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IN SEARCH OF NEW THERAPEUTIC TARGETS AND STRATEGIES FOR HEART FAILURE: RECENT ADVANCES IN BASIC SCIENCE

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Introduction

Chronic heart failure (CHF) is a complex clinical syndrome that arises secondary to inherited or acquired abnormalities of cardiac structure and/or function that impair the ability of the heart to fill or eject blood. Common causes include conditions that chronically increase cardiac workload, such as loss of muscle due to myocardial infarction (MI) or pressure overload due to hypertension. The cardiac response to such stresses involves complex remodelling of cardiomyocytes and the non-myocyte compartment which may initially be adaptive but eventually progresses to contractile dysfunction, ventricular dilatation and arrhythmias. Here, we review recent advances in deciphering the molecular mechanisms underlying cardiac remodelling, focusing on CHF with depressed systolic function rather than the less defined condition of heart failure with preserved ejection fraction.

The remodelling phenotype

A prominent feature of the remodelling heart is cardiomyocyte hypertrophy but substantial changes also occur in myocyte electrical properties, calcium handling, energy metabolism, contractile function and cell viability¹. Extracellular matrix remodelling involves both fibrosis and activation of collagenolytic enzymes termed matrix metalloproteinases that lead to chamber dilatation, as well as significant changes in the myocardial vasculature. Although this broad phenotype is common to diverse causes of CHF, studies in gene-modified mouse models show that different phenotypic components can be independently regulated. For example, hypertrophy without contractile dysfunction, fibrosis or dilatation – mimicking “physiological” hypertrophy in athletes – is observed in some models – suggesting that specific pathways may drive adaptive versus maladaptive remodelling^{2,3}.

Hypertrophic signalling

The insulin-like growth factor (IGF)/phosphatidylinositol 3-kinase alpha (PI3K α)/protein kinase B (PKB or Akt) pathway is strongly implicated in physiological hypertrophy³ and recent work suggests an important role for the transcription factor C/EBP β ⁴. In contrast, pathological remodelling involves multiple overlapping pathways^{5,6,7} (Figure 1). G-protein-coupled receptor (GPCR) agonists such as catecholamines, angiotensin II and endothelin activate protein kinases including protein kinase A (PKA), protein kinase C (PKC), protein kinase D (PKD) and mitogen-activated protein kinases (MAPKs) that switch

on pro-hypertrophic programmes. These involve the synergistic action of transcription factors such as myocyte enhancer factor-2 (MEF2), nuclear factor of activated T cells (NFAT), GATA4 and serum response factor (SRF). Elevated intracellular calcium levels activate PKC isoforms and calcium/calmodulin-dependent kinase II (CAMKII), as well as calcineurin, a phosphatase that activates NFAT. Additionally, there may be a role for mechanosensitive signalling pathways activated at sarcomeric level ⁸.

These cascades do not simply operate in parallel but in fact display significant cross-talk and key points of convergence that may be especially amenable to therapeutic targeting. For example, the calcineurin/NFAT pathway is regulated not only by calcium but also by kinases that inhibit NFAT activity and interactions with sarcomeric proteins.

Pharmacological inhibitors of calcineurin (e.g. cyclosporine A) are clinically used as immunosuppressants but are unsuitable for use in CHF due to their side-effects. However, agents targeting other components of this pathway, e.g. regulators of calcineurin, may prove more useful. Another crucial signalling convergence point is the activity of histone deacetylases (HDACs), which inhibit transcription factor access to DNA ⁹. Phosphorylation of Class II HDACs by kinases such as PKC, PKD and CAMKII relieves this inhibition and de-represses the transcriptional activity of MEF2. HDAC kinases are regulated by receptor-activated pathways and calcium, as well as signals such as reactive oxygen species (ROS), and may integrate these different inputs and fine-tune the hypertrophic response. Class I HDACs appear to have opposite effects to Class II HDACs ¹⁰ so that any therapeutic targeting of this pathway would need to be selective. Other pathways can also act as a brake to pro-hypertrophic signalling. For example, the activation of cGMP-dependent protein kinase (PKG) by nitric oxide (NO) and natriuretic peptides inhibits hypertrophic signalling pathways at multiple levels ¹. Indeed, cGMP phosphodiesterase inhibitors (e.g. sildenafil) that elevate cGMP and enhance PKG signalling are beneficial in animal models of CHF and are in current clinical trials ^{11, 12}.

