Molecular Remodeling in the Failing Human Heart

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Heart failure is a progressive and fatal disease process. Arrhythmias and progressive cardiac dysfunction account for most of the morbidity and mortality in patients with heart failure. In general, the physiologic mechanisms responsible for progressive myocyte dysfunction, remodeling, and arrhythmias involve signaling mechanisms that alter myocardial gene expression. These changes in gene expression are complex and involve contractile proteins, ion channels, Ca++ handling, apoptosis, cell metabolism, the extracellular matrix, signal transduction pathways, and growth factors.

Introduction

Despite advances in medical and device therapy, heart failure remains a disease process with a high morbidity and mortality. Recent clinical trials suggest that the annual mortality from heart failure remains in the range of 6% to 12% (Table 1). There is a strong association between remodeling of the ventricle and mortality. Remodeling in heart failure is clinically characterized by a decline in systolic function and an increase in end-diastolic volumes. Myocardial injury results in activation of multiple signaling pathways that are involved in this remodeling (Fig. 1). Activation of the adrenergic nervous system, the renin-aldosterone-angiotensin system, increased wall stress, and cytokine production have all been implicated in this process. Remodeling increases myocardial oxygen demand, worsens mitral regurgitation, and predisposes patients to progressive myocardial dysfunction and arrhythmias. Therapies that inhibit or reverse remodeling, such as β-blockers, angiotensin-converting enzyme inhibitors, and aldosterone inhibitors, in general improve survival and delay disease progression in heart failure. There are multiple molecular mechanisms involved in myocardial remodeling.

The Human Genome Project has deciphered 3 billion base pairs that encode more than 35,000 genes of the human genome. It is important to note that the function of 50% of these genes remains unknown. It is the differential expression of these genes that ultimately determines cell type. Alterations of gene expression within cell types by disease processes contribute to disease progression. Advances in quantitative real-time reverse transcriptase polymerase chain reaction and gene chip microarray technology allow for the rapid measurement of thousands of different genes within a sample. The human heart expresses approximately 40% to 50% of the genes within the human genome. Gene expression studies involving explanted human hearts obtained during cardiac transplant and endomyocardial biopsies from patients with heart failure suggest that disease progression involves alterations in hundreds of different genes involved in myocyte function. This review discusses the alterations in gene expression that may contribute to the progressive contractile dysfunction and arrhythmias observed in patients with heart failure.

Fetal Gene Expression and Contractile Dysfunction

A hallmark of progression to heart failure is a decrease in myosin heavy chain (MHC) or myofibrillar ATPase activity. The functional myosin molecule is composed of two light chains and two MHCs. There are two MHC isoforms in the human heart (α and β). The α-MHC isoform has approximately three times the ATPase activity as the β-MHC isoform. Higher levels of β-MHC are associated with more rapid contractile velocity and force generation.

In the nonfailing human heart, α-MHC mRNA represents approximately 20% to 30% of total MHC expression [1,2]. In the failing human heart, α-MHC mRNA is downregulated and represents less than 2% of total MHC mRNA. When α-MHC mRNA is downregulated, β-MHC mRNA undergoes reciprocal upregulation. There is a strong association between protein levels and mRNA abundance for β-MHC and α-MHC consistent with transcriptional regulation for both these genes. At the protein level in the nonfailing heart, α-MHC represents approximately 10% of

Table 1. Mortality in HF

Trial	Therapy	Patients, *n*	NYHA class	Follow-up, *mo*	Mortality (treatment group)	Annualized mortality (treatment group)
Digoxin HF	Digoxin	6800	I–IV	37	34.8%	11.3%
Carvedilol HF	Carvedilol	1094	II–IV	6.5	3.2%	5.9%
MERIT-HF	Metoprolol XL	3991	II–IV	12	7.2%	7.2%
VAL-HeFT	Valsartan	5010	II–IV	23	19.7%	10.2%
EPHESUS	Eplerenone	6642	I–IV	16	14.4%	10.8%
COMPANION	BiV-AICD	1520	III–IV	14.8	12%	9.8%

BiV-AICD—biventricular implantable cardioverter-defibrillator; COMPANION—Comparison of Medical Therapy, Pacing, and Defibrillation in Heart Failure; EPHESUS—Eplerenone Post-acute Myocardial Infarction Heart Failure Efficacy and Survival Study; HF—heart failure; MERIT-HF—Metoprolol CR/XL Randomized Intervention Trial in Congestive Heart Failure; NYHA—New York Heart Association; VAL-HeFT—Valsartan Heart Failure Trial.

total MHC protein expression, and in the failing human heart α-MHC protein is downregulated and undetectable [3]. The mechanisms controlling MHC isoform shifts in humans remain to be elucidated. In animal models, GATA4, NFAT3, TEF, MEF2, and thyroid response elements are important for upregulation of α-MHC, whereas YYI and the KU protein complex appear to play a role in repression of α-MHC [4].

