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Review

Myocyte hypertrophy and apoptosis: a balancing act

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Abstract

In response to a variety of extrinsic and intrinsic stimuli that impose increased biomechanical stress the heart responds by enlarging the individual myofibers. Even though myocardial hypertrophy can normalize wall tension, it instigates an unfavorable outcome and threatens affected patients with sudden death or progression to overt heart failure, suggesting that in most instances hypertrophy is a maladaptive process. Increasing evidence suggests that several of the signaling cascades controling myocyte growth in the adult heart also function to enhance survival of the myocyte population in response to pleiotropic death stimuli. In this review, we summarize recent insights into hypertrophic signaling pathways and their ability to control the balance between myocyte life and death. As modulation of myocardial growth by antagonizing intracellular signaling pathways is increasingly recognized as a potentially auspicious approach to prevent and treat heart failure, the design of such therapies should respect the dichotomous action of pathways that dictate a balance between myocyte hypertrophy, survival and death.

Keywords: Apoptosis; Hypertrophy; Heart failure; Mitochondria

1. Introduction

Heart failure is a leading cause of mortality worldwide [1]. Patients receive symptomatic treatment, and future biologically targeted therapy will depend on the discovery of new pathways that initiate, promote or potentially reverse the onset of heart muscle failure in response to stress [2]. In response to diverse load conditions (pressure, volume, etc.), heart muscle cells typically hypertrophy or commit suicide in a process commonly referred to as left ventricular remodeling. Due to the suspected maladaptive character of myocyte hypertrophy [3] and limited capacity of self-renewal [4,5], the biological processes leading to myocyte hypertrophy and apoptosis remain in the center of attention for future biologically targeted therapies. Traditionally, these two processes have been approached as reinforcing biological circuits that are not necessarily mutually exclusive.

A classical example of this line of interpretation involves signaling through the alpha subunit of the heterotrimeric guanine nucleotide-binding proteins (G proteins) of the G_q family ($G\alpha_q$), which transduce signals from a variety of widely expressed membrane receptors to generate diverse, tissue-specific effects (Figs. 1 and 2) [6]. In many target tissues, receptor-mediated activation of $G\alpha_q$ regulates diverse physiological responses such as contraction, secretion, growth and death. In cardiomyocytes G-protein-coupled receptor (GPCR) agonists, such as catecholamines, angiotensin II, prostaglandin $F_{2\alpha}$ or endothelin-1, bind to transmembrane GPCRs which leads to activation of cytoplasmic signaling. Overexpression of moderate (~ 4-fold) levels of wildtype $G\alpha_q$ to the cardiomyocyte population induces a stable form of hypertrophy with normal cardiac function in mice, while expression of higher levels (~ 25-fold) was associated with the onset of remarkable chamber dilation, a high incidence of myocyte apoptosis and congestive heart failure in response to stress during peripartum pregnancy in female mice [7] or after pressure overload [8]. Consistent with these observations, targeted overexpression of a constitutively active rather than wildtype form of $G\alpha_q$ in mice induces a pathological form of hypertrophy associated with excessive programmed cell death [9]. In fact, adenoviral expression of constitutively active $G\alpha_q$ in cultured neonatal cardiomyocytes directly leads to loss of the mitochondrial

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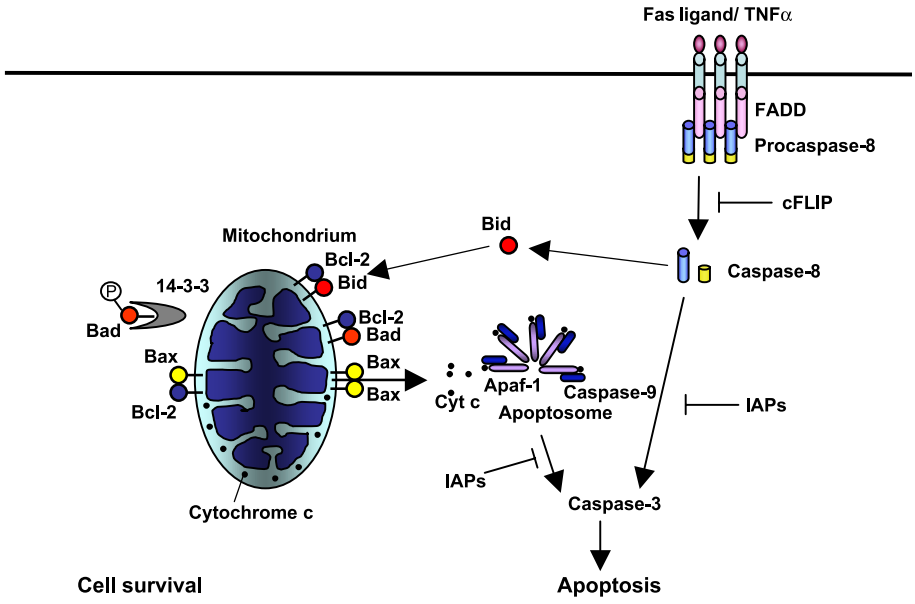