MicroRNAs (miRNAs or miRs) are short, highly conserved, non-coding RNAs that regulate gene expression at the post-transcriptional level by inhibiting translation or promoting degradation of target mRNAs. The extant literature suggests that miRNAs are differentially regulated in the failing heart, and play a key role in the pathogenesis of heart failure through their ability to negatively regulate expression levels of networks of genes that govern adaptive and maladaptive cardiac remodelling ¹³ (Figure 2). Both gain- and loss- of function experiments in mice have shown that miRNAs modulate various aspects of the CHF phenotype, including cardiac myocyte hypertrophy, excitation-contraction coupling, apoptotic cell death and myocardial fibrosis ^{14, 15, 16, 13}. RNA interference-based technologies are currently being developed as clinical therapeutics to antagonize specific miRNAs that have been associated with the development of a heart failure phenotype in small and large animal models of cardiac injury ¹⁷. In addition, there is significant interest in the use of plasma miRNAs or miRNA signatures as disease biomarkers ^{18, 19}.

Calcium dysregulation

Excitation-contraction coupling (ECC) involves calcium influx through sarcolemmal L-type channels, calcium-induced calcium release from the sarcoplasmic reticulum (SR) through ryanodine receptor channels (RyR), and the binding of calcium to myofilaments to initiate contraction. The process is reversed by calcium reuptake into the SR via a calcium ATPase pump (SERCA2a) and cellular efflux via the sodium/calcium exchanger. The failing myocyte has a reduced calcium transient amplitude and increased diastolic calcium level due to several abnormalities, including impaired SERCA2a function and increased calcium leak through RyR ²⁰ (Figure 3). The decrease in calcium transient contributes to reduced contractile force while increased SR leak and elevated diastolic calcium may cause

arrhythmia and diastolic dysfunction. Defective SERCA2a function is related to decreased protein levels as well as altered phosphorylation of phospholamban - the regulator of SERCA activity—which may involve perturbations in phosphatase activity^{21, 22}. The increased SR calcium leak has been variably attributed to RyR hyperphosphorylation (by PKA and/or CAMKII) or defects in RyR stabilisation^{23, 24}. Intracellular sodium may also be increased in failing myocytes and promotes arrhythmia as well as causing a reduction in mitochondrial calcium levels that compromises antioxidant capacity and enhances mitochondrial ROS production²⁵.

Beyond contractile dysfunction and propensity to arrhythmia, myocyte calcium dysregulation impacts on pro-hypertrophic and cell survival signalling pathways and on mitochondrial function and energy production. Studies indicate that calcium levels in specific subcellular microdomains of the failing myocyte, for example plasmalemmal or perinuclear compartments, may be differentially regulated and may influence distinct processes such as contraction and transcription respectively^{26–28}. Approaches to correct abnormal ECC in experimental animal models can prevent maladaptive remodelling, suggesting an important role for abnormal ECC in the pathogenesis of CHF.

Several potential therapies to target abnormal ECC are being developed²⁹. Enhancement of SERCA2a activity may be a promising approach and small clinical trials of SERCA2a gene transfer to increase protein levels have been conducted. Another approach is to inhibit RyR calcium leak, for example with CAMKII inhibitors or agents that stabilise the RyR, which could be especially efficacious in reducing arrhythmias. Increased intracellular sodium due to augmented late sodium currents might be treatable with the drug ranolazine, which is already available for use in patients with angina – although this agent may also have other actions. Recently, a positively inotropic small molecule – omecamtiv mecabril – has been reported that directly activates cardiac myosin (i.e without altering calcium levels) without increasing energy demand or arrhythmia, unlike beta-adrenergic agonists³⁰. However, the long-term effects of this agent in CHF remain to be established.

Myocyte survival or death

A low level but progressive loss of myocytes in the chronically overloaded heart is believed to contribute to the development of cardiac remodelling and contractile failure³¹. Apoptosis is an important mode of death that may be triggered by GPCR activation, cytokines and increased ROS production. GPCR-induced apoptosis involves kinases such as apoptosis signal-regulating kinase 1 (Ask1), p38MAPK, JNK and CAMKII as well as PKC-dependent transcriptional upregulation of a pro-apoptotic protein, Nix, which targets to mitochondria and activates a death pathway³². CAMKII may be one point of convergence of pro-apoptotic signalling as it is activated both by calcium and the regulated production of NADPH oxidase-derived ROS, downstream of angiotensin II-induced GPCR stimulation³³. Cell death is counteracted by pro-survival pathways such as the activation of Akt and Pim-1 kinases and the inactivation of GSK3 β ³⁴.

