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Recent Developments in Heart Failure

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Despite significant advances in cardiovascular medicine during the late 20th century, heart failure (HF) remains a leading cause of death in the United States and much of the rest of the world¹. Although improvements in acute management of cardiovascular disease have reduced death rates, efforts to halt the inexorable deterioration are largely futile. The current clinical approach focuses on disease management rather than curing heart failure – because there is presently no cure². Primary treatment consists of angiotensin-converting enzyme inhibitors, beta-blockers, and mineralocorticoids antagonists. And, although a new class of agents (i.e. neprilysin inhibitor) has shown promise in a Phase 3 clinical trial³, collectively, these drugs only delay disease progression and death due to heart failure. Given the general stagnation in the progress of clinical treatment of heart failure, we must undertake more daring and high-risk preclinical studies to achieve the collective dream of curing heart failure. This “Developments” will highlight some recent progress in understanding the pathobiology of heart failure and advances in conceptual approaches for future treatments. The goal is to focus the readers' attention on some of the more exciting and daring areas of cardiovascular research, which will likely dictate advances in the 21st century.

The central element of heart failure relates to the heart's inability to pump sufficient blood to meet the metabolic demands of the body. Although various factors can contribute to such a defect, myocardial infarction is the most frequent cause of heart failure. Following an infarction, the significant loss of cardiomyocytes is replaced with akinetic scar tissue, rather than contracting cardiomyocytes. Such ‘wound healing’ satisfies the short-term goal of retaining ventricular integrity; however, the chronic implications include progressive fibrosis, stiffness, and dilation of the ventricle. Thus, improving mechanical performance and limiting remodeling represent two key areas to address in the failing heart.

Defects in Ion Handling

The basic contractile function of the heart is centrally regulated by ion exchange; in particular, the entry and exit of sodium and calcium, and propagation of action potentials. Although one could argue that elevated calcium during heart failure could augment cardiac function⁴, elevated Ca²⁺ can be detrimental and can lead to the development of arrhythmias, hypertrophy, and apoptosis^{5, 6}. More detailed discussion of calcium handling during heart failure can be found in a recent review⁷. Of course, Na⁺/K⁺ ATPase, the primary ionic

regulator in cardiomyocytes, has a long history as a target in heart failure (remember cardiac glycosides?). The familiar Na^+/K^+ ATPase has two subunits ($\alpha 1$, $\alpha 2$), which can couple with a sodium-calcium exchanger (NCX1) in T-tubules. Moreover, inhibition of the $\alpha 2$ isoform can increase calcium transients indicating a possible link between Na^+/K^+ ATPase $\alpha 2$ and NCX1. Correll *et al.* recently examined the contribution of Na^+/K^+ ATPase $\alpha 2$ in regulation of disease progression⁸.

Indeed, calcium handling plays a central role in the failing heart, and the ryanodine receptor has long been the focus of heart failure studies. Calcium leak through ryanodine receptors (RyR2) contributes to atrial fibrillation⁹, and abnormal termination of Ca^{2+} release is a common defect of RyR2 mutations associated with – albeit rarely – cardiomyopathies¹⁰. Interestingly, CaMKII activity increases in HF and regulates RyR2 through phosphorylation of residue S2814¹¹, and CaMKII phosphorylation of RyR2 is sufficient to increase sarcoplasmic reticulum (SR) Ca^{2+} leak in mice and to contribute to cardiomyopathy. And, although there has been some disagreement¹², hyperphosphorylation at a nearby site (S2808) does not appear to be a likely contributor to heart failure¹³.

Inflammation and Remodeling

The induction of the inflammatory response is a critical step in mediating cardiac repair following MI. Robust infiltration of leukocytes mediates removal of necrotic cardiomyocytes and initiates wound healing. Recruitment of these inflammatory cells results from release of chemoattractants from damaged and dying myocardium. These cells become recruited to both infarcted and non-infarcted areas. The inflammatory response also helps mediate extracellular matrix (ECM) synthesis through promoting fibroblast activation, ECM degradation, and altering matrix metalloproteinase (MMP) activity; however, chronic inflammation results in exacerbation of cardiomyocyte damage and cardiac dysfunction through adverse remodeling¹⁴. Thus, the regulation of the inflammatory response and its mediators may provide new therapeutic targets in HF.

