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Molecular Mechanisms in Heart Failure

Focus on Cardiac Hypertrophy, Inflammation, Angiogenesis, and Apoptosis

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Heart failure is a final common pathway in cardiovascular disease, as a result of sustained pressure overload (i.e., hypertension), myocardial ischemia or infarction, volume overload (i.e., mitral regurgitation), or inherited and acquired cardiomyopathies. Heart failure is a major health care burden, and despite significant therapeutic advances, morbidity and mortality in heart failure remain unacceptably high. Therefore, novel insights into pathophysiology and molecular mechanisms of heart failure are required to develop novel therapeutic approaches. In this review we highlight several advances in the understanding of molecular pathways involved in cardiac hypertrophy, inflammatory signaling (i.e., tumor necrosis factor-α, interleukin-6), and oxidant stress that may play a key role in altering transcriptional regulatory networks regulating adaptation or maladaptation, and consequently, the transition to overt heart failure. In this respect we focus on paracrine mechanisms (vascular endothelial growth factor, CCN1) and intracellular signaling (interleukin-6–glycoprotein 130–signal transducer and activator of transcription-3). In addition, we highlight the impact of current treatment options on these molecular pathways and their potential impact on progression of heart failure. (J Am Coll Cardiol 2006;48:A56–66)

Heart failure is a leading cause of hospitalization and mortality worldwide. Despite significant therapeutic advances, the morbidity and mortality of heart failure remain unacceptably high. Heart failure is a final common pathway of various cardiovascular diseases, including sustained pressure overload (i.e., hypertension), myocardial ischemia or infarction, volume overload (i.e., valvular heart disease), and inherited or acquired cardiomyopathies.

Myocardial hypertrophy is a major predictor of progressive heart disease and an adverse prognosis. Myocardial hypertrophy is a response of cardiac muscle to altered conditions caused by a large number of physiological and pathological stimuli. "Physiological cardiac hypertrophy" occurs in response to growth, exercise, or pregnancy, in which cardiomyocyte hypertrophy is paralleled by a proportional growth of the vasculature and the capillary network and is usually not accompanied by cardiac fibrosis (1). At the molecular levels, physiological hypertrophy seems to be characterized by increased expression of adult isoforms of sarcomeric genes (i.e., α-myocin heavy chain [MHC], cardiac α-actin) (1,2). Pathological hypertrophy in response to pathophysiological stress signals (e.g., neurohormonal activation, aortic stenosis, inflammation or cardiac injury) may be initially adaptive in terms of muscular economy, normalizing wall stress, and preserving contractile performance, but can proceed to decompensation and heart failure. Notably, at the molecular level pathological hypertrophy is characterized by the activation of gene expression patterns of the fetal stage, which includes an up-regulation of fetal isoforms of genes whose products regulate cardiac contractility and calcium handling, which is often paralleled by a down-regulation of their adult isoforms (i.e., up-regulation of β-MHC vs. down-regulation of α-MHC) (1,2). Pathological hypertrophy is often associated with impaired myocardial vascularization, unfavorable changes in the extracellular matrix composition, and fibrosis (3,4). More recently, evidence has been presented showing that pathophysiological stresses also interfere with the normal cell turnover in the heart, leading to an unfavorable ratio of cardiac apoptosis and regeneration from circulating and cardiac progenitor cells (5).

The last decade has seen a major advance in the understanding of molecular mechanisms of adaptive and maladaptive hypertrophy and heart failure in response to stress signals and has shown that a multitude of extracellular factors and signaling pathways are involved. In this regard, adaptive myocardial hypertrophy has been associated with growth hormone/insulin-like growth factor-1, interleukin-6 (IL-6) cytokines/glycoprotein 130 (gp130) and biomechanical stretch signaling and seems to involve the activation of the phosphatidylinositol 3-kinase (PI3-K)/protein kinase B (Akt)/glycogen synthase kinase 3β cascade, the signal transducer and activator of transcription 3 (STAT3), and/or activation of extracellular signal-regulated kinases (ERK) (6–9). These signaling pathways have been implicated in adaptive hypertrophy because their overexpression or constitutive activation leads to increased cardiomyocyte size and concentric hypertrophy in the absence of fibrosis, with preserved systolic function (6,8,9). In contrast, maladaptive myocardial hypertrophy has been associated with angiotensin II, tumor necrosis factor-α (TNF-α), and catecholamines (7,10) and seems to involve downstream signaling pathways, c-Jun N-terminal kinase and p38, which promote cardiac fibrosis and apoptosis (11).

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Abbreviations and Acronyms

Akt	= protein kinase B
ERK	= extracellular signal-regulated kinases
gp130	= glycoprotein 130
GSK	= glycogen synthase kinase
IL-6	= interleukin-6
JAK	= Janus kinase
LIF	= leukemia inhibitory factor
LV	= left ventricle/ventricular
MHC	= myosin heavy chain
MLP	= muscle limb protein
NF-kappaB	= nuclear factor kappaB
NO	= nitric oxide
PI3-K	= phosphatidylinositol 3-kinase
ROS	= reactive oxygen species
STAT3	= signal transducer and activator of transcription
TNF-α	= tumor necrosis factor-α
VEGF	= vascular endothelial growth factor

Conceivably, sustained pathophysiological stress may promote the transition of initially adaptive hypertrophy and remodeling into a decompensated stage with cardiac contractile dysfunction, clinical signs of heart failure, and premature death. This process, termed maladaptive remodeling, has been linked to biological processes provided by activated stress pathways.

At the Symposium on Cardiovascular Disease held by the Fondation Leducq in Paris, France, October 2005, we highlighted recent mechanistic findings in some molecular pathways related to heart failure such as cardiac hypertrophy, inflammation, angiogenesis, and apoptosis. Because of limits in space, other important aspects, such as excitation-contraction coupling, calcium signaling, and the beta-adrenergic receptor pathway are not discussed and the reader is referred to excellent reviews on these topics (12–14).