Fig. 1. Two major apoptotic pathways are active in mammalian cells, including the cardiac muscle cell. The mitochondrial death pathway is used extensively in response to extracellular cues and internal insults such as DNA damage. These diverse response pathways converge on mitochondria, often through the activation of a pro-apoptotic member of the Bcl-2 family. Unlike Bcl-2, which seems to spend most, if not all, of its life attached to intracellular membranes, many group II and group III members, including Bax, Bad, Bim and Bid, can shuttle between the cytosol and organelles. Pro-apoptotic signals redirect these proteins to the mitochondria, where the fight for the cell's fate will take place. Activation of pro-apoptotic members can occur through proteolysis, dephosphorylation and de novo gene transcription (e.g. Nix). Pro- and anti-apoptotic Bcl-2 family members meet at the surface of mitochondria, where they compete to regulate cytochrome *c* exit by a mechanism that is still debated. If the pro-apoptotic camp wins, an array of molecules is released from the mitochondrial compartment. Principal among these is cytochrome *c*, which associates with Apaf-1 and then procaspase-9 (and possibly other proteins) to form the apoptosome to eventually activate caspase-3 whose activity is antagonized by the IAP (inhibitor of apoptosis) proteins, which themselves are antagonized by the Smac/DIABLO protein released from mitochondria. Another cell death pathway includes death-receptors, which are triggered by binding of members of the death-receptor superfamily, such as Fas or CD95 and TNF-α, to their cognate receptors, which induces receptor clustering, formation of a death-inducing signalling complex, and finally, caspase-8 and caspase-3 activation. Downstream of caspase-3, the apoptotic programme branches into a multitude of subprogrammes, the sum of which results in the ordered dismantling and removal of the cell.

membrane potential ($\Delta\Psi_m$) and cytoplasmic release of cytochrome *c*, which initiates activation of the apoptosome and onset of proteolytic activity by caspase-3 [10]. This phenomenon appeared to be dependent on the ability of $G\alpha_q$ to activate a pro-apoptotic Bcl-2 family member, designated Nix, which activates the mitochondrial death pathway [11] as this effect could be inhibited by bonkrekic acid, an inhibitor of the mitochondrial permeability transition pore, while conversely, subcutaneuous administration of the poly-casapase inhibitor IDN-1965 rescued peripartum myocyte apoptosis and heart failure in $G\alpha_q$ transgenic mice [12]. Collectively, these studies support a model in which noxious GPCR-coupled hypertrophic cascades instigate myocyte enlargement and, depending on the intensity of the signal, myocyte apoptosis, both of which processes are demonstrably known to have an independent negative impact upon load-induced left ventricular remodeling [3,13]. According to this classical model, myocyte hypertrophy and apoptosis are not interpreted as opposing forces [14–17].

The ongoing identification of additional signaling routes that are intricately involved in myocyte enlargement have provided an alternative view, in which the majority of signaling cascades uncovered to date fulfill a dichotomous role facilitating myocyte hypertrophy and signaling strong cell-survival cues. Accordingly, a more nuanced mechanistic interpretation for left ventricular decompensation may now emerge, in which initial forward signaling through these cascades exerts an early hypertrophic remodeling phase, often characterized with stable cardiac function, and that failure of these hypertrophy/survival pathways to inhibit myocyte apoptosis ultimately signals a critical step towards *decompensatio cordis* due to dramatic loss of contractile units, dilation of the ventricles and, finally, loss of contractile force.