Ischemic cardiomyocytes release ATP, which can serve as a chemoattractant to recruit phagocytes to sites of inflammation to clear dead cells and debris¹⁵. In addition, ATP can be released from neutrophils to guide other neutrophils to inflammatory sites. Extracellular ATP is rapidly dephosphorylated to ADP, AMP and adenosine. These resultant products may play an integral role in modulating immune cell infiltration and cardiac healing/remodeling subsequent to I/R. Recent studies¹⁶ have identified CD39 as an ectonuclease responsible for the hydrolysis of ATP. Ecto-5'-nucleotidase (CD73) further hydrolyzes AMP to adenosine. Adenosine can subsequently induce both pro and anti-inflammatory responses. Immune cells in the unstressed heart exhibit marked levels of CD39 and lack CD73; however, 3 d after I/R invading leukocytes demonstrated significant upregulation of CD73.

Post-MI matrix metalloproteinase (MMP) activity increases in response to a host of inflammatory cytokines¹⁷. Activation of MMPs leads to degradation of myocardial matrix and cardiac remodeling, and pharmacological inhibition of MMPs has demonstrated attenuation of LV dilatation^{18, 19}. In addition to MMPs, the heart also expresses tissue inhibitors of matrix metalloproteinases (TIMPs), whose expression is actually reduced after

MI. Thus, our general understanding has held that a relative imbalance exists between MMPs and TIMPs. And, although recent insights derived from cardiac expression of TIMP-4 demonstrates clearly this idea²⁰, not all MMPs mediate maladaptive remodeling. MMP-28 appears to be necessary for proadaptive remodeling¹⁷. MMP-28 is expressed in cardiomyocytes in normal conditions; however, following MI, macrophages become a prominent source of MMP-28¹⁷. Loss of MMP-28 leads to adverse LV remodeling and dysfunction, which is accompanied by increased death and rates of ventricular rupture¹⁷. When the phenotype of the macrophages was examined, it appeared that both pro-inflammatory factors and M2 macrophage polarization were reduced with MMP-28 deletion. In addition, extracellular matrix deposition and cross-linking, and myofibroblast numbers, were all reduced in MMP-28 KO mice¹⁷.

Clearly, macrophages play an important role in post-infarct wound healing, in part, through transition from M1 to M2 phenotype to reduce inflammation and mediate fibrosis. The necessity of macrophages in wound healing is even more evident in the neonatal heart. Macrophages play a pivotal part in cardiac regeneration and neoangiogenesis following myocardial injury²¹. Thus, their utility in mediating cardiac remodeling in the adult heart should be further explored. To this end, several elegant studies were published addressing primarily the role of macrophages in heart failure, and secondarily other aspects of inflammation. Ismahil et al²² developed the novel hypothesis that post-MI alterations of splenic function contribute to LV remodeling. In their study, they found that splenectomy blocked post-infarct LV remodeling, while reconstitution of naïve mice with heart failure-derived macrophages largely recapitulated LV remodeling. The origin of cardiac macrophages has been somewhat enigmatic; however, recent discoveries indicate that whereas homeostatic maintenance of cardiac macrophages occurs via proliferation of existing cardiac macrophages, bone marrow-derived monocytes contribute to post-infarct cardiac macrophages²³. Another question to be answered is what regulates the distribution of Ly-6C^{high} (inflammatory) and Ly-6C^{low} (reparative) monocytes? A study from Hilgendorf and colleagues²⁴ addressed this important issue and found that Nr4a1 (nuclear receptor subfamily 4) expression was associated with suppression of inflammation, and deletion of Nr4a1 promoted adverse cardiac remodeling. Of course, macrophages are not solo actors in post-infarct inflammation; T lymphocytes may also exert critical regulatory effects on cardiac inflammation. Ablation of T_{reg} cells before MI exacerbates inflammation, promotes M1 macrophage polarization, and depresses cardiac function in the failing heart²⁵.