NEUROHORMONAL STIMULATION OF CARDIAC HYPERTROPHY AND DYSFUNCTION

The understanding of the important role of neurohormonal activation in the pathophysiology of heart failure has paved the way for the current medical treatment to improve morbidity and mortality in patients with heart failure (15). Besides angiotensin II–induced signaling mediated by the AT-1 receptor and sympathetic activation, increased aldosterone production, at least in part angiotensin II independent, has been suggested to play a major role in left ventricular (LV) remodeling and LV dysfunction. Accordingly, recent experimental data support the concept that aldosterone blockade exerts beneficial effects in addition to effective renin-angiotensin system blockade. In $AT\text{-}1_A$-receptor-deficient mice, cardiac gene expression levels of aldosterone synthase were elevated after myocardial infarction and LV remodeling and dysfunction were attenuated by spironolactone in these mice (16). Furthermore, Fiebeler et al. (17) have recently provided experimental evidence showing that increased cardiac damage caused by aldosterone may be secondary to increased adrenal aldosterone production, because both adrenalectomy and aldosterone synthase (CYP11B2) inhibition reduced cardiac aldosterone levels and prevented cardiac hypertrophy and death in renin-angiotensin transgenic rats. Aldosterone is one of the stimulators of cardiomyocyte reactive oxygen species (ROS) production (18) that has been implicated in the development of cardiac hypertrophy and dysfunction in response to both biomechanical and neurohormonal stimuli.

INCREASED OXIDANT SIGNALING IN HEART FAILURE

Numerous studies have reported increased myocardial production of ROS (superoxide, hydrogen peroxide, hydroxyl radical) in experimental and clinical heart failure (19–21). Recent work has characterized potential sources of increased ROS production in failing myocardium, including NAD(P)H and xanthine oxidase, dysfunctional nitric oxide synthase, and the mitochondrial electron transport chain (22–24). Two recent studies have reported increased NAD(P)H oxidase activity in human failing compared with nonfailing left ventricles (22,25). Maack et al. (22) have further provided evidence for an increased expression of the NAD(P)H oxidase subunit p47phox and increased activity of the small G protein rac-1, thought to be important for activation of the NAD(P)H oxidase enzyme complex, in patients with heart failure. Bendall et al. (26) have shown that angiotensin II–induced cardiac hypertrophy after 2-week treatment was dependent on NAD(P)H oxidase activation. Interestingly, increased NAD(P)H oxidase activation may therefore represent a potential treatment target for preventing cardiomyocyte hypertrophy in heart failure. Of note, statins prevent membrane translocation of the small G protein Rac-1 (because of inhibited isoprenylation) and are thought to thereby inhibit NAD(P)H oxidase. This may contribute to the prevention of cardiac hypertrophy and dysfunction after statin treatment that has been observed in numerous experimental studies (27–30). Recent in vitro and in vivo studies have provided evidence for an important role of antioxidant effects of statins for their effect on cardiac hypertrophy (27) and vascular dysfunction in patients with heart failure (Fig. 1) (29).

Increased myocardial and vascular xanthine oxidase activity has been observed in human heart failure (31,32). Experimental studies have suggested beneficial effects of high-dose treatment with allopurinol, a xanthine oxidase inhibitor, on LV dysfunction and remodeling (33) and potentially increased survival in mice after myocardial infarction (34). In patients with chronic heart failure, increased serum levels of uric acid, the product of xanthine oxidase, have been associated with an adverse prognosis (35). In a small clinical study, acute xanthine oxidase inhibition with intracoronary allopurinol improved LV efficiency in patients with idiopathic dilated cardiomyopathy (31). A larger clinical study has been performed in 405 patients with class III

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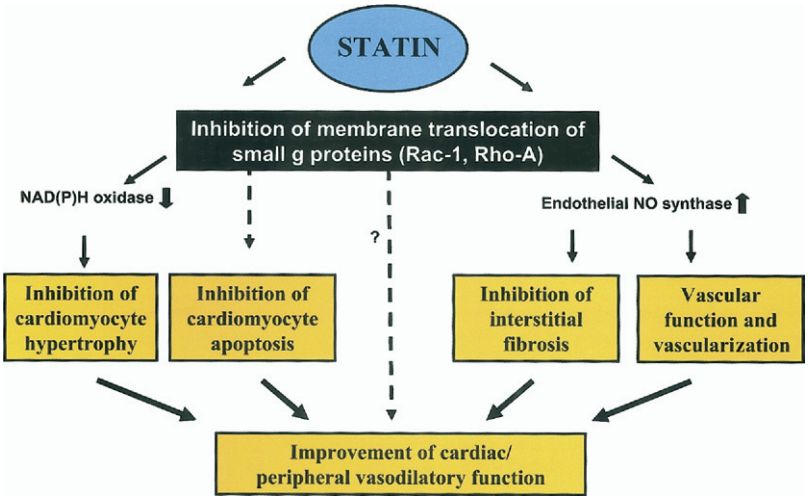

Figure 1. Statin therapy may represent a potentially novel treatment strategy for preventing cardiac hypertrophy and for improving myocardial vascularization. Statins may exert beneficial effects in heart failure independent of cholesterol lowering by prevention of isoprenylation of small g proteins, such as Rac-1 or Rho-A, which have been shown to be critically involved in the activation of the oxidant enzyme NAD(P)H oxidase and the regulation of endothelial nitric oxide (NO) synthase expression. Prevention of NAD(P)H oxidase activation and an increased endothelial NO synthase-dependent NO availability may reduce cardiomyocyte hypertrophy and improve vascular (i.e., endothelial) function.

to IV heart failure over 24 weeks (OPT-CHF [Controlled Efficacy and Safety Study of Oxypurinal Added to Standard Therapy in Patients with New York Heart Association class III to IV Congestive Heart Failure] trial) using oral xanthine oxidase inhibition by oxypurinol; however, the results of this study have not been published.