This pattern of myosin isoform switching, decreased α-MHC and increased β-MHC, is similar to that observed in the fetus during development. Atrial natriuretic peptide and brain natriuretic peptide are also expressed in the fetus, diminished in the adult, but reactivated in heart failure. Several other genes that may affect cardiac contractility recapitulate fetal gene patterns in association with the development of heart failure. Sarcoplasmic-reticulum ATPase, as well as GLUT4, mGS, and mCPT-I, are considered "adult genes" that are diminished in heart failure similar to levels in the nonfailing fetal heart. These patterns are consistent with a general hypothesis that the progression of myocyte dysfunction observed over time in heart failure is associated with a reversal to fetal gene expression patterns.

The molecular changes and contractile dysfunction observed in the failing human heart are reversible to some degree. It has been shown that β-blockers are capable of improving contractility, chamber geometry, and survival while decreasing cardiac hypertrophy in patients with heart failure [5]. This improvement in outcomes is seen in patients with ischemic and nonischemic cardiomyopathies. The molecular mechanisms by which β-blockers exert their effects are still being investigated but appear attributed in part to the reversal of this pattern of fetal gene expression. We have previously studied gene expression in 53 patients with idiopathic dilated cardiomyopathy randomized to placebo or β-blocker, using serial endomyocardial biopsies. Patients who improved their ejection fraction over time had a significant increase in sarcoplasmic-reticulum ATPase mRNA and α-MHC mRNA, and a significant decrease in β-MHC mRNA [6•].

Ion Channels and Arrhythmias

Cardiac action potentials are the composite result of depolarization and repolarization currents in myocyte cells. Regulation of these currents is the product of coordination of multiple ion channels, pumps, and ion handling. Disturbances of the normal timing and sequence of the action potential repolarization ultimately can be arrhythmogenic [7]. The heritable channelopathies have shown that a mutation in a single ion channel can result in an arrhythmogenic substrate. Prolongation of the action potential and changes in the electrical system associated with a pathologic process are termed electrical remodeling [8•]. Prolongation of the action potential in the setting of heart failure has been documented in human myocardial tissue [9] and in myocyte cells [10]. Electrical remodeling may be the result of changes in ion channels at the level of mRNA expression, protein expression, post-translational modification, changes in trafficking, splice variants, phosphorylation, or channel function. These changes may occur in a homogenous fashion throughout the myocardium or can vary from region to region. Much of the focus in electrical remodeling has been on changes of ion channel function and calcium handling.

Repolarization of the action potential is primarily the result of potassium channels [8•]. There are several types of potassium channels found in cardiac cells, with only two highlighted for the purposes of this review. IK_1 is primarily responsible for the resting membrane potential of the cell. IK_{to} is important in early repolarization during phase 1 of the action potential [8•]. Measurements of the various potassium channels by current density, mRNA expression, current kinetics, or current voltage have had varying conclusions. In general, there is thought to be a downregulation in potassium channels in the setting of heart failure. Reduction of IK_{to} has been the most consistent finding in the heart failure phenotype of all the ion channels [8•]. There has been documentation of a decreased current density of IK_{to} [11,12]. Moreover, there has been correlation between measurement in mRNA expression and current density [11]. The density of IK_{to}

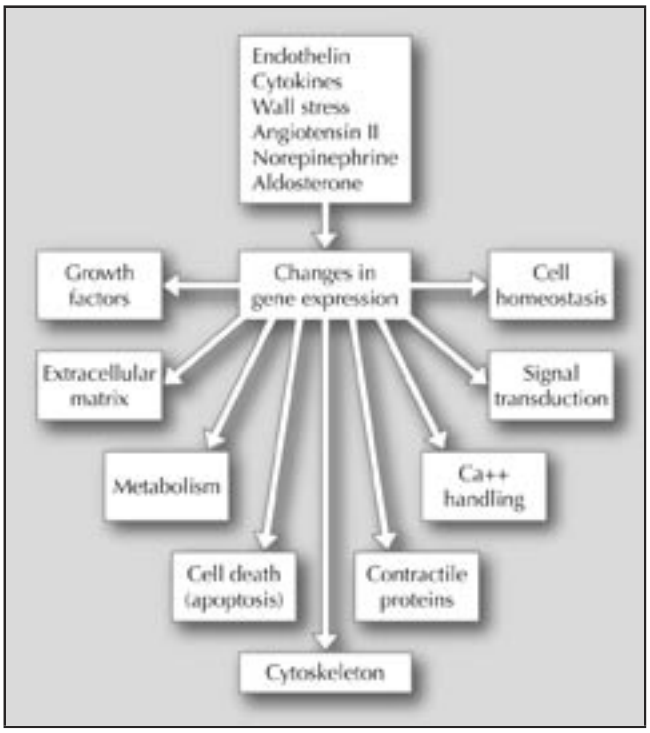

Figure 1. Signal transduction pathways and gene expression changes in heart failure.

varies regionally and transmurally. There are two genes that encode for IK_{to}, which are expressed in a specific pattern across the myocardial wall [13].