Regulation of Cardiac Hypertrophy

Following cardiac injury, compensatory mechanisms, such as cardiac hypertrophy, maintain cardiac function. Cardiac hypertrophy serves primarily as an adaptive response to increased workload; however, chronically, hypertrophy is associated with increased interstitial fibrosis, apoptosis, and eventually heart failure. Hypertrophic growth requires gene regulation at multiple levels: epigenetic, transcriptional, post-transcriptional, and translational regulation. Regulation of hypertrophy in HF, especially the induction of the fetal gene program, has been studied extensively; however, new regulators have been identified.

For example, G-protein-coupled receptors that result in the activation of Gq and its downstream effects stimulate hypertrophy. Among these downstream effectors are histone deacetylases (HDACs), which normally suppress expression of hypertrophic genes. Class II HDACs, including HDAC4 and HDAC5, interact with key transcription factors that mediate hypertrophy including nuclear factor of activated T cells (NFAT) and myocyte enhancer factor-2 (MEF2), and inhibit their activity through histone deacetylation. Yet, hypertrophic stimuli can activate HDAC kinases, such as protein kinase D, CamKII, and the recently discovered G-protein-coupled receptor kinase 5 (GRK5), which results in the phosphorylation and cytoplasmic translocation of class II HDACs and removal of their inhibitory effect on hypertrophic transcription factors. GRK5 overexpression promotes NFAT-induced activation of hypertrophic gene transcription, whereas GRK5 deficiency limits NFAT activation during pressure overload²⁶. Thus, limiting GRK5 expression may protect against maladaptive cardiac growth and HF development.

In addition to HDAC kinases, oxidation of specific serine residues of class II HDAC can also result in their cytoplasmic translocation. Sources of oxidation include reactive oxygen species (ROS) that naturally result from physiological and pathological processes. In particular, NADPH oxidases are major sources of superoxide formation. Recently, NADPH oxidase 4 (Nox4) has been shown to increase ROS in response to hypertrophic stimuli, resulting in cysteine oxidation and nuclear export of HDAC4, a class II HDAC²⁷. Similarly, deletion of Nox4 attenuates HDAC4 oxidation and cardiac hypertrophy two weeks following pressure overload²⁷. Clearly, HDACs play an important role in suppressing hypertrophy, and regulating their activity may regulate the transition to decompensated HF²⁸.

Controlling the intensity and duration of hypertrophic signaling may provide therapeutic benefit in HF. Interestingly, the Sussman group may have found a novel target for the regulation of cardiac hypertrophy in Pin1. Pin1, a proline directed isomerase extensively studied in cancer where it regulates proliferation, cell survival, lineage commitment, and aging, now appears to orchestrate cardiac hypertrophy. Pin1 is upregulated after pressure overload²⁹, and loss of Pin1 limits cardiomyocyte hypertrophy through the inhibition of Akt and MEK activation. Pin1 overexpression preserves cardiac function after pressure overload, possibly through inhibition of MEK but not Akt²⁹. Because attenuation of the hypertrophic response was attributed to both overexpression and loss of Pin1, Toko *et al* surmised that Pin1 operates within a restricted range. Thus, Pin1 appears to facilitate an appropriate hypertrophic response through regulation of intensity and duration of hypertrophic signaling^{29, 30}.

Preserving cardiac function is the ultimate goal, and technological advances in mechanical circulatory support have led to the development of left ventricular assist devices (LVAD). These devices serve as a last resort therapeutic option for patients with end-stage heart failure to provide a bridge to transplantation. Considering the universal shortage of donor organs, LVADs offer decreased mortality and morbidity for those on a waiting list³¹. Following 30 d of LVAD implementation, patients exhibit recovery of ejection fraction³²; however, such improvement often regresses to the pre-LVAD level upon withdrawal of LVAD³². As expected, cardiac tissue examined prior to and post-LVAD demonstrate

reduced myocyte size and fibrosis without an obligatory improvement in function³³. Such findings provide some limited promise for LVADs and re-emphasize the bidirectional nature of myocardial remodeling. Thus, understanding the underlying mechanisms may guide our ability to mend the failing heart.

