression of selected genes are excellent models for establishing the functional importance of a particular gene product. Homologous recombination using embryonic stem cells (gene targeting) is a powerful investigative tool being widely used in many disciplines. At present, the mouse is the only mammalian species in which gene targeting can first be performed and then be successfully transmitted to the germ line. In addition to studies of loss of gene function, these animals often represent models of human inherited or acquired diseases. The phenotypes resulting from such alterations have ranged from the predictable to the surprising. In some cases, mice carrying a disrupted gene appear phenotypically normal. Findings such as the latter may result from redundancy in the genome and/or the expression of compensatory mechanisms.

One of the great strengths of the gene-targeting technique is that it can overcome many problems present in other, more commonly used models. This includes the limited specificity of pharmacological agents (e.g., enzyme inhibitors or receptor antagonists). A good example of such a limitation in relation to vascular biology is the study of the NO system using inhibitors of NO synthase (the enzyme that converts L-arginine and molecular oxygen to NO and L-citrulline). It is now known that there are three major isoforms of NO synthase. These enzymes are products of separate genes and are designated neuronal, endothelial, and inducible isoforms of NO synthase (nNOS, eNOS, and iNOS, respectively). Although pharmacological inhibitors of NO synthases such as N^G-monomethyl-L-arginine have been very useful in examining the role of NO in vascular biology, a major limitation exists because this analogue of L-arginine (and most inhibitors of NO synthase) nonselectively inhibits all isoforms of the enzyme (eNOS, iNOS, and nNOS). For example, there are no selective inhibitors of eNOS. Thus, it is difficult to study the effects of selective inhibition of single isoforms of NO synthase. In addition, when inhibitors of NO synthase are used, there are often uncertainties regarding tissue or cellular access as well as the extent of enzyme inhibition. A major strength of the gene-targeting approach is that it allows the use of a precise genetic alteration to study complex responses in blood vessels or in intact animals. Gene targeting offers a level of specificity that traditional pharmacology can rarely, if ever, achieve.

In addition to studies of gene deletion in mice generated through gene targeting, the generation of transgenic mice that overexpress a selected gene is also a

common approach. With this approach, one can study the effects of overexpression of a candidate gene that may contribute to normal vascular regulation or vascular dysfunction under pathophysiological conditions. A transgenic animal is one that contains a segment of exogenous genetic material that is stably incorporated into the recipient genome. In contrast to deletion of a gene through gene targeting, overexpression of genes through the use of transgenic models can be performed in other species.

Limitations in using genetically altered mice. There are limitations in the use of genetically altered mice for experimental studies. For example, a potential limitation exists in that compensation may occur in the animal either during development or later in response to deletion of the targeted gene. For example, another redundant gene product may replace the function of the gene that was disrupted. As a result, deletion of one gene product may not result in any detectable change in phenotype.

The relatively small body size of mice is an additional obvious limitation for studies of blood vessels. The most commonly used method for *in vitro* studies of vascular function is to use vessel rings suspended in an organ bath. To date, the aorta has been by far the most frequently studied blood vessel in mice, although some studies have been performed with other mouse arteries. As the aorta is not a resistance blood vessel, data obtained with the use of the aorta may not always be representative of mechanisms present in smaller blood vessels or in specific vascular beds.

References

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