Mechanisms of Cardiovascular Homeostasis and Pathophysiology – From Gene Expression, Signal Transduction to Cellular Communication –

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When confronted with myriad and often harsh external stimulations and environmental changes, organisms mobilize every possible means to maintain homeostasis and hence survival. Differentiated cardiomyocytes carry the pump function of the heart, and are equipped with an ability to adjust the pump performance moment by moment in response to the demand for systemic blood supply. The heart may encounter an “index event” that damages myocardium, with a consequence of cardiomyocyte loss. 

This index event includes cases of an abrupt or acute onset such as myocardial infarction (MI) and viral infection. It has been reported that cardiac failure is produced by a segmental loss of 40–50% of cardiomyocytes after coronary artery occlusion in rats. 

In contrast, a diffuse loss of 10–20% of cardiomyocytes is sufficient to produce symptomatic heart failure (HF) in transgenic mice expressing human diphtheria toxin receptor in the heart after injection of diphtheria toxin. 

The index event also includes cases of subacute or chronic onset such as endocrine abnormalities and valvular abnormalities leading to pressure or volume overload. Regardless of the nature of the index event, the heart adapts by activating a variety of compensatory mechanisms such as the sympathetic nervous system and the renin-angiotensin system (RAS). These stress responses are beneficial, and contribute to the maintenance of functional homeostasis in the cardiovascular system over the short term. However, with the passage of time, excessive and sustained activation of these stress responses leads to worsening of left ventricular (LV) or atrial remodeling and subsequent cardiac decompensation and arrhythmias. For example, after MI, the border zone expands in response to increased wall stress, and causes further LV dilatation and contractile dysfunction. The Ca\(^2+\)-dependent intracellular cysteine proteases, the calpains, are activated at the border zone of MI hearts in the chronic phase, and further activation of calpains by genetic disruption of the Cast gene encoding calpastatin, the specific endogenous inhibitory protein for calpains, exacerbates LV remodeling after MI. 

Therefore, it is an important subject of cardiovascular research to understand how cardiomyocytes sense and respond to the variety of stresses imposed on the heart.

My group’s research interest has focused on transcriptional regulation of the cardiac gene program, signal transduction underlying physiological hypertrophy and load-induced pathological hypertrophy, and inflammatory infiltration in atrial remodeling and fibrillation. In this review, the contributions to understanding the molecular mechanisms of cardiovascular
stress responses are described, with an emphasis on their effect on cardiovascular homeostasis and pathophysiology.

**Cardiac Transcription Factors in Cardiac Development and Pathophysiology**

Cardiomyocytes undergo hypertrophic growth in response to alterations in the hemodynamic workload.\(^5\)\(^-\)\(^7\) Cellular responses during cardiac hypertrophy include an increase in cell size and reprogramming of the fetal cardiac genes. It is well known that some of the contractile proteins, ion channels and metabolic enzymes have both fetal and adult isoforms. Switching of gene expression from the normally expressed adult isofrom to the fetal isoform of sarcomeric myosin heavy chain is regarded as an adaptive response, because it decreases the speed of sarcomeric shortening but improves the efficacy of contraction. Ventricular expression of the \(Nppa\) gene encoding atrial natriuretic peptide (ANP) is reactivated in hypertrophied hearts, although it is silenced postnatally. Given that ANP provides natriuretic, diuretic and vasodilatory effects, upregulation of \(Nppa\) during cardiac hypertrophy is also considered an adaptive response. Indeed, some elements of fetal gene activation are salutary adaptations to hemodynamic stress, but aberrant expression of fetal genes also causes maladaptive changes in cardiac structure, function, and metabolism, and leads to progression of adverse cardiac remodeling and HF.\(^6\)\(^,\)\(^8\)