Gene/Molecular Therapy

Because heart failure is not a single loss-of-function genetic abnormality, fine-tuning gene expression may represent another feasible avenue via microRNAs (miRs). These small, noncoding RNAs are responsible for genetic regulation at the post-transcriptional level. MiRs can directly modulate cardiac transcription and indirectly regulate other miRs. Thousands of miRs have already been identified and many regulate cardiac development³⁴, pathological remodeling³⁵, and cardiomyocyte hypertrophy³⁶. One such regulator of hypertrophy, miR-22, mediates a compensatory hypertrophic response following pressure overload, and loss of miR-22 accelerates the progression of dilated cardiomyopathy³⁷. Likewise, miR-24 expression increases during hypertrophy, and it suppresses both junctophilin-2 (JP2) expression and attenuates excitation-contraction coupling³⁸. *In vivo* treatment with antagomir against miR-24 preserved excitation-contraction coupling and prevented transition to decompensation³⁸. Likewise, miR-25, which is a potent inhibitor of SERCA2a, can be blocked (via antagomiR) to improve contractility during HF^{39, 40}.

Multiple additional effects of miRs have also been identified in HF. Not only can miRs direct cardiomyocyte remodeling, they can also affect resident fibroblasts. Interestingly, circulating miRs appear may be potential biomarkers of cardiovascular disease⁴¹, and, miR-1, miR-133a, and miR-208a are present in serum following myocardial infarction^{42, 43}. Matsumoto *et al.* found several, p53-responsive miRs (miR-192, miR-194, miR-34a) are upregulated after acute MI and may contribute to the development of HF⁴¹. These markers, however, have short half-lives and do not predict future HF after acute myocardial infarction. Of course, there are cautionary notes with miRs as therapeutic targets, as pointed out by Matkovich *et al*⁴⁴.

Although once heralded as a definitive treatment for monogenic disorders, gene therapy has been slow to gain traction in cardiovascular medicine; however, several groups have recently reinvigorated interest in gene therapy to mitigate HF. Defective calcium handling is a key aspect of heart failure. Of the multiple regulators of calcium handling in heart failure, SERCA2 has received significant attention because of its primary role in calcium re-uptake to the sarcoplasmic reticulum. Indeed, SERCA2's activity is diminished in the failing heart⁴⁵, and adenoviral administration of SERCA2a in failing cardiomyocytes improves contractility in various animal models of HF⁴⁶⁻⁴⁹. The resulting clinical trial, CUPID, demonstrated safety⁵⁰ and efficacy⁵¹ of intracoronary infusion of recombinant AAV1/SERCA2a. Through three years of follow up, gene therapy caused no adverse events, even though the SERCA2a transgene was present in patients up to 31 months post transfusion⁵². Gene therapy appears to be a feasible, clinical therapeutic option.

Ultimately, while preserving and enhancing cardiomyocyte function serves to potentiate the adverse effects of the failing heart, the need to address the loss of cardiomyocytes and their

limited renewal still looms. Replacing the cardiomyocyte pool appears to be the limiting factor in mediating cardiovascular disease as most treatments are geared toward maintaining cardiomyocyte survival and function. Many are excited by the potential for cardiomyogenesis through gene transfer. Recent studies demonstrate the ability to reprogram cells into cardiomyocyte-like cells. It remains to be seen whether reprogramming resident fibroblasts would provide clear clinical benefit. Subpopulations of resident cardiac fibroblasts do indeed have a cardiac-specific gene expression profile and may be primed for transdifferentiation⁵³; however, cardiac fibroblasts, in general, do not contribute substantially to myocardial regeneration following injury⁵³.