A detailed understanding of the pathophysiologically relevant sources of ROSs and their role in heart failure will be necessary to possibly limit cardiac hypertrophy and dysfunction. Another major consequence of increased ROS production in heart failure is a loss of endothelial nitric oxide synthase (NOS)-derived nitric oxide (NO), which reacts very rapidly with superoxide, leading to formation of the toxic peroxynitrite, and may contribute to impaired peripheral vascular function and coronary perfusion (36,37). In this respect we and others have observed an improved endothelium-dependent, NO-mediated vasodilation after administration of a high dose of the antioxidant vitamin C in patients with heart failure (37) or after treatment with the superoxide scavenger superoxide dismutase in experimental heart failure (36). In addition, accumulating experimental data suggest an important role of reduced endothelial nitric oxide synthase-dependent NO availability in heart failure that may augment LV remodeling and dysfunction (30,38–40). In addition, several recent clinical studies have closely linked the degree of endothelial dysfunction, thought to indicate a reduced endothelial NO availability, with an adverse clinical outcome in patients with heart failure (41–43). Notably, recent data have implicated a protective role of the neuronal NO synthase, because neural NOS-deficient mice had an augmented LV remodeling in experimental heart failure (40,44).

In summary, increased ROS production is likely involved in key aspects relevant to the development and the progression of heart failure, such as cardiomyocyte hypertrophy, ventricular dysfunction, and endothelial dysfunction. A therapy that may exert beneficial effects in heart failure by reducing oxidant stress and increasing endothelial NO availability is statin treatment. Statins have been shown to inhibit NADPH oxidase activation in the cardiomyocyte (45) and at the same time to increase endothelial cell NO production (46). The proposed mechanisms by which statins may exert beneficial effects in heart failure independent of cholesterol lowering are shown in Figure 1.

INFLAMMATORY SIGNALING IN HEART FAILURE: WHAT IS DOWNSTREAM OF TNF-α?

It is well established that increased levels of TNF-α appear in the circulation of patients with heart failure, and together with the soluble TNF-α receptors 1 and 2 serve as prognostic markers in these patients (47,48). The observation that mice harboring a transgene that selectively overexpresses TNF-α in the heart developed cardiac hypertrophy and dilated cardiomyopathy implicated a detrimental role of this cytokine in the heart (49). In addition, TNF-α exerts strong direct effects on cardiomyocytes, as it induces apoptosis, depression of contractility, and down-regulation of sarcomeric proteins in cardiomyocytes in vitro (50–52). Therefore, a causal role of TNF-α in heart failure seemed to be obvious and clearance of TNF-α as a novel therapeutic strategy was hoped to improve heart failure. Clinical pilot trials in small patient collectives showed that clearance of TNF-α improved cardiac performance in heart failure (reviewed by Torre-Amione [48]). However, the outcomes of 2 large-scale clinical trials (with etanercept and infliximab) that directly antagonized TNF-α have been rather disappointing and suggested that counterbalancing this cytokine alone may not be sufficient (53).

More recently, novel promising strategies have emerged from experimental work targeting specifically downstream

signaling pathways of TNF-α. In this regard, the nuclear factor kappaB (NF-kappaB) was robustly activated in mice with cardiac-specific activation of TNF-α, and this activation was mediated via the TNF-α receptor 1 (54). In mice double mutant for the TNF-α transgene and a knockout of the p50 subunit of NF-kappaB, cardiac function and survival was significantly improved compared with mice harboring the TNF-α transgene alone (55). Thus, NF-kappaB seems to be an important mediator of the detrimental effects of TNF-α signaling in the heart. Further evidence for a negative role of activated NF-kappaB in the stressed heart was obtained from experiments using NF-kappaB inhibiting decoy oligonucleotides, which significantly reduced the infarct size after experimental myocardial infarction (56). NF-kappaB is also activated in failing human hearts of various etiologies, implicating a role of this transcription factor in the pathophysiology of human heart failure (57,58). However, care must be taken because NF-kappaB activation seems not always detrimental for the heart. In this regard, pharmacologic inhibition of NF-kappaB abolished the protective effect of ischemic preconditioning (short periods of ischemia and reperfusion) on reduction of the infarct size during reperfusion (59).

The role of TNF-α signaling in the heart is complex and seems to depend on the nature of stress and the interplay of downstream inflammatory mediators. Therefore, detailed analysis and understanding of the signaling networks underlying TNF-α– mediated inflammatory processes in cardiomyopathies of different etiologies is essential for developing novel therapies targeting specifically the detrimental biological effects of TNF-α.

