Dilated Cardiomyopathy
—— Concepts Derived From Gene Deficient and Transgenic Animal Models ——

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Genetic forms of human dilated cardiomyopathy (DCM) are briefly discussed, and a variety of animal models of genetic DCM are presented, some of which are caused by the gene mutations that also cause DCM in humans. The forms of DCM related to mutations or deletion of genes coding for extrasarcomeric or intrasarcomeric proteins, as well as to overexpression or knockout of genes in the $\alpha$-adrenergic signaling pathway, are included. Finally, novel approaches to treatment in experimental animal models are discussed, including double transgenesis and newer recombination methods, as well as in vivo somatic gene transfer which, based on initial experiments in animals, seems likely to find eventual application in human cardiac failure. (Circ J 2002; 66: 219 – 224)

Key Words: $\alpha$-adrenergic signaling pathway; Cardiac failure; Double transgenesis; Extrasarcomeric proteins; Gene mutations; In vivo somatic gene transfer; Intrasarcomeric proteins; Recombination

In this brief review, the focus will be on dilated cardiomyopathy (DCM), particularly the genetically determined animal models of DCM, some of which relate to human DCM. In humans, hereditary forms of DCM are present in about 25% of patients and several of these gene mutations have now been identified and will be reviewed briefly.

Genetic DCM in Humans

The forms of hereditary DCM in humans in which the genetic basis has been identified include mutations in the intermediate filament protein desmin, responsible for familial cardiac and skeletal myopathy (desmin-related myopathy) which also can be caused by a mutation in the $\alpha$-crystalline gene associated with lens cataract. A mutation in another intermediate filament protein gene, nuclear lamin A/C, and in a gene coding for the protein tafazzin (Barth syndrome) also are associated with DCM. Genetic DCM frequently occurs in association with Duchenne’s muscular dystrophy caused by mutations in the dystrophin gene as well as in X-linked forms of dystrophin deficiency. Dystrophin is a component of the transmembrane dystrophin–glycoprotein complex that links intracellular actin with lamin in the extracellular matrix and which thereby may contribute to membrane stability and perhaps to signaling. The genetic deficiency of other components of this complex, particularly the sarcoglycans, also can cause DCM; typical hereditary DCM was recently reported in families with mutations in the $\delta$-sarcoglycan gene and other sarcoglycan deficiencies in patients with limb girdle muscular dystrophy can be associated with milder forms of DCM. Mutations in the cardiac $\alpha$-cardiac actin gene have been found in patients with DCM; these mutations were identified close to the fixed end of actin near the Z-disc, and it was hypothesized that DCM may result primarily from deficiency in force-bearing proteins in contrast to mutations in the force-generating end of actin and other intrasarcomeric proteins which comprise the thick and thin filaments and are often responsible for hypertrophic cardiomyopathy (HCM). The muscle LIM protein (MLP) is associated with the actin cytoskeleton at the Z-disc, and its potential role in DCM was first identified in MLP null mice (discussed later). Deficiency of this protein has been reported in patients with heart failure from DCM of various causes and recently a specific mutation in the MLP gene was identified in several of a large number of patients with DCM screened by DNA sequencing of the MLP coding region (Knoell R, Chien KC; personal communication). Thus, several cytoskeletal gene mutations involving intrasarcomeric ($\alpha$-actin, MLP) and extrasarcomeric proteins (eg, desmin, dystrophin, sarcoglycans) observed in DCM appear to occur in genes involved in structural support, and potentially in signaling between the intracellular and extracellular compartments, and it has been proposed that defects in the cytoskeleton are primarily responsible for many forms of DCM.

Animal Models of DCM

Reports on animal models of DCM in some instances preceded the recognition of a mutation in the same gene in humans and contributed to the discovery of genetic forms of human DCM (eg, desmin, $\delta$-sarcoglycan, and MLP). In other instances gene defects discovered in humans with DCM were used to create animal models in order to confirm causality and investigate disease mechanisms, as discussed later and which have been reviewed in detail elsewhere.