A different form of cell death, termed programmed necrosis, has recently been recognised to also be of importance in heart disease³⁵. In contrast to apoptosis, necrosis is accompanied by early loss of plasma membrane and organelle integrity and marked inflammation. The latter may contribute to extracellular remodelling as well as the development of contractile failure. The defining molecular feature of programmed necrosis is the opening of the mitochondrial permeability transition pore (MPTP) in response to elevated mitochondrial calcium levels and perhaps oxidative stress. The opening of this channel causes collapse of the mitochondrial membrane potential and ATP production and triggers execution of necrosis. Studies in gene-modified mice lacking cyclophilin D, a regulator of the MPTP,

suggest that this pathway is important in acute MI^{36,37}. Necrosis contributes to heart failure in a genetic model of myocardial calcium overload³⁸ and in a model where the protein Nix is targeted to the endoplasmic reticulum instead of mitochondria³⁹, but its contribution to CHF induced by increased workload remains to be defined.

A third process that may affect myocyte survival is autophagy (or self-digestion), an evolutionarily conserved mechanism for the bulk degradation and recycling of long-lived proteins and organelles within cells during starvation. Autophagy is activated in the haemodynamically overloaded heart and after ischaemia/reperfusion⁴⁰. Studies in gene-modified mice deficient in Atg5, a protein required for autophagy, suggest that it plays an adaptive role – perhaps by removing abnormal protein aggregates and increasing cellular energy supply⁴¹. Indeed, protein quality control by other cellular pathways such as the ubiquitin-proteasome system may also be beneficial in the failing heart⁴². However, some authors have reported that autophagy was detrimental during haemodynamic overload⁴³; the reasons for this discrepancy remain to be established.

Myocardial perfusion and energetics

The maintenance of an oxygen supply-demand balance is critical for normal heart function and recent work shows that myocardial capillary density is a key determinant of the remodelling response even in non-ischaemic haemodynamically overloaded hearts⁴⁴. An insufficient increase in capillary density relative to increasing muscle mass promotes pathological remodelling with fibrosis, dilatation and contractile failure. Important stimuli for myocardial capillarisation in this setting are the transcriptional factors Hif1 α and GATA4, which induce the production and release of vascular endothelial growth factor (VEGF) from cardiomyocytes to exert paracrine effects on myocardial vessels^{45,46}. A protein called p53 was found to antagonise Hif1 α activation⁴⁵ whereas a ROS-generating enzyme, Nox4, was recently found to be a positive driver of Hif1 α activation during pressure overload⁴⁷. The latter data are also of interest in that they identify a protective pathway mediated by ROS in contrast to ROS-mediated detrimental effects, and suggest that therapeutic approaches may need to target specific ROS sources and pathways rather than the non-specific antioxidants that have failed to date in clinical trials.

Energy production and metabolism within the cardiomyocyte are also of major importance in the failing heart. The mitochondria take centre stage in this process and there is substantial remodelling of mitochondrial structure and function as the heart itself remodels. The utilisation of substrates (glucose versus fatty acids), ATP synthesis and handling, energy efficiency and antioxidant reserve are all altered⁴⁸. A by-product of these changes is usually a significant increase in mitochondrial ROS which exerts detrimental effects both within and outside mitochondria⁴⁹. Mitochondrial remodelling is largely driven by a complex transcriptional programme in which a key player is PGC1 α (peroxisome proliferator-activated receptor- γ (PPAR- γ) coactivator 1)⁵⁰. PGC1 α induces and interacts with other transcription factors and also drives an increase in mitochondrial number (biogenesis). An important question is to what extent the alterations in mitochondrial function are a manifestation of the remodelled heart or whether they accelerate the process of remodelling. Regardless of the answer, drugs that can modulate substrate utilisation (e.g. etomoxir, ranolazine) are being studied as therapies for heart failure⁵¹. A more detailed discussion of this area is beyond the scope of this article but may be found in recent comprehensive reviews^{52,50,51,48}.