Recent studies have identified several transcription factors that regulate the expression of myriad cardiac genes not only during cardiogenesis, but also during the adaptive and maladaptive processes against hemodynamic stress.\(^5\)\(^,\)\(^8\) These transcription factors include nuclear factor of activated T cells (NFAT), serum response factor, nuclear factor \(\kappa B\) (NF \(\kappa B\)), Smad transcription factors, and cardiac transcription factors such as myocyte enhancer factor 2 (MEF2) transcription factors, GATA family transcription factors, and Nkx homeobox transcription factors.\(^5\)\(^,\)\(^8\) Epigenetic modifiers and noncoding RNAs are also involved in the combinatorial networks governing embryonic and postnatal regulation of cardiac genes.\(^9\) Of these transcriptional regulators, I am focusing on cardiac transcription factor Nkx2-5, a member of the NK homeobox protein family that acts as a DNA-binding transcriptional activator.\(^5\)\(^,\)\(^10\) The Nkx2-5 gene was identified as a mammalian homolog of \(Drosophila\) tinman (NK4), which is essential for \(Drosophila\) heart formation.\(^11\) Tinman-related proteins have been identified in various species of vertebrates from Xenopus to humans.\(^10\)\(^-\)\(^12\) During embryogenesis, Nkx2-5 expression is activated in the cardiac mesoderm under the influence of multiple inducing signals such as BMP signaling and stage-specific canonical Wnt signaling.\(^13\)\(^-\)\(^15\) Nkx2-5 strongly transactivates the promoter of a variety of cardiac genes such as \(Nppa\),\(^12\) and the transcriptional activity of Nkx2-5 is enhanced through physical interaction with other transcription factors such as GATA-4\(^16\) and transcriptional co-activators such as Migfilin.\(^17\) Mammalian NK homeobox proteins play an essential role in cell-type specification and organogenesis.\(^5\)\(^,\)\(^8\) For example, the \(Bapx1\) gene is a mammalian homolog of \(Drosophila\) bagpipe (NK3), which is essential for \(Drosophila\) midgut musculature, and gene targeting in mice demonstrated that Bapx1 is indispensable for normal development of the vertebral column, craniofacial bones, spleen and gastroduodenal tract.\(^18\) Nkx2-5-deficient mice are lethal around E9–10 because of arrested loop morphogenesis of the heart tube,\(^19\) indicating an essential role of Nkx2-5 in cardiogenesis. In humans, mutations in NKX2-5 are etiologically crucial in nonsyndromic congenital heart diseases, including secundum atrial septal defect and atrioventricular conduction disturbance.\(^10\) Most mutations suppressed the transcriptional activity of NKX2-5 by a dominant-negative mechanism or by haploinsufficiency.\(^20\) However, one mutation enhanced the transcriptional activity and increased susceptibility to apoptosis in cardiomyocytes.\(^20\)\(^,\)\(^21\)

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**Figure 1.** Cardiac transcription factor Nkx2-5 in the regulation of cardiac development and pathophysiology. Nkx2-5 plays a crucial role in the transcriptional regulation essential for normal cardiac development and homeostasis of the postnatal heart.
Besides regulating the gene program for cardiogenesis, Nkx2-5 is involved in transcriptional regulation of the cardiac gene program in hypertrophied hearts. Although Nkx2-5 is upregulated in response to hypertrophic stimulation, transgenic mice overexpressing Nkx2-5 under the control of the cytomegalovirus enhancer/chicken β-actin promoter exhibited normal-sized hearts despite upregulation of cardiac genes such as Nppa, Nppb, and Myl2. These studies suggest that Nkx2-5 is not sufficient for the generation of cardiac hypertrophy but that it controls the cardiac gene program in adult hearts as well as in embryonic hearts. Interestingly, overexpression of Nkx2-5 prevented H2O2- or doxorubicin-induced apoptosis in cultured cardiomyocytes, and doxorubicin-induced cardiac dysfunction was more severe in transgenic mice overexpressing a dominant-negative mutant of Nkx2-5, while it was alleviated in transgenic mice overexpressing wild-type Nkx2-5. My group’s studies have provided mechanistic insights into the molecular framework of how Nkx2-5 controls cardiac development and homeostasis of postnatal hearts (Figure 1).