In the next sections, this dualistic model of cellular signaling that simultaneously facilitates hypertrophy and survival of the cardiac myocyte population will be exemplified by the phenotypic particulars of four distinct signaling paradigms, notably (1) signals utilizing the gp130 receptor, (2) the IGF-1–PI3K–Akt route, (3) calcium-dependent signaling through calcineurin and NFAT, and (4) signals activating NF-κB.

2. Gp130 receptor-coupled signaling

Cytokines play a critical role in the control of mammalian physiology in multiple organ systems [18,19]. For

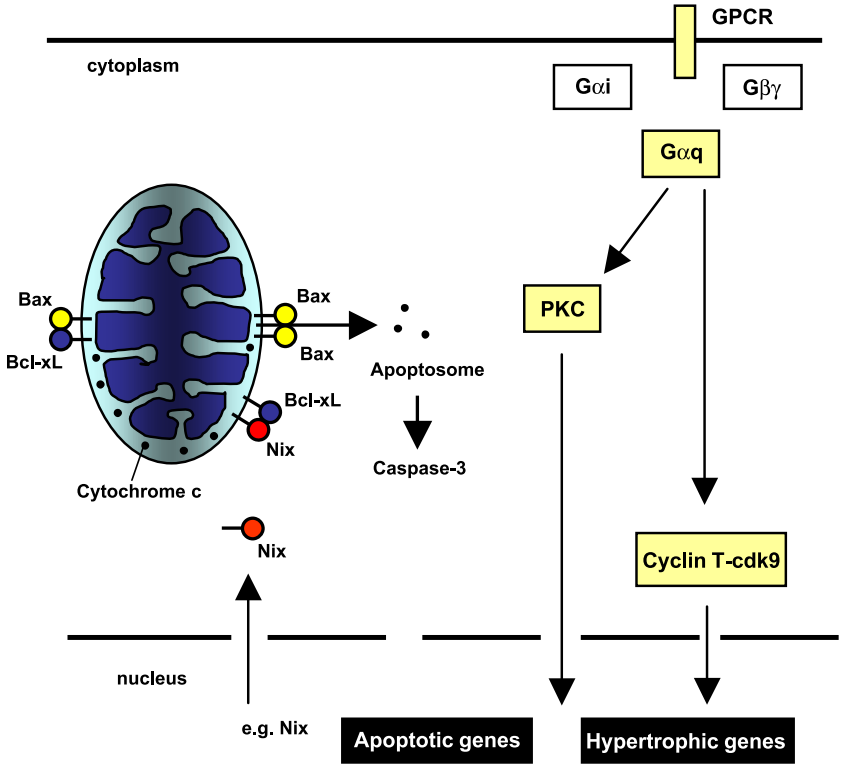

Fig. 2. G-protein-coupled receptor (GPCR) agonists (e.g. catecholamines, angiotensin II, prostaglandin $F_{2\alpha}$ or endothelin-1), bind to transmembrane GPCRs which leads to activation of cytoplasmic signaling through the alpha subunit of the heterotrimeric guanine nucleotide-binding proteins (G proteins) of the G_q family ($G\alpha_q$), which activate protein kinase C (PKC) members and transcriptionally activate a pro-apoptotic Bcl-2 member called Nix. In addition, $G\alpha_q$ is a potent hypertrophic signal transducer, and enhances (as calcineurin, see Fig. 5) transcript initiation via a cyclin T-cdk9 complex that phosphorylates RNA polymerase II.

example, mice that harbor a complete deficiency in individual members of the interleukin 6 (IL-6) family of cytokines (IL-6, leukemia inhibitory factor [LIF], ciliary neurotrophic factor [CNTF], or IL-11 receptor α) or their downstream gp130-dependent signaling components can display multiple organ defects, including disorders of the immune system, hepatic function, bone metabolism, neurological function, and hematopoiesis [20–26]. gp130 has been identified as co-receptor for the IL-6 family of cytokines. Ligand binding of cytokines to their cognate receptors induces heterodimerization with gp130, leading to activation of Janus kinases (JAKs), which in turn phosphorylate downstream substrates such as the transcription factor signal transducer and activator of transcription (STAT), most notably STAT3. Activated (phosphorylated) STAT3 translocates into the nucleus and directly activates genes involved in hypertrophy (*c-fos*, *ANP*), cell survival (*BCL-xL*, *MnSOD*) and angiogenesis (*VEGF*) (Fig. 3).