Changes in the extracellular matrix

It has long been recognized that alterations in the extra-myocyte compartment, leading to fibrosis, dilatation and shape change, are a major component of cardiac remodelling and

current therapies that reduce mortality in patients with CHF (e.g. ACE inhibitors) have a significant impact on these abnormalities. It is also recognised that enhanced matrix turnover due to the activation of matrix metalloproteases (MMPs) is important, although MMP inhibitors have failed to advance to clinical trial stage. However, the precise inter-relationship between alterations that are ongoing in the extra-myocyte compartment and those occurring within cardiomyocytes in the remodelling heart remains incompletely understood. The stressed or failing cardiomyocyte signals to fibroblasts and other cells within the matrix through the release of factors such as connective tissue growth factor (CTGF) and transforming growth factor- β (TGF β)⁵³. However, it has become evident that fibroblasts also signal to the myocyte in the reverse direction. The paracrine secretion of IGF1 from fibroblasts to myocytes contributes to adaptive hypertrophy during haemodynamic overload in a mouse model⁵⁴. Likewise, signalling involving mammalian sterile 20-like kinase 1 (Mst1) within fibroblasts inhibits TNF α release and is protective through this mechanism⁵⁵. Inflammatory cell influx within the myocardium is also involved in remodelling, not only after MI or in myocarditis but also in the haemodynamically overloaded heart⁵⁶. As mentioned earlier, an important stimulus that attracts inflammatory cells may be the occurrence of programmed myocyte necrosis within the heart, which releases damage associated molecular patterns (DAMPs) from the cytosol that are capable of provoking an inflammatory response through activation of the innate immune system^{57, 58}. Deciphering the complexity of paracrine cross-talk among the different cell types within the myocardium may lead to novel therapies that specifically target the fibroblast or inflammatory cell.

Gaps in understanding

The striking progress that has occurred in basic cardiovascular science over the past decade has dramatically increased the number of possible therapeutic targets for treating CHF. However, with the exception of the bradycardic agent ivabradine⁵⁹, this proliferation of targets has not resulted in the development of new therapies for treating heart failure with depressed ejection fraction. Moreover, there are no effective therapies for acute decompensated heart failure, nor for patients with heart failure with preserved ejection fraction, despite extensive efforts to develop new therapies in these areas. We next address some of the issues that should be considered in order to close the widening gap between target discovery and viable heart failure therapies.

Target Identification

Our current approach to treating CHF has targeted cell-surface receptors or the intracellular mineralocorticoid receptors. While this approach has worked well for antagonizing components of the adrenergic and the renin-angiotensin-aldosterone systems, this type of “reductionist” approach has not worked well for antagonizing other systems (e.g. endothelin, adenosine, tumor necrosis factor). As is discussed later, newer approaches designed to modulate gene networks (e.g. antagonizing miRNAs) and/or specific intracellular signalling pathways (e.g. kinase inhibitors) have the potential to expand upon existing therapeutic strategies. Second, the classical approach that has been taken to identify new therapeutic targets is focused on preventing the development of a CHF phenotype after cardiac injury, rather than reversing the phenotype. While the former approach is relevant to target development for therapies for acute MI, it may work less well when it comes to impacting on disease pathogenesis once CHF is fully established. Indeed, one of the many lessons gleaned from CHF trials is that therapies that reverse the heart failure phenotype (i.e. myocardial recovery) are also accompanied by improved patient outcomes⁶⁰. Given how little we know about the biology of myocardial recovery at the gene, cell and organ level, this represents a significant opportunity for discovery and potential target development.

Methodological issues

Given that the clinical syndrome of CHF represents the summation of the changes in gene expression in the cardiac myocyte, quantitative and qualitative changes in cell types and the composition of the extracellular matrix, as well as changes in the geometry of the left ventricle that evolve over years, it has been challenging to develop experimental systems that accurately model clinical CHF. Indeed, it is sobering to realize that therapies such as beta-blockers were developed largely based on observations from small clinical studies. The discussion that follows is intended to focus attention on the methodological gaps in our approach to studying the pathobiology of heart failure rather than to suggest the superiority of one model system over another, with the multiple model systems being complementary.