Several murine models of DCM have not yet been shown to be related to human DCM including cardiac expression of a dominant negative CREB transcription factor and cardiac overexpression of tropomodulin and...
homozygous expression in mice of a mutant form of the sarcomeric protein myosin-binding protein C, which has both titin and myosin heavy chain binding sites and causes DCM that is present at birth. 

Mice with knockout of the Δ-SG gene develop DCM, whereas Δ-sarcoglycan deficiency does not cause DCM and those lacking Δ-SG develop HCM; the DCM in Δ-SG null mice is associated with deficiency of this protein in vascular smooth muscle as well as in striated muscle, and there is evidence that occlusions of small coronary vessels cause ischemic microinfarcts in the ventricular myocardium. 

The progressive cardiomyocyte dropout in the hamster DCM also has been attributed, at least in part, to loss of sarcomembran integrity. 

It is known that severe cardiac hypertrophy caused by mechanical overload (such as aortic banding or myocardial infarction), or by overexpression of certain signaling pathways such as expression of activated calcineurin or abnormal G protein signaling (discussed subsequently), can lead to cardiac hypertrophy followed by chamber dilation and heart failure. However, the current discussion will be largely limited to DCM caused by gene mutations or deletions that result in specific protein deficiencies shown to be related to genetic DCM in humans, although selected transgenic mouse models involving Δ-adrenergic receptors and G proteins also will be mentioned. Finally, some novel approaches to the treatment of DCM will be discussed.

Desmin

Desmin, an intermediate filament protein, links the sarcomere to the cell membrane. Mice with missense mutations in the desmin gene, which cause DCM (as well as skeletal myopathy and other disorders), were described before identification of the genetic human forms; these mice exhibit severe disorganization of muscle architecture associated with degeneration. As mentioned earlier, there is a desmin-related myopathy associated with DCM in humans, and mice with a mutation in the crystallin (a molecular chaperone heat shock protein essential for cytoskeletal integrity) mimic desmin-related myopathy.

Cardiac Actin

In mice, a null mutation of the sarcomeric protein cardiac Δ-actin gene results in early postnatal death, associated with both a reduced number of actin filaments and myofilament disarray. 

Dystrophin

Dystrophin null mice do not develop DCM, but mice with deficiency of both dystrophin and urotropin exhibit both DCM and severe muscular dystrophy. These findings probably are explained by compensatory upregulation of urotropin in mice with isolated disruption of the dystrophin gene.

Sarcoglycans (SG)

The naturally occurring genetic form of cardiomyopathy in the hamster, which also is associated with mild skeletal myopathy, has been studied for many years and a component of the dystrophin/dystroglycan complex, Δ-SG, recently was shown to be deficient in that animal model of heart failure, caused by a mutation in the ΔSG gene. Other sarcoglycans also are downregulated in association with Δ-SG deficiency in several types of hamster DCM, and myocyte loss is associated with replacement fibrosis and progression to severe heart failure and death at age 10–12 months.

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The progressive cardiomyocyte dropout in the hamster DCM also has been attributed, at least in part, to loss of sarcomembran integrity and it is possible that both mechanisms are involved.

Muscle LIM Protein (MLP)

Among the first murine models of DCM described was that related to knockout of MLP, which has a phenotype typical of heart failure in humans, including cardiac chamber dilation, relative wall thinning (but with increased cardiac mass), depressed systolic function and myocardial contractility, pulmonary congestion, and impaired Δ-adrenergic responsiveness. In addition, the left ventricular (LV) dilation and dysfunction became progressively worse during serial follow-up by echocardiography over a number of months. This model has been used to study the effects of potential therapeutic agents; for example, improvement of the echocardiographic and hemodynamic variables occurred during short term rhGH (growth hormone) administration. MLP interacts with the Z disc protein Δ-actinin, which in turn interacts with titin, a large protein spanning the half sarcomere from the Z line to the M line, which is associated with HCM and may be involved in force transmission.

Cardiac Δ-Adrenergic Receptor Pathway

The adverse effects of prolonged sympathetic nervous system activation are known to be associated with Δ-adrenergic receptor (Δ-AR) downregulation in human heart failure and prevention of cardiotoxic effects on the myocardium from prolonged Δ-AR stimulation acting via cyclic-AMP are considered to underlie many of the beneficial effects of Δ-AR blockade on morbidity and mortality in the clinical setting.