Growth Signals in Physiological Cardiac Hypertrophy

Mammalian cardiomyocytes are long-lived and their ability to proliferate is strongly restrained soon after birth, although recent studies showed a low level of human cardiomyocyte turnover throughout life. Physiological growth of cardiomyocytes in size without cell division contributes to the postnatal growth of the heart from birth to adulthood and the growth of the heart in trained athletes. During physiological hypertrophy, cardiac structure and function are preserved with induction of myocardial angiogenesis and without induction of replacement fibrosis. Physiological growth signaling is mediated by tyrosine kinase receptors and nuclear receptors, and the most crucial signaling pathway downstream of tyrosine kinase receptors is the phosphoinositide 3-kinase (PI3-K)/3-phosphoinositide-dependent kinase-1 (PDK1) pathway. PDK1 is a member of the AGC serine/threonine kinase family (so named because it includes protein kinase A, G, and C), which shares the PIF-pocket, a regulatory domain serving as a docking site for substrates. PDK1 functions downstream of PI3-K and activates several AGC kinases, including Akt, p70 ribosomal S6 kinase, serum- and glucocorticoid-induced protein kinase 1, and protein kinase C isofoms. The PI3-K/PDK1 pathway regulates the cellular growth in multiple cell lineages, and mice lacking Pdk1 gene expression in skeletal muscle and heart die of HF by 11 weeks of age. Interestingly, these mice show attenuation of cardiomyocyte cell growth and impairment of LV contraction, indicating that PDK1 is essential for normal regulation of cell size in the heart. However, it remains unclear whether retardation of cardiomyocyte growth is the primary cause of cardiac dysfunction. When Pdk1 is disrupted in the hearts of tamoxifen-inducible and heart-specific Pdk1 knockout mice, they show severe and lethal cardiac dysfunction in spite of the unchanged size of cardiomyocytes. Mechanistically, loss of Pdk1 enhanced the susceptibility of cardiomyocytes to apoptosis, and enhanced PI3-K activity, which consequently led to robust downregulation of β-adrenergic receptor and contractile dysfunction. Therefore, besides the fundamental role in promoting cell growth and survival, PDK1 plays a unique and essential role in accommodating the β-adrenergic response to prevent cardiac decompensation (Figure 2). Because cardiac expression of Pdk1 is significantly decreased in murine models of HF, it raises the possibility that functional alterations of PDK1 may be implicated in the pathogenesis of HF, although it remains unclear how PDK1 expression and function are regulated in the stressed heart.
Mechanical stress, accompanied by neurohumoral factors, is the primary stimulus for induction of cardiac hypertrophy, and it evokes a variety of cellular responses that alter gene expression, protein synthesis, sarcomere assembly, ion channel function, and energy metabolism. Indeed, hemodynamic overload induces cardiac hypertrophy in part through the actions of catecholamines. However, hypertrophic responses were observed when passive stretch was imposed on cardiomyocytes cultured upon deformable silicone rubber dishes in serum-free media, indicating that mechanical stress per se induces hypertrophic responses primarily by stretching cardiomyocytes without the involvement of neurohumoral factors. Utilizing the device for stretching cultured cells, my group and others have demonstrated that mechanical stretching of cultured cardiomyocytes induces hypertrophic responses by activation of multiple signaling pathways, including protein kinases such as extracellular signal-regulated protein kinases (ERKs), integrin signaling, reactive oxygen species, and calcium signaling involving calcineurin and calmodulin-dependent kinase II (Figure 3). In vivo, pharmacological blockade of L-type calcium channels effectively suppressed the development of cardiac hypertrophy in spontaneously hypertensive rats through inhibition of calcineurin activity.

Several molecules have been identified that function as mechanosensors, such as muscle LIM protein within the Z-disc, integrin-linked kinase, and melusin within the costameres (band-like structures linking the sarcolemmal membrane to Z-discs), stretch-sensitive ion channels, G protein-coupled...
receptors (GPCR) such as angiotensin II (Ang II) type 1 (AT1) receptor\(^{40,43}\) and APJ\(^{44}\) and other membrane proteins such as polycystin-1.\(^{45}\) However, the precise mechanisms of mechanosensation and mechanotransduction involving these sensor molecules remain undetermined, especially during the development of load-induced cardiac hypertrophy.

**Mechanical Stress-Induced Activation of the AT\(_1\) Receptor in Pathological Cardiac Hypertrophy**

The RAS plays a crucial role in the regulation of blood pressure and electrolyte balance. In the classical pathway of RAS, angiotensinogen is converted to Ang II via a 2-step proteolytic process by renin and angiotensin-converting enzyme. Ang II is the pivotal bioactive molecule of the RAS, and induces vasoconstriction, sodium and water retention, activation of sympathetic nervous system, and anorexic behavior.\(^{58}\) In cardiovascular tissues, RAS also exerts deleterious effects as a form of cardiovascular remodeling, through local activation of the RAS with autocrine and paracrine mechanisms.\(^{42,47,48}\) Most of the pathophysiological actions of Ang II in the cardiovascular system are mediated through the 7 transmembrane TM\(_3\) spanning AT1 receptor, which is a member of the GPCR family.\(^{43,47,49}\)