Cardiomyocyte culture systems provide relatively high throughput means for identifying and validating potentially important signal transduction pathways, but the extant neonatal and adult cell culture models have well-recognized limitations in relation to their potential utility for identifying relevant pathways in human CHF. The ability to derive human cardiomyocytes through the use of induced pluripotent stem cells (e.g. from skin fibroblasts) may help to overcome some of these limitations, as well as enable patient-specific studies^{61, 62, 63}. Mouse models to study LV structure and function in vivo following targeted genetic manipulations of various pathways have been critical in advancing the field⁶⁴, although the results of such studies are not always unambiguous⁶⁵. Another limitation of mouse models vis-à-vis target identification in CHF is that aspects of the physiology (e.g. calcium handling) differ significantly compared to humans⁶⁴. Thus, targets that are identified in mouse models may not necessarily be germane to human physiology, and require validation in large animal models or humans. Another limitation is that investigators have tended to employ mouse strains and models of injury (e.g. acute severe pressure overload) that develop the most dramatic heart failure phenotype. However, it is not clear how closely this mimics the human remodelling response and whether this inherent experimental bias may lead to false positive target identification. Large animal models offer the advantage of physiology that more closely resembles humans, and recapitulate the heart failure phenotype in a variety of injury models (reviewed in⁶⁶). However, although some models have high predictive accuracy in clinical trials⁶⁷, there is no animal model that reliably predicts outcomes in phase III studies. An obvious reason is that large animal studies conducted over months largely focus on safety and potential efficacy with respect to outcomes such as prevention of remodelling, whereas human CHF studies are conducted over years and focus on “hard endpoints” such as death or heart failure hospitalization, which are difficult to study in large animal models. The renewed emphasis on integrative physiology in many universities may help to better inform the training of MDs, PhDs and MD/PhDs who wish to engage in translational research and use multiple complementary models to this end.

Newer approaches to discovery

Research into the basic mechanisms responsible for the development and progression of heart failure has highlighted the enormous complexity of the molecular and cellular interactions that govern the process of cardiac remodelling and reverse remodeling^{60, 1}. As discussed earlier, this type of “reductionist” approach has not been successful in targeting other biological systems beyond the adrenergic and renin-angiotensin-aldosterone systems. Moreover, these types of studies have led to only limited understanding of the basic mechanisms that underlie myocardial recovery. It is likely that future therapeutic advances will require a more comprehensive understanding and analysis of heart failure pathobiology, as well as the complex interactions that govern the process of myocardial recovery. The emerging field of systems biology may allow investigators to accelerate the pace of novel target identification, as well as potentially improve the likelihood of success in clinical trials

(Figure 4A). In contrast to “reductionist” experimental approaches that seek to establish causal associations between distinct molecular or cellular entities and phenotypes, systems biology attempts to understand how the interactions of multiple components of the cell (i.e., genome, transcriptome, proteome, metabolome) govern its function. Systems biology uses “network theory” to describe how the interrelationships between genes, proteins and metabolites determine functional changes at the level of the cell, tissue and organ. Networks are typically represented graphically and conceptually as a series of nodes (e.g. a gene, protein or metabolite), that are connected to each other by “edges” that represent the interaction (activation or suppression) between the nodes of interest (Figure 4B). Although the majority of nodes in a network have very few edges, some nodes termed “hubs” have many edges, suggesting that they are potentially involved in critical regulatory processes (Figure 4C). Our understanding of how these networks and hubs are modulated in heart failure (e.g. “rewiring”) and how they are affected by existing heart failure therapies is embryonic at present, but is beginning to be studied^{68, 69, 70, 71}. Such approaches have revealed specific cellular interactions that would not necessarily be obvious and/or predicted from the extant literature. Moreover, network modelling allows for the identification of pathways that are not targeted by existing therapies, but that may be synergistic with such pathways – a potentially valuable attribute in relation to the likelihood that new therapeutic agents will have to be added “on top” of existing therapies in clinical trials. Finally, network modelling readily lends itself to examining changes within the components of the network in relation to changes in cell phenotype/function following a given therapeutic intervention. Targeting cellular function as a system rather than as a single target within the biological system may improve the chances of progressing from novel basic advances to viable therapies. Although systems biology has not been applied broadly to developing new heart failure therapeutics, the approach has been successful in areas such as oncology^{72, 73}. Further advances in the field of systems biology in heart failure will require the continued development of methodologies for obtaining high fidelity and comprehensive datasets (e.g. newer sequencing technologies and ‘omics methods), analyzing high-dimensional data sources, annotating interactions, and the development of interactive platforms that facilitate the layering of different types of “omics” datasets (e.g. transcriptomics and proteomics or data obtained from different model systems). This will likely prove challenging both for economic reasons and with respect to the multidisciplinary skillsets that are required, but will ultimately prove to be priceless if these modern biological approaches facilitate the development of new therapeutics to treat the growing pandemic of heart failure.