THE MANY FACETS OF CARDIOPROTECTION BY IL-6–gp130–STAT3 SIGNALING

Some years ago it was reported that IL-6 cytokine levels are elevated in patients with heart failure and that IL-6 is a strong prognostic marker for the morbidity and mortality in patients with heart failure or after myocardial infarction (60,61). More recently, our laboratory has explored the IL-6–gp130–Janus kinase (JAK)–STAT signaling cascade in patients with end-stage heart failure and showed that this signaling cascade is altered at each level in failing human hearts (62) (Fig. 2). For example, the cardiac expression of IL-6 (Fig. 2) is reduced, whereas the expression of IL-6 family member leukemia inhibitory factor (LIF) is elevated. The expression of cardiotrophin-1, another member of the IL-6 cytokine family, is not altered (62). At the receptor level we observed that gp130 expression is not different, but failing hearts show enhanced gp130 activation (tyrosine phosphorylation) (Fig. 2). The expression of JAK2, the next downstream signaling molecule of the gp130 receptor, is also not altered, but surprisingly its activation state (tyrosine phosphorylation) is diminished in failing hearts (Fig. 2). Most strikingly, the effector signaling molecule of the IL-6–gp130 cascade, STAT3, is severely reduced in failing hearts (Fig. 2) (62). Although these observations do not identify the exact role of individual factors within the IL-6–gp130–JAK–STAT signaling system, a growing body of experimental work indicates that IL-6–related cytokine signaling contributes to compensatory hypertrophy, provides cardioprotection, and promotes neovascularization in the stressed heart (63–68). In particular, the analysis of transgenic mice harboring knockouts for specific components of the IL-6–gp130–JAK–STAT cascade sheds light on the role of this signaling pathway in the pathophysiology of the heart. For example, mice with a systemic deficiency of IL-6 were not different from wild types regarding their basal cardiac phenotype, respectively concerning infarct size, cardiac function, and survival after myocardial infarction (Fig. 2), most likely because IL-6 cytokines are redundant, and other proinflammatory cytokines such as LIF can compensate for the lack of IL-6 (69). However, infusion of an IL-6/soluble IL-6 receptor complex inhibited cardiomyocyte apoptosis and limited infarct size after ischemia/reperfusion (70), suggesting a rather protective role of IL-6. LIF induces hypertrophy and promotes survival of cardiomyocytes in vitro and in vivo (71,72). Furthermore, injection of LIF promoted healing after myocardial infarction (73). Cardiotrophin-1 also stimulates cardiomyocyte hypertrophy and seems to be involved in wound healing after myocardial infarction (74,75). Importantly, IL-6 cytokine signaling via gp130 involves activation of 3 major pathways: MEK/ERK; JAK/STAT, and PI3-K/Akt, and each of these pathways seems to be involved in hypertrophic and protective signaling in the heart in vivo and in vitro (63,76). Although IL-6 cytokines might be redundant in exerting cardioprotective effects, apparently they differ in the induction of the activation pattern of the gp130 downstream signaling. For example, gp130-mediated activation of the mitogen-activated protein kinase (MEK)/ERK signaling pathway by cardiotrophin-1 induces hypertrophy (75), whereas LIF promotes cardiac hypertrophy and protection via gp130-mediated activation of the JAK2/STAT3 pathway (77).

Notably, activation of STAT3, via the gp130 receptor system is essential for cardiomyocyte hypertrophy and cytoprotection in cardiomyocytes subjected to ischemia or toxic stress (76–78). Moreover, STAT3 is necessary for the cardioprotective effects induced by ischemic preconditioning and various pharmacologic preconditioning programs (79), and STAT3 is also required to protect the heart from ischemia/reperfusion injury and myocardial infarction (Fig. 2) (67). Recent data from our laboratory and others provide evidence that STAT3 plays a crucial role in the protection of the heart during aging (67,68) (Fig. 2). One reason why STAT3 could provide protection in response to such a wide array of physiological and pathophysiological stresses may result from its ability as a transcription factor to directly induce the expression of antiapoptotic and cytoprotective