In several experimental studies in transgenic mice, prolonged overexpression of Δ-AR has induced late onset DCM. Other recent studies indicate that although marked overexpression of the Δ-AR (≥100-fold background) caused rapidly progressive DCM lower expression levels (60-fold) enhanced myocardial contractility and cardiac function and these effects persisted for more than 1 year without mortality or detrimental effects. Recent evidence has shown that signaling downstream of the Δ-AR and cAMP (which normally activates protein kinase A, PKA) can cause DCM; thus, adult transgenic mice with overexpression of the catalytic subunit of PKA developed DCM, mild fibrosis and arrhythmias associated with hyperphosphorylation of the ryanodine receptor (RyanR2) and phospholamban, without activation of Δ-AR signaling. It was suggested that the main mechanism of PKA-induced DCM may relate to RyanR2 phosphorylation associated with reduced binding of FKBP12.6 to RyanR2 resulting in abnormal sarcoplasmatic reticulum (SR) Ca2+ fluxes (abnormalities that have also been noted in human heart failure).

G Proteins (GTP-Binding Proteins)

Prolonged overexpression of the stimulatory G protein,
adenylyl cyclase (AC), and the AC 6 isoform has been the PLB-/- / MLP-/- mice. Reductions in the Ca2+ transient in MLP-/- mice having DCM; the offspring were then inhibitor of the failing heart. Transgenic mice that express a peptide approach has been used in mice to enhance the function of the Gs protein, causing its phosphorylation accompanied by some investigators to be rate-limiting in signaling. Overexpression of a synthetic Gi-coupled receptor causes DCM, are discussed in more detail later.

**Approaches to Therapy in Animal Models of DCM**

It is well known that long-term therapeutic use of ß-AR agonists or phosphodiesterase inhibitors, including prolonged intravenous infusion of dobutamine, has unfavorable effects in patients with congestive heart failure. However, recent experiments in animals concerning components of the ß-AR signaling pathway that operate downstream of the receptors, including phospholamban, G-protein receptor kinases and adenylyl cyclase, suggest potential usefulness of other strategies for improving the function of the failing heart.

**Double Transgenic Mice**

The MLP knockout mouse model of DCM, discussed earlier, has been useful for examining the importance of abnormal Ca2+ regulatory mechanisms in sustaining the DCM phenotype. Crossbreeding experiments between mice with knockout of the phospholamban (PLB) gene (PLP-/-), which exhibit hypercontractility and normal life expectancy, and MLP knockout (MLP-/-) mice have generated double knockout offspring that show complete prevention of the DCM phenotype. Also, in these PLP-/-/MLP-/- mice, LV contractility, expressed as LVdP/dtmax, using high fidelity micromanometry, was high and not significantly different from that in PLP-/- mice; the LVdP/dtmax level was much lower than normal in control MLP-/- mice, despite a higher LV end-diastolic pressure, and the markedly impaired response to ß-AR stimulation with dobutamine in MLP-/- mice became supranormal in the PLB-/-/MLP-/- mice. Reductions in the Ca2+ transient and in SR Ca2+ storage in the MLP-/- mice also were restored in the double transgenic mice, suggesting that defective Ca2+ cycling was involved in maintenance of the DCM phenotype.

When ß-ARs are occupied by an agonist, ß-AR kinase (ß-ARK, a member of the G-protein coupled receptor kinase family) when activated interacts with the ß submit of the Gs protein, causing its phosphorylation accompanied by ß-AR desensitization. Therefore, inhibition of ß-ARK would be expected to enhance ß-AR function, and this approach has been used in mice to enhance the function of the failing heart. Transgenic mice that express a peptide inhibitor of ß-ARK 1 were created and then crossed with MLP-/- mice having DCM; the offspring were then compared with control MLP mice. The transgenic mice showed markedly higher echocardiographic and hemodynamic measures of cardiac function than the MLP-/- mice, including improved ß-AR responsiveness to near-normal levels. These findings suggest a therapeutic potential for this approach.