IK_1 has been reported as being downregulated, but the results of several studies are not consistent. IK_1 plays a prominent role in maintaining the resting potential of cell membrane. Measurements of IK_1 current density in patients with heart failure have shown to be decreased in comparison with nonfailing controls [9]. Conversely, measurements of IK_1 mRNA were no different between heart failure patients and nonfailing controls [11].

Calcium handling is integral in the electrical contraction coupling, and abnormalities in this process can result in derangements in the electrical conduction system. L-type Ca+ currents contribute in the inward current, which aids in prolonging the action potential. L-type calcium channels current density is thought to be decreased or show no change in the setting of heart failure in comparison with nonfailing controls. Changes in mRNA or in dihydropyridine binding sites may result in the decrease in current density [14]. However, studies evaluating measurements of mRNA by different methods have had varying conclusions. One study found a change in the L-type Ca+ current decay kinetics, which would change excitation-contraction coupling [14].

The sodium calcium exchanger (NCX) also contributes to extrusion of cellular calcium. NCX appears to be upregulated in the setting of heart failure by measurement of mRNA and protein [15,16]. It has been theorized that upregulation of NCX would compensate for a decrease of calcium extrusion secondary to the decrease in sarcoplasmic reticulum (SR) Ca^{2+}-ATPase function in heart failure. Ultimately, the combination of an increase in NCX and a decrease in SR Ca^{2+} could result in a decrease in the available calcium pool and ultimately result in an increase in the depolarizing current [17]. However, a recent study showed that upregulation of the NCX protein may be related to diastolic function. Specifically, upregulation of the NCX protein in the setting of decreased SR Ca^{2+} ATPase protein correlated with left ventricular systolic dysfunction and preservation of diastolic function. This was in direct contrast with the alternative phenotype of left ventricular systolic dysfunction coupled with diastolic dysfunction, in which a decrease in SR Ca^{2+} ATPase was measured along with unchanged measurements of NCX protein [18].

Recently, I_f or the pacemaker channel has gained attention. Initially, I_f was thought to be confined to the electrical system but more recently has been located in failing ventricles [19]. In human myocytes, I_f is overexpressed in the failing phenotype in comparison with controls [20]. In a separate study there was a trend to increased I_f current density [21]. Although the role of I_f in perpetuating an arrhythmogenic substrate is not conclusive, upregulation of I_f coupled with downregulation of IK_1 could result in increased depolarization potential in the heart failure phenotype [22].

The mechanism that is responsible for the changes in ion channels is not certain. Increases of circulating catecholamines are theorized to be the initiating factor. More recently, increased pressure/volume is thought to play a pivotal role in eliciting remodeling [23]. This theory has been evaluated in recent studies by comparing gene expression and protein measurements from failing, nonfailing, and post–left ventricular assist device patients [24]. The pathologic regulation of ion channels ultimately appears to result in heterogeneous repolarization, afterdepolarizations, and ultimately, ventricular tachyarrhythmias. An understanding of the mechanisms that are responsible for electrical remodeling and discovery of treatments that reverse this process will be instrumental in improving the foundation of knowledge and treatment opportunities in the heart failure realm.

Genomics and Heart Failure

High-density DNA microarrays now allow for broad-spectrum analysis of mRNA expression. This technique involves robotic microfabrication of gene chips by binding tens of thousands of short specific synthetic DNA strands to a glass slide. Messenger RNA is isolated, modified with a fluorescent tag, and used to label the chips. Several observational cross-sectional and serial studies have been conducted using gene chips and explanted end-stage human heart tissue [25,26•,27]. These studies suggest that the molecular mechanisms involved in cardiac remodeling are more than

changes in contractile proteins or ion channels. It appears cardiac remodeling involves altered expression of hundreds of different genes involved in cardiac metabolism, cell death, the extracellular matrix, signal transduction, myocardial growth, and cell homeostasis (Fig. 1).

However, there are limitations to broad-spectrum transcriptional analysis. Whenever analyzing thousands of different genes, it is possible to discover large differences in expression by type I statistical error alone. Changes in gene expression at the mRNA level also do not necessarily correlate with alterations in protein abundance or activation. The significance of alterations observed in gene chip studies requires confirmation by more quantitative techniques and proteomic analysis. The biological importance of these changes will ultimately require experiments in genetically engineered animals and trials in humans targeted to specific pathways.

Conclusions

The prevalence of chronic left ventricular systolic dysfunction continues to increase. According to the Heart Failure Society of America, an estimated 400,000 to 700,000 new cases of heart failure are diagnosed each year. The number of deaths in the United States from this condition has more than doubled since 1979, averaging 250,000 annually. The mechanisms responsible for what are ultimately detrimental remodeling processes are numerous and complex. The two most common causes of death in the heart failure patient population are progression to refractory congestive heart failure and sudden death, which is often the result of malignant arrhythmias. A theme for remodeling appears to be a return to the fetal gene expression pattern. Newer technologies including microarray chips are aiding in further defining the numerous changes that occur in heart failure. Ultimately, a clearer understanding of the pathways that lead to these maladaptive changes and the processes involved in these changes will result in new treatments for patients with heart failure.

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