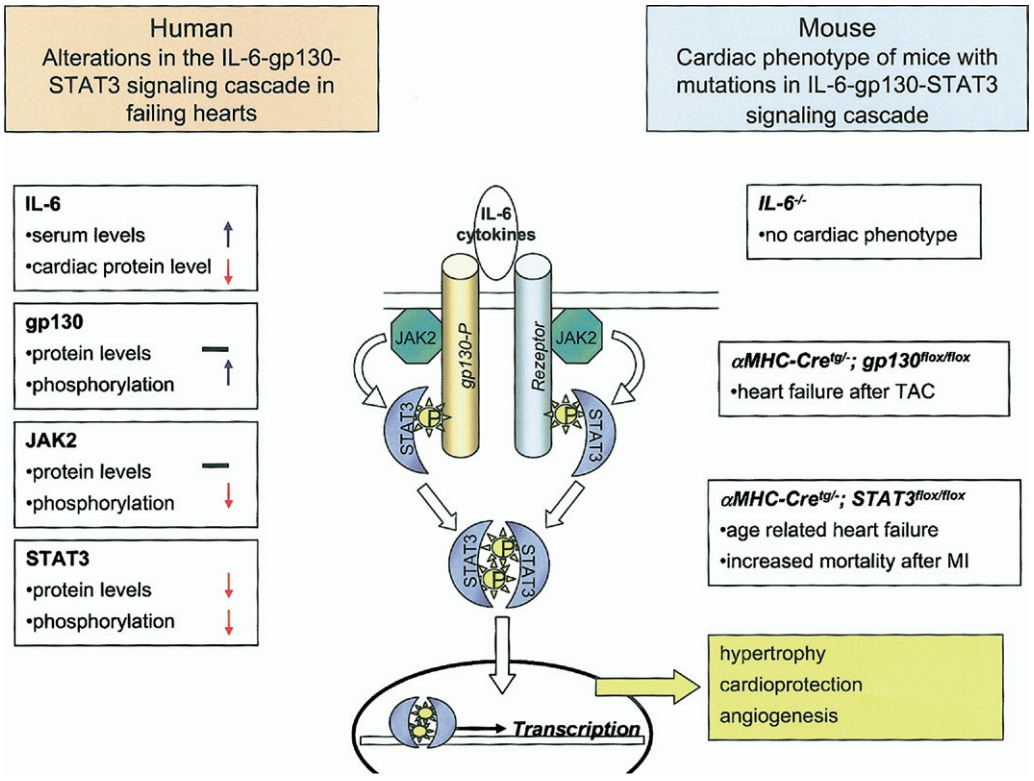

Figure 2. Comparison of the interleukin (IL)-6–glycoprotein 130 (gp130)–Janus kinase (JAK)–signal transducer and activator of transcription 3 (STAT3) signal cascade in end-stage failing human hearts with the cardiac phenotype of mice harboring systemic or cardiac-restricted knockouts in this cascade. The **left side** indicates the alterations in the IL-6–gp130–JAK–STAT3 cascade in end-stage human dilated cardiomyopathy in comparison with normal myocardium: Serum levels of IL-6 are upregulated and myocardial IL-6 protein levels are reduced; gp130 protein levels are not altered, but their activation stage (tyrosine phosphorylation) is enhanced; JAK2 activation (tyrosine phosphorylation) is diminished with no alteration in protein expression; STAT3 is reduced at both the protein level and the activation stage (tyrosine phosphorylation) (60–62). The **right side** summarizes the cardiac phenotypes of mice with mutations in the IL-6–gp130–JAK–STAT3 signaling cascade. Mice with systemic deletion of IL-6 (IL-$6^{-/-}$) have no apparent cardiac phenotype at baseline or after myocardial infarction (69); mice with cardiac-restricted deletion of gp130 (α-myosin heavy chain [MHC]-$Cre^{tg/-}$; $gp130^{flox/flox}$) appear normal at baseline but show early cardiac failure and enhanced mortality after pressure overload induced by thoracic aortic constriction (TAC) (63). Mice with cardiac-restricted deletion of STAT3 (α-MHC-$Cre^{tg/-}$; $STAT3^{flox/flox}$) develop an age-related dilated cardiomyopathy (67,68) and are more susceptible to ischemic injury (67). In summary, the gp130–STAT3 cascade seems to promote hypertrophy, cardioprotection, and angiogenesis in the stressed heart (4,63–66,68).

proteins, such as Bcl-xL, heat shock protein 70, and manganese superoxide dismutase, in cardiomyocytes (66,67,77).

Taken together, the IL-6–gp130–STAT3 signaling cascade in human failing hearts is deregulated at all levels. Analysis in various knockout mouse models showed that the gp130 receptor system and specifically its downstream mediator STAT3 plays a key functional role for cardioprotection against physiological and pathophysiological stress by promoting cardiomyocyte survival, inducing compensatory hypertrophy and preserving cardiac function (Fig. 2). Thus, in the light of these experimental data sets, elevated IL-6 serum levels in patients with heart failure seem to be protective rather than detrimental with respect to gp130 signaling.

NOVEL INSIGHTS INTO BIOMECHANICAL SIGNALING IN HEART FAILURE

Mechanical stress is considered a major trigger of cardiomyocyte growth in response to pressure overload. The transduction of mechanical stress into biomechanical signals is thought to be largely mediated by a group of surface receptors called integrins (Fig. 3) that link the extracellular matrix to the cellular cytoskeleton, thus providing physical integration between the outside and inside of the cell. The Z-disc, a multiprotein complex located at the interface of the cytoskeleton, the contractile apparatus, and the sarcolemma in cardiomyocytes are thought to be essential for biomechanical stress signaling in the heart (80,81).

Activation of integrins by different ligands in the extracellular matrix (e.g., collagen, fibronectin laminin) initiates signaling in multiple intracellular pathways through integrin-bound proteins (Fig. 3). One of the biomechanical sensors in this pathway is melusin (Fig. 3), a muscle-specific protein that interacts with the integrin β1 cytoplasmic component. Brancaccio et al. (80) have shown that melusin is required to promote adaptive hypertrophy in response to mechanical but not to hormonal stress. Furthermore, it has been shown that the intracellular signaling pathways regulated by melusin involve ERK1/2, PI3-K/Akt and glycogen synthase kinase 3-beta (GSK-3β) (Fig. 3) (80). A deficiency of melusin has resulted in an early onset of cardiac dilation and dysfunction after pressure overload, suggesting that melusin is required for the compensatory cardiac hypertro-