A large number of experiments have demonstrated that activation of the AT1 receptor stimulates G-protein-dependent diverse signaling pathways and G-protein-independent signaling pathways such as the Jak/STAT pathway and the β-arrestin-mediated pathway, and evokes hypertrophic responses in cardiomyocytes.\(^{42,43,50}\) Interestingly, recent studies revealed that mechanical stress, as well as systemically and locally generated Ang II, induces cardiac hypertrophy through the activation of the AT1 receptor (Figure 4).\(^{42,47,51}\) First evidence for mechanical stress-induced activation of AT1 receptor was obtained from experiments demonstrating that pretreatment with the AT1 receptor blocker (ARB), candesartan, significantly inhibited Ang II-induced ERK activation,\(^{42}\) indicating that Ang II, even if secreted from stretched cardiomyocytes, plays a marginal role in stretch-induced ERK activation. Furthermore, mechanical stretch did not evoke hypertrophic responses in HEK293 cells with no detectable expression of angiotensinogen or AT1 receptor or in cultured cardiomyocytes deficient for the Agt gene encoding angiotensinogen, but forced expression of AT1 receptor conferred the ability to respond to stretch on these cells. Mechanical stress-induced AT1 receptor activation contributes to cardiac hypertrophy in vivo. Pressure overload for 2 weeks induced cardiac hypertrophy in Agt\(^{-/-}\) mice, as well as in wild-type mice, which was significantly inhibited by treatment with candesartan.\(^{40}\) These results suggest that mechanical stress can induce cardiac hypertrophy in vivo through activation of the AT1 receptor without the involvement of Ang II.\(^{40,42,43,47,55,56}\) According to a previous paper, granular staining of Ang II in cardiomyocytes disappeared after aortic banding in pigs,\(^{57}\) indicating a possibility that mechanical stress might trigger release of preformed Ang II in the heart. However, it remains unclear whether locally released Ang II has a significant effect on the development of load-induced cardiac hypertrophy in wild-type mice.

The AT1 receptor undergoes a conformational switch upon activation by mechanical stress, as well as binding to Ang II. According to the results of substituted cysteine accessibility mapping that probes relative conformational changes of the receptor by validating the presence of Cys residues within the ligand-binding pocket, TM7 undergoes a counterclockwise rotation and a shift into the ligand-binding pocket in response to mechanical stretch.\(^{41}\) We assume that the stabilizing interactions involving TM7 in the AT1 receptor are disrupted by mechanical stress independently and that counterclockwise rotation of TM7 may cause activation of intracellular signaling pathways.

It was reported that inflation of balloon placed in the LV activated β-arrestin-biased signaling downstream of the AT1 receptor.\(^{58}\) Intriguingly, β-arrestin-biased agonism of the AT1 receptor provided inotropic and anti-apoptotic effects in isolated cardiomyocytes,\(^{59,60}\) and a β-arrestin-biased ligand for AT1 receptor enhanced contractility and promoted cardiomyocyte survival during ex vivo balloon stretch, and another ARB, losartan, even increased cardiomyocyte apoptosis.\(^{59}\) It remains unclear how the AT1 receptor senses mechanical stress and undergoes a conformational switch, leading to differential activation of selective intracellular signaling mediators. Recently, crystallizing of the AT1 receptor has determined its structure in complex with its selective antagonist ZD7155 at 2.9 Å resolution,\(^{61}\) and crystal structural information of the AT1 receptor will deepen our understanding of the mechanisms underlying activation, inactivation, and modification of the receptor at the molecular level.
Constitutive Activity of the AT1 Receptor in Pathological Cardiac Hypertrophy

The classical concept that receptors switch by a simple “on-off” mechanism has been challenged since the discovery of spontaneous and constitutive activity of GPCRs in the absence of an agonist. Simultaneously, some ligands have been found to produce negative efficacy, and are classified as “inverse agonists”. An inverse agonist is defined as a ligand that stabilizes inactive conformation of the receptor and reduces constitutive activity of the receptor or antagonistic independent receptor activity in a dose-dependent manner.