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Figure 1. Overview of cellular signalling pathways involved in cardiomyocyte hypertrophy

Multiple pathways can regulate cardiomyocyte growth, acting through a complex network of intracellular signalling cascades. Agonists for α -adrenergic, angiotensin, and endothelin receptors couple to phospholipase C (PLC) and calcium influx channels (CC) by way of G-proteins ($G\alpha$ and $G\gamma$). Activation of PLC results in the generation of two second messengers, inositol triphosphate (IP_3) and diacylglycerol (DAG). IP_3 causes the release of calcium from intracellular stores, and DAG activates protein kinase C (PKC). Changes in intracellular calcium stores can activate calcium-calmodulin-dependent kinases (CaMK), as well as calcineurin, which affect gene expression in multiple ways. The modulation of other membrane transporters (such as the Na^+-H^+ exchanger which regulates cellular pH) and the activation of mitogen-activated protein kinase (MAPK) cascades are also involved. Histone deacetylase complexes (HDACs) are emerging as important negative regulators of genes involved in cardiac hypertrophy. Cytokines and peptide growth factors can be elaborated by various cells within the heart and may act in an autocrine or paracrine manner, generally via tyrosine kinase (TK) receptors that are coupled to downstream protein kinase signalling cascades. Mechanical forces can also affect hypertrophy through several pathways, including direct effects at the level of the sarcomere, alterations in matrix-integrin interactions and the autocrine action of released agonists such as angiotensin. Both nitric oxide and reactive oxygen species are capable of modulating distinct signalling pathways within the cell, either in a negative or positive fashion depending upon circumstances. The net effects of such intracellular signalling include myocyte hypertrophy, changes in contractile function and altered cell viability. (Adapted from Mann DL, Pathophysiology of Heart Failure. In Bonow RO, Mann DL, Zipes DP, Libby P [eds]: Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine, 9th ed, pp ???, 2010).

Figure 2.

Candidate miRNAs with suggested roles in the cardiac remodelling process are depicted with respect to the how they might influence the process of LV remodelling. (From Topkara VK, Mann DL: Clinical applications of miRNAs in cardiac remodeling and heart failure. *Personalized Medicine* 2010;7:531–548).

Figure 3.

Major pumps, channels and regulatory proteins involved in excitation-contraction coupling in the cardiac myocyte. Calcium (Ca) influx through L-type channels triggers the release of further Ca from the sarcoplasmic reticulum (SR) via ryanodine receptor channels (RyR). In addition to Ca-myofilament interaction (which regulates contraction and relaxation), Ca also regulates mitochondrial functions and may have effects in other organelles such as the nucleus. Calcium re-uptake into the SR occurs via the calcium ATPase pump (SERCA), which is regulated by phospholamban. Other membrane pumps and channels also contribute to Ca homeostasis. Dysfunction at different levels of this process contributes to contractile dysfunction in the failing myocyte and the molecular abnormalities underlying this dysfunction may define potential therapeutic targets. (Adapted from Mann DL, Pathophysiology of Heart Failure. In Bonow RO, Mann DL, Zipes DP, Libby P *eds+: Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine, 9th ed, pp ????, 2010).

Figure 4.

Application of a systems biology approach to understanding the pathogenesis of heart failure. A, Systems biology involves a series of steps traditionally beginning with highly sophisticated characterisation of genomic, transcriptomic, proteomic, and metabolomic data sets, which can then be analyzed using a variety of bioinformatic approaches. Systems biology places emphasis on defining interactions, delineating networks linking proteins, genes or metabolites, and describing functional units or sets to provide testable mechanistic models of clinical phenotypes. B, Visual illustration of a simple “scale free” gene network, which is comprised of nodes (circles) with a large number of links or “edges” (depicted by lines) which represent the interaction (activation or suppression) between the nodes. C, Visual illustration of a complex scale free network, with the majority of nodes having one or two links and a few nodes (illustrated in red) having a large number of links. Nodes with a high degree of connectivity are referred to as “hubs.” The high degree of connectivity guarantees that the system is fully connected. (Adapted from Adams KF. Systems biology and heart failure: concepts, methods, and potential implications. *Heart Fail Rev* 2010;15:371–98).