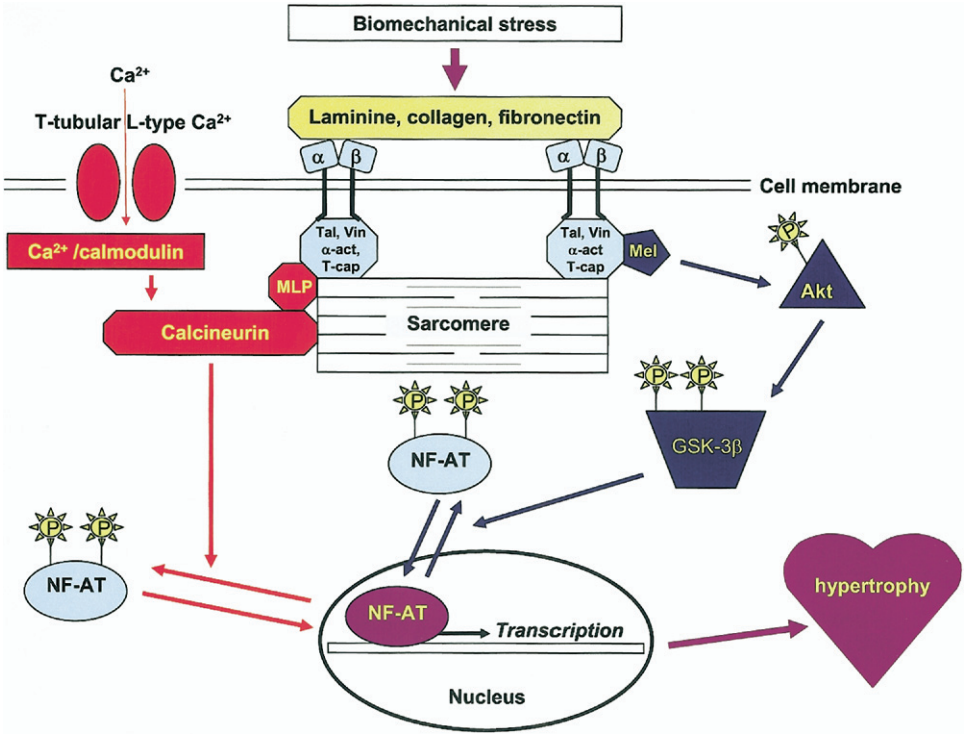

Figure 3. Sensing and transmission of stress signaling in cardiomyocyte exposed to biomechanical stretch. Mechanical strain induces activation of integrins (α,β) by different ligands in the extracellular matrix (e.g., collagen, fibronectin, laminin), initiating signaling of multiple intracellular pathways. Melusin (Mel), an integrin-bound protein, transduces the stress signal from the cell membrane to the nucleus by activating protein kinase B (Akt)-glycogen synthase kinase (GSK)-3β, thereby promoting the dephosphorylation (activation and nuclear location) of the prohypertrophic transcription factor nuclear factor of activated T-cells (NF-AT3). NF-AT3 then contributes to the induction of a prohypertrophic gene program and subsequent cardiomyocyte hypertrophy (80,87,93). A second stress-sensing pathway involves muscle LIM protein (MLP), a Z-disc protein, which with others, such as α-actinin (α-act), telethonin (T-cap), vinculin (Vin), and talin (Tal), is anchored to integrins (α,β) at the plasma membrane and to the sarcomere. This molecule complex connects the contractile machinery to the extracellular matrix proteins laminin and collagen (87). MLP acts as an anchoring protein for other Z-disc proteins, e.g., for T-cap, and seems to be necessary to keep these proteins in place during contraction (81). In addition, MLP functions as an anchoring protein for calcineurin at the Z-disc, where it brings calcineurin in close approximation with T-tubular L-type Ca^{2+}-channels, which are implicated in calcineurin activation by Ca^{2+}/calmodulin after Ca^{2+} influx. Activation of calcineurin then promotes the activation of NF-AT3, resulting in hypertrophic gene expression (91,115).

phy initially preventing ventricular dilation. Vice versa, cardiac overexpression of melusin reduced ventricular dilation in response to pressure overload and resulted in a prolonged compensatory hypertrophy (82). A potential beneficial role of downstream Akt signaling in terms of adaptive hypertrophy is supported by transgenic mice with a moderate cardiac specific overexpression of Akt, which develop a concentric myocardial hypertrophy in the absence of fibrosis with preserved systolic function (83). In turn, the importance of down-regulating GSK-3β for adaptive hypertrophy is manifested by the observation that cardiac hypertrophy is inhibited in mice with a cardiomyocyte-specific overexpression of a constitutively active form of GSK-3β in response to pressure overload (84). Similarly, a lack of Fas receptor-induced inhibition of GSK-3β by pressure overload coincided with a rapid onset of left ventricular dilatation and heart failure caused by the absence of compensatory hypertrophy (85). One mechanism by which GSK-3β attenuates cardiac hypertrophy lies in its ability to phosphorylate the hypertrophy-associated transcription factors nuclear factor of activated T-cells (NF-AT) and GATA4, thereby causing their nuclear exit with the consequence that hypertrophic gene expression in response to hypertrophic stimuli is lowered (86). Therefore, it has been proposed that melusin via activating Akt signaling and subsequent silencing of GSK-3β activates a prohypertrophic gene program required for compensatory hypertrophy in the heart exposed to increased biomechanical stress (Fig. 3). Thus, selective activation of melusin may protect the chronically pressure-overloaded heart from the transition toward dilation and failure and should be explored therapeutically.

The contractile machinery of the cardiomyocyte, the sarcomere, is anchored to integrins at the plasma membrane via a number of Z-disc proteins, including, among others, α-actinin, vinculin, and talin (87). The Z-disc functions as a physical anchor for myofilament and cytoskeletal proteins and as a coordination center for the reception and transduction of mechanical and biochemical stress signals. It has been shown that mutations in Z-disc proteins, which affect both the structural integrity of the Z-disc and/or the transmission of intracellular signals, promote dilated cardiomyopathy in mice (81,88,89). For example, muscle LIM protein (MLP), which is tethered to the Z-disc via its interacting partners α-actinin and telethonin, has been identified as an essential part of the mechanical stretch sensor machinery and is likely to be involved in the transmission of biochemical growth signals in

cardiomyocytes (81,90). Knoll et al. (81) have shown that MLP knockout mice spontaneously develop dilated cardiomyopathy within a few weeks after birth. Moreover, MLP-deficient cardiomyocytes display severe defects of tension development in response to mechanical stretch (81). Our group (91) has recently shown that MLP acts as an anchoring protein for calcineurin at the Z-disc (92) (Fig. 3) and that MLP is required for calcineurin activation after myocardial infarction. Z-disc anchorage brings calcineurin in close proximity with T-tubular L-type Ca^{2+}-channels, which have previously been implicated in calcineurin activation in cardiomyocytes (93). Notably, it has been shown that calcineurin via dephosphorylation of NF-AT3 induces cardiomyocyte hypertrophy (94), suggesting that MLP via calcineurin and NF-AT may provide an additional pathway for adaptive hypertrophy in response to mechanical stress (Fig. 3).

MLP plays an important role in the human heart because loss of function mutations in MLP have been linked to rare, hereditary forms of dilated and hypertrophic cardiomyopathy in humans (81). Interestingly, myocardial MLP expression levels are reduced by about 50% in patients with heart failure after myocardial infarction, suggesting that reduced MLP expression may also play a role in more common, acquired forms of heart failure (95).

Thus, mechano-transduction signaling in cardiomyocytes is likely to operate via the melusin–PI3-K/Akt and the MLP–calcineurin signaling pathways and promotes hypertrophy via activation of NF-AT3 (81,87,91,94) (Fig. 3). Gene mutations aborting one of these pathways result in an inadequate hypertrophic response and early cardiac failure in response to mechanical stress (81,87,91), implying that the activation of these 2 signaling pathways is important for promoting adaptive hypertrophy and for preventing early dilation and failure during hemodynamic overload.