The constitutive activity of the wild-type AT1 receptor under basal conditions is relatively low, but can be detected when the AT1 receptor is overexpressed in heterologous cells even without endogenous expression of angiotensinogen. Because expression of the AT1 receptor is upregulated in the heart, the AT1 receptor may contribute to the progression of pathological cardiac hypertrophy. In the hearts of transgenic mice overexpressing the AT1 receptor under the control of the Mvh6 gene promoter in an Atg−/− background, the AT1 receptor was constitutively activated in the absence of Ang II. As a consequence, these mice showed spontaneous LV dysfunction and chamber dilatation, indicating that constitutive activity of the AT1 receptor promotes cardiac remodeling independently of Ang II when the AT1 receptor is upregulated in the heart (Figure 4). In addition, progression of cardiac remodeling was prevented by treatment with an inverse agonist for the AT1 receptor, but not by its derivative deficient of inverse agonism.

Genetic blockade of AT1 receptor signaling has attenuated the progression of HF in several mouse models. Mice deficient for Agr1a encoding AT1 receptor showed better survival at 4 weeks after MI, with milder LV dilatation, systolic dysfunction, and cardiac fibrosis, than control mice. The Agr1a−/− mice also showed milder HF phenotypes than the control mice in cardiomyopathic models of doxorubicin-induced cardiotoxicity and genetic disruption of Marcks11 encoding the muscle LIM protein. ARBs are a highly effective and well-tolerated class of drugs for the management of hypertension. Clinical trials have demonstrated that ARBs are beneficial in patients with cardiovascular and metabolic complications, and provide cardiovascular protection and reduce death and hospitalization in patients with HF. ARBs share biphenyl-tetrazol and imidazole groups in structure, but have the drug-specific property of inverse agonist activity that can inhibit Ang II-independent constitutive activity and mechanical stress-induced receptor activation. The clinical importance of the inverse agonist activity of ARBs is still speculative, and future studies are awaited that address their therapeutic benefits in the prevention of cardiovascular diseases.

Mast Cell Infiltration in the Pathogenesis of Atrial Remodeling and Fibrillation

Atrial fibrillation (AF), a common but ominous arrhythmia, gives rise to an increased risk of stroke and HF. Because pharmacological antiarrhythmic approaches yield poor efficacy, the “upstream” therapy, targeting atrial structural remodeling underlying a susceptible AF substrate, has increasingly become the focus of attention. Although the pathophysiology of AF remains incompletely understood, clinical and experimental studies have suggested that inflammation underlies a susceptible AF substrate.

In a mouse model of AF, mast cells played a crucial role in the development of atrial fibrosis and AF in pressure-overloaded hearts (Figure 5). Pressure overload induced mast cell infiltration and interstitial fibrosis in the atrium, and electrical stimulation of the right atria of pressure-overloaded hearts evoked AF under Langendorff perfusion. Importantly, both atrial fibrosis and AF inducibility were remarkably attenuated by pharmacological stabilization of mast cells with cromolyn or by genetic depletion of mast cells using bone marrow reconstitution from mast cell-deficient WBB6F1-Kitw/W− mice. Furthermore, infiltrating mast cells induced upregulation of Pdgfa encoding platelet-derived growth factor (PDGF)-A in the atrium, and systemic administration of PDGF-A protein promoted atrial fibrosis and enhanced AF susceptibility in normal hearts. It was reported that pharmacological inhibition of PDGF receptor signaling prevented the development of interstitial fibrosis of the infarcted heart of rats. Likewise, administration of a neutralizing antibody against PDGF α-receptor prevented atrial fibrosis and AF inducibility, indicating a pivotal role of PDGF-A in mediating mast cell-triggered evolution of an AF substrate. Because mast cells, key effector cells in allergic and immune responses, play a pathogenic role in AF, the mast cell-PDGF-A axis is a promising target for “upstream” prevention of AF in stressed hearts.

Concluding Remarks

The contributions to elucidating the molecular mechanisms of cardiovascular homeostasis and pathophysiology were introduced in this review article. Regulatory and signaling circuits in molecular networks are involved in exquisite processes whereby cardiomyocytes sense and respond to the stresses. Of course, we just get only a small fraction of how cardiomyocytes truly behave in response to pathological biomechanical stress to maintain cardiovascular homeostasis. Substantial research must be continued to pursue the definitive goal of uncovering the full scope of disease processes and developing novel diagnostic and therapeutic tools for cardiovascular diseases.
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