The key to genetic regulation of neocardiomyogenesis may revolve around regulation of the cardiomyocyte cell cycle to induce cardiomyocyte proliferation. Adult cardiomyocytes are terminally differentiated and have likely exited the cell cycle, diminishing any significant capacity for proliferation. Recently, Chen *et al.* studied the miR-17-92 cluster, a human oncogene that induces proliferation in the heart. Deletion of this cluster from embryonic and postnatal hearts reduced cardiomyocyte proliferation, and *in vitro* overexpression of the miR-17-19 cluster appears sufficient to induce cardiomyocyte proliferation⁵⁴. Furthermore, overexpression of this cluster following MI modestly improved cardiac function, decreased scar formation, and induced cardiomyocyte proliferation⁵⁴. Elucidating the molecular signaling mediated by this cluster may provide necessary molecular targets to mediate robust *de novo* cardiomyocyte formation in the context of cardiac necrosis. Additionally, at least one group has already overexpressed the Stem Cell Factor gene into infarcted myocardium⁵⁵. Induction of SCF resulted in dramatic increase in progenitor cell recruitment to the myocardium. Combining recruitment of reparative cells and induction of cardiomyogenesis via gene therapy may prove to be a useful adjunct to induce cardiac regeneration⁵⁶.

Cell Therapy

The heart was believed to be a post-mitotic organ with limited (if any) capacity for regeneration; however, discovery of putative stem/progenitor cells in the heart ushered in a relative renaissance in cardiovascular research. The most active – and certainly controversial⁵⁷⁻⁶¹ – area of investigation in cardiovascular research is cell therapy. Regardless of the origin and identity of the cells used, there is a general consensus that most cells “work” (i.e. improve cardiac function) – candidly, it seems a bit surprising that so many supposedly varied cell types can actually improve function. Nevertheless, the data indicate that cell therapy improves cardiac function in multiple animal models from multiple laboratories, as well as preliminary studies of patients.

Given the ever-increasing number of clinical trials completed, ongoing, and being planned to harness the promise of cell to repair the failing heart⁶², it is not surprising that the field continues to experience remarkable growth. Although several recently completed trials are varied in cell type and outcomes, they all exhibit limited adverse effects in patients indicating a suitable vehicle for further experimentation; that is, at minimum, cell therapy appears to be safe⁶³⁻⁶⁷. Currently, there is a race to find the most effective cell type to mediate cardiac regeneration and, eventually, improve clinical outcomes. The race to find

the most therapeutic cell requires stringent and thorough examination of the regenerative potential and appropriate product control along the way. There are obstacles that still need to be surmounted, including developing high throughput methods for testing the cells. Purifying, selecting, and injecting potential cardiac progenitors to measure functional outcomes following HF without properly addressing the cells' functional and regenerative capacity is a timely and costly endeavor. In addition, there are a multitude of cell markers that may be attributable to therapeutic potential. Studying the efficacy of these cells in high throughput *in vitro* settings may be beneficial to the future of the field.

If, however, optimization of autologous cells does not prove successful, investigators may need to turn to allogeneic cell populations. It appears that immunogenic response to allogeneic stem cells may be modulated through programmed death ligand 1(PD-L1)⁶⁸. Thus, cell populations that exhibit PD-L1 expression may serve as a selection criterion for allogeneic cardiac stem cells. Furthermore, mesenchymal precursor cells (MPCs) that are stromal precursor antigen 3+ (Stro-3+), considered to be immune privileged, appear effective, safe, and feasible, and represent an allogeneic source of cells to treat acute MI in large animal models⁶⁹.

This is an exciting time for the field of cell therapy. Although some of the cells may turn out to be unique subsets of fibroblasts, which many have speculated, they appear collectively to be effective in the hands of numerous investigators.

Summary

Advances in understanding cellular pathophysiology and elucidating mechanisms of self-renewal lie at the crux alleviating HF. Establishing a panacea for HF is unlikely in the short term; however, a combination of adjunctive therapies, including pharmacological management, genetic regulation, and cell therapy may be the key to reinvigorating the failing heart.

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