VASCULARIZATION IN THE STRESSED HEART

Myocardial perfusion is a key requisite for myocardial homeostasis. Pathophysiological stresses such as myocardial infarction or pressure overload enhance oxygen demand (3). Therefore, failure to induce sufficient neovascularization results in deficient oxygen supply and subsequently in loss and degeneration of cardiomyocytes, atrophy, and interstitial fibrosis, and may represent a major cause of myocardial dysfunction and heart failure (3,4,96,97). In line with this hypothesis, fewer capillaries have been observed in many forms of human adaptive cardiomyopathies, including end-stage dilated cardiomyopathy (3,4,98,99). Thus, impaired capillary growth and the resultant metabolic imbalance may be an important contributor to the transition to heart failure (3,99).

Among the signaling molecules involved in the expression of proangiogenic factors in cardiomyocytes, STAT3 seems to play a crucial role. Mice with cardiomyocyte-specific overexpression of STAT3 present increased capillary density accompanied by enhanced expression of the proangiogenic factors vascular endothelial growth factor (VEGF) and VE-cadherin (100). In contrast, mice with a cardiomyocyte-specific deletion of STAT3 show a subsequent reduction in myocardial capillary density and cardiac failure (67) (Fig. 4). In the absence of STAT3, an antiangiogenic gene program emerges that involves the upregulation of multiple antiangiogenic factors (67), implicating a key functional role of STAT3 in the regulation of angiogenic circuits in the mature heart (4).

Mice lacking JunD, a transcription factor activator protein-1 transcription factor, show a high rate of mortality in response to severe pressure overload, associated with an increase in cardiac apoptosis (101), most likely as a consequence of enhanced oxidative stress (101). Remarkably, chronic moderate pressure overload in JunD knockout mice evoked a more pronounced hypertrophy and strongly promoted expression of VEGF and myocardial vascularization (Fig. 4B) without adverse effects on survival and cardiac function. Thus, JunD exerts protection in the acutely pressure overloaded heart, but it attenuates neovascularization in the chronic stage of pressure overload and may thereby cause hypoxia and promote the transition into the decompensated stage (101).

Both STAT3 and JunD regulate the expression of the proangiogenic secreted factor VEGF, pointing to a key role of this protein for postnatal myocardial angiogenesis, in which cardiomyocytes themselves play an important role as a source for VEGF. In fact, mice with a cardiomyocyte-specific deletion of VEGF show less coronary microvascularization and display an induction of hypoxia-responsive genes (102). In line with these findings, application of VEGF has been shown to induce angiogenesis in many animal models (reviewed by Isner and Losordo [103]). However, transgenic mice with an inducible VEGF transgene showed abnormal vascular trees, chaotic connections with the existing network, and formation of irregularly shaped sac-like vessels. The VEGF also caused a massive and highly disruptive edema in transgenic mice (104). Thus, the therapeutic potential of VEGF to induce sustained cardiac neovascularization is limited, and its application in humans has yielded equivocal long-term results (105).

We have recently shown that a second proangiogenic factor, CCN1, is induced in the heart in vivo and in cardiomyocytes in vitro by various extracellular stress-related stimuli such as neurohumoral activation, cytokines, mechanical stress, and ischemia (106). CCN1 is also highly expressed in cardiomyocytes and vascular cells of human failing hearts with ischemic cardiomyopathy (106) (Fig. 4C) and acts as a proangiogenic factor by promoting integrin-mediated migration and adhesion as well as survival of endothelial cells, vascular smooth muscle cells, fibroblasts, and monocytes (107). We have shown that cardiomyocytes secrete CCN1 and that this secreted protein is able to induce migration of smooth muscle cells in vitro (106). Thus, CCN1 could induce a more complete set of angiogenic stimuli than VEGF and may therefore lead to a more mature vessel type. The potential therapeutic effects of

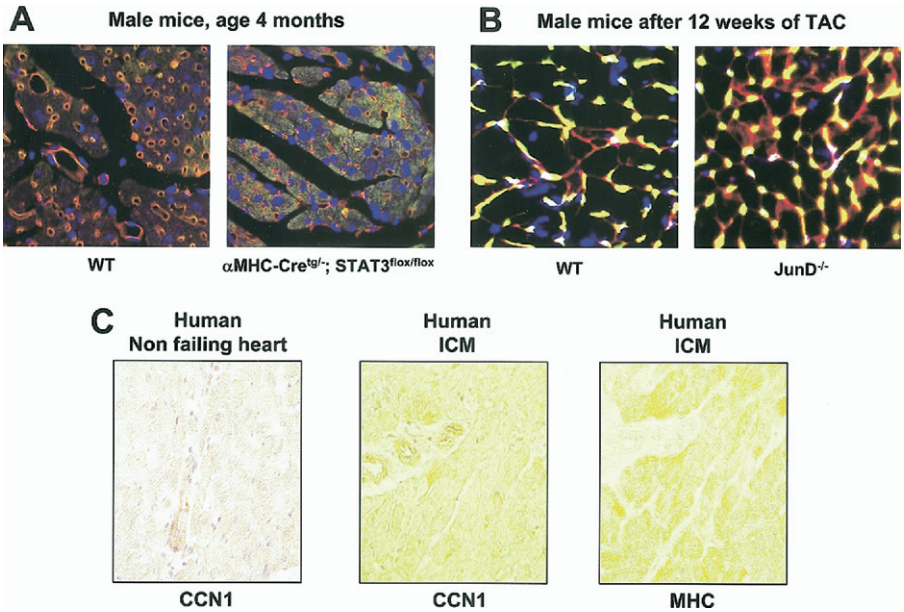

Figure 4. Angiogenic circuits in the heart. **(A)** Immunohistochemical staining for isolectin B4 in in-situ fixed myocardial cross-section shows reduced capillary density in 4-month-old α-MHC-Cre$^{tg/-}$; STAT3$^{flox/flox}$ male mice compared with age-matched wild-type (WT) sibling males (67). **(B)** Chronic pressure overload augments capillary density in JunD knockout male mice (JunD$^{-/-}$) to a higher degree than in WT sibling male mice, as shown by triple staining for isolectin B4 **(yellow)**, cell membrane marker wheat germ agglutinin **(red)**, and nuclear stain Hoechst **(blue)** (101). **(C)** Immunohistochemical staining shows high expression of CCN1 protein in cardiomyocytes and blood vessels of a left ventricular (LV) section from a patient with end-stage heart failure caused by ischemic cardiomyopathy (ICM) compared with low CCN1 staining in a LV section from a nonfailing human heart. The MHC staining of a serial ICM section with an antibody-detecting sarcomeric myosin heavy chain identifies cardiomyocytes as a source of CCN1 (106). Other abbreviations as in Figure 3.