Another downstream component in the ß-AR pathway is adenylyl cyclase (AC), and the AC6 isoform has been shown by some investigators to be rate-limiting in ß-AR signaling. Overexpression of AC6 in otherwise normal mice causes enhanced ß-AR responsiveness without appar-
lems, and application of recombination techniques to achieve temporal tissue specific regulation for evaluating therapeutic strategies is complex. An alternative approach to genetic manipulation, which seems more likely to find eventual application in human cardiac failure, is somatic gene transfer.

Several years ago, such studies were begun by developing a new method to achieve high efficiency gene transfer into the myocardium of rodents, with initial studies in the hamster. The current method involves total body hypothermia by immersion to 25–26°C, a small high thoracic incision to allow placement of snares on the ascending aorta and main pulmonary artery, and placement of a catheter above the aortic valve via the carotid artery. The aorta and the pulmonary artery are then occluded for approximately 3 min, cardioplegic solution containing a permeabilizing agent (histamine or substance P) is injected into the aortic root, followed by injection of a viral vector in the same solution; the snares on the great vessels are then released and the heart resuscitated by compression and transient dobutamine (or dopamine) infusion; the animal is then warmed and allowed to recover. This approach for intracoronary gene transfer to the myocardium has been applied successfully with low mortality (<5%) in normal hamsters, with more than 70% of left ventricular myocytes showing nuclear targeted LacZ expression, associated with high expression in the right ventricle as well. Recently, similar results in ongoing studies have been obtained in normal rats.

The initial application of this approach has been in the cardiomyopathic (CM) hamster. An adenoviral (Adv)−sgS gene in the CM hamster. An adenoviral (Adv)−sgS vector was used in 6-week old hamsters (Bio 14.6 strain) to produce short term transduction of the −sgS protein in the heart. Highly efficient expression was achieved at 1 week with demonstration of diffuse staining of −sgS protein in 50–60% of cardiomyocyte sarcolemmal membranes throughout the left ventricle. There was also restoration of other sarcoglycans that are downregulated in the CM hamster. As expected, there was marked reduction at 3 weeks of the high LacZ expression observed 2 weeks’ earlier. However, even more uniform and extensive staining of all dystroglycans was observed at 3 weeks, together with improvement of LV systolic function on echocardiography. Thus, persistent high-efficiency cardiac expression of a missing structural component of the myocardial sarclemma was demonstrated. Kawata et al have also recently reported transfer of the −sgS gene in the CM hamster using direct myocardial injection into the LV apex of an adenovirus-associated viral (AAV)−sgS vector, and at 10 weeks cardiac expression was limited to the apex and mid LV (41% and 27% efficiency, respectively); hemodynamic variables that improved were LV relaxation and ventricular filling pressures.

Other studies have explored the use of gene transfer by other methods in models of LV dysfunction in the rat and rabbit shown improvement after transduction of the SERCA-2 gene.

Recently, the in vivo somatic gene transfer technique was used as a means of testing new approaches to the treatment of established DCM. As part of the study cited earlier on double transgenic PLB−/−/MLP−/− mice it was found that AdV transfection of cardiomyocytes from MLP−/− mice with a pseudophosphorylated mutant PLB peptide acted effectively to prevent binding of PLB to the SERCA-2 calcium pump and reduce Ca2+ uptake by the SR; the mutant peptide restored basal contractile function and Ca2+ stores to the cardiomyocytes. In recently completed studies, an rAAV vector was constructed for use in gene transfer using a CMV promoter and a slightly different PLB mutant. Studies at 5 weeks and at 6 months after gene transfer in CM hamsters using this AAV/S16E PLB vector show significantly less deterioration of cardiac function by echocardiography and improved contractility (assessed by LVdP/dtmax) compared with rAAV/LacZ treated animals (Hoshishima M, Ikeda Y, Iwanaga Y, Minamisawa S, Date M, Li M, et al personal communication). This study using an rAAV vector to achieve long-term, high-efficiency in vivo gene delivery via the coronary arteries demonstrates the feasibility of successful gene therapy in established DCM by improving excitation-contraction coupling through a mechanism that is independent of cAMP effects.

Together, these experimental observations offer the possibility that in vivo gene transfer will eventually be applied to the treatment of human heart failure.

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References