CCN1 on myocardial angiogenesis are currently being explored in our laboratory.

In summary, cardiac neovascularization is not dependent on one single gene or factor, but relies on the regulation of multiple factors by various signaling pathways. Moreover, the cardiomyocyte seems to be an important source for proangiogenic factors. Novel therapeutic strategies applying directly proangiogenic factors or promoting the endogenous secretion of angiogenic factors to enhance neovascularization in injured, overloaded, and failing hearts are warranted.

APOPTOSIS AND HEART FAILURE

Apoptosis is involved in cell loss of cardiomyocytes during evolving heart failure. Apoptotic pathways are widely induced in cardiomyocytes in the context of ischemia/reperfusion injury over a limited time frame (108,109). In contrast to high rates of apoptosis in acute ischemia-reperfusion, heart failure is characterized by very low—but abnormal—levels of cardiomyocyte apoptosis that persist for months to years. In this regard, apoptosis rates of 0.08% to 0.25% in patients with end-stage dilated cardiomyopathy have been described, compared with 0.001% to 0.002% in nonfailing control hearts (110). Although apoptosis rates in failing hearts are significantly increased, their principal functional role for the development of heart failure has remained elusive. However, there is now evidence showing that apoptosis may contribute to the development of heart failure. In this respect, Wenker et al. (111) have addressed this issue in transgenic mice with a heart-restricted expression of a procaspase-8 fusion protein, whose dimerization and activation could be pharmacologically induced. The activation of caspase-8 is a central step in apoptosis initiated by the activation of cell surface death receptors (e.g., Fas/FasL) (110). On activation, caspase-8 subsequently cleaves and activates among others procaspases-3, which in turn activates downstream proapoptotic effector proteins such as Bax and Bad and subsequently the release of cytochrome *c* (cyt *c*) and other apoptogens from mitochondria (reviewed by Foo et al. [110]). Not surprisingly, therefore, activation of the procaspase-8 transgene in mice has led to high mortality within a few hours after pharmacologic stimulation. Surprisingly, however, even without the activating drug procaspase-8, transgenic mice showed a slightly increased ongoing rate of apoptosis (about 10-fold higher than in wild-type siblings), which was associated with a profound dilated cardiomyopathy and a high long-term mortality rate (111). Notably, treatment with a broad-spectrum caspase inhibitor, starting before cardiac decompensation, prevented dilation and attenuated cardiac dysfunction in procaspase-8 transgenic mice (111). The broad-spectrum caspase inhibitor also attenuated heart failure in a second transgenic mouse model with peripartum cardiomyopathy due to overexpressing of a subunit of the heterotrimeric guanine nucleotide-binding (G) proteins (112). Moreover, caspase inhibitors reduced infarct size after myocardial infarction (113). Notably, these effects of caspase inhibition always correlated with inhibition of cardiomyocyte death, suggesting that the major benefit of this treatment results from attenuating apoptosis (111–113).

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Caspases are also known to have additional roles aside from induction of apoptosis; particularly, they cleave myocardial contractile proteins and promote systolic dysfunction (113). Consistent with this property of caspases, treatment with caspase inhibitors after myocardial infarction has been shown to preserve myocardial contractile proteins, reduce systolic dysfunction, and attenuate adverse ventricular remodeling (113). Thus, caspases are not only responsible for cell loss by apoptosis but seem in addition to promote cardiac dysfunction and adverse remodeling.

Beyond the stress-mediated apoptosis, stress-dependent deregulation of survival pathways may affect the balance of normal myocyte apoptosis and self-renewal, leading to a continuous cell loss in the adult heart. In light of the recent identification of resident cardiac stem cells, it is therefore conceivable that a diminished self-renewal and "stemness" of these cells with ageing or in response to stress factors may tip the balance toward a greater loss of contractile cells, resulting in cardiac dysfunction (5). Recent investigations have shown that cardiac hepatocyte growth factor/insulin-like growth factor-1 signaling plays a crucial role for migration, proliferation, and differentiation of residual cardiac stem cells, suggesting that these factors might be able to tip the balance from cardiac cell loss toward cardiac regeneration (114,115).

Taken together, there is increasing evidence showing that sustained loss of cardiac cells by apoptosis, even when occurring in a low-grade manner, can cause cardiac dysfunction and ultimately lead to heart failure. In addition, apoptotic pathways seem to have direct effects on cardiomyocyte contractility and remodeling. Although drug therapies targeting potential upstream signaling mediators of apoptosis such as β-adrenergic receptor blockers and inhibitors of the angiotensin II axis are standard in heart failure therapy, novel approaches such caspase inhibitors and regenerative factors in addition to standard heart failure therapy are likely to emerge as components of novel optimized treatment of heart failure.

Conclusions. The last decade has seen tremendous progress in the understanding of the molecular mechanisms of heart failure. This article highlights some recent advances in the analysis of molecular mechanisms related to cardiac hypertrophy, oxidative stress, inflammation, angiogenesis, and apoptosis. Some of these findings may have important implications for the development of novel treatment strategies in heart failure. In-depth understanding of molecular mechanisms of heart failure will finally provide valuable information in the design of novel treatment strategies that promote protective signaling pathways and prevent maladaptive responses, such as advanced hypertrophy, insufficient vascularization, and apoptosis.

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