Cardiotrophin-1 (CT-1), a gp130 receptor-dependent cytokine, was isolated from a mouse embryonic stem-cell model of cardiogenesis. Within minutes of aortic constriction, gp130 ligands such as CT-1 and leukemia inhibitory factor (LIF) bind to their cognate receptors and induce heterodimerization with gp130. STAT expression is upregulated in human hearts with dilated cardiomyopathy [27], while LIF and CT-1 are both known for their ability to induce hypertrophy. For example, LIF expression is upregulated in the failing canine heart [28], CT-1/gp130/JAK activity is increased in cardiomyocytes in response to hypertrophic stimuli such as stretching or pressure overload [29,30], while continuous activation of the gp130 pathway causes cardiac hypertrophy in mice [31]. These findings, however, do not necessarily convey any proof whether JAK–STAT signaling is also facilitating cardiac dysfunction nor whether it possesses an additional protective role against the onset of cardiac failure.

Evidence for a cytoprotective role for gp130 signaling was first provided by the conditional targeting of the gp130 receptor using Cre-*lox*P technology, as conventional targeting of the receptor results in embryonic lethality at E6.5 due to defects in diverse embryonic compartments [32]. Mice deficient in cardiac gp130 demonstrated normal cardiac function and whole body function under baseline conditions. In response to a mild pressure stimulus, wildtype mice developed concentric hypertrophy without deleterious functional, histological or clinical signs. In contrast, mice lacking gp130 in the myocyte population displayed a rapid onset of dilated cardiomyopathy accompanied with an increased myocyte apoptotic index, lethal ventricular arrhythmias and concomitant shortened survival in response to the same

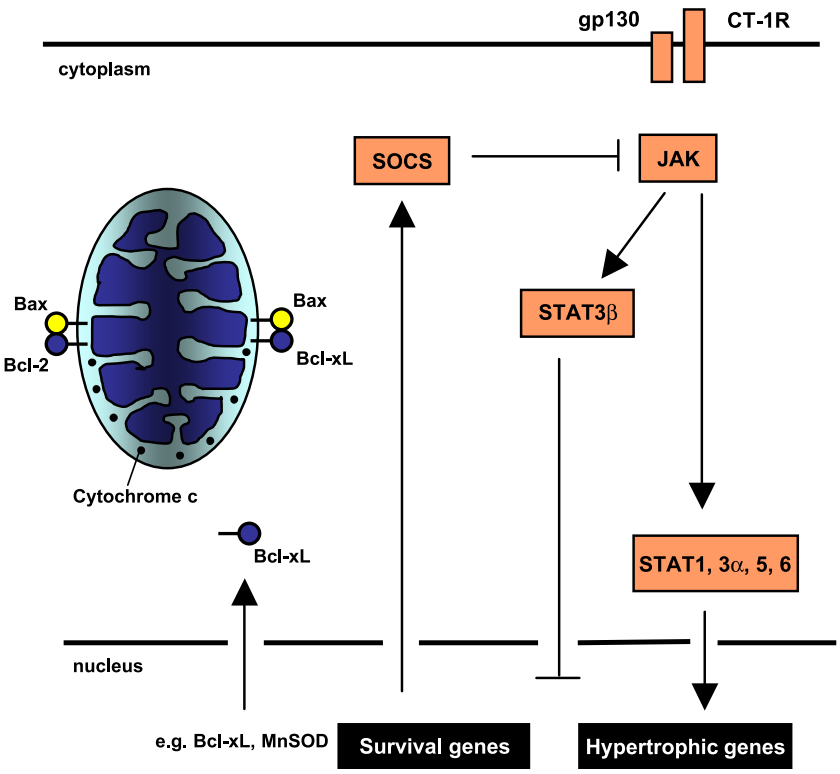

Fig. 3. gp130 transduces pro-hypertrophic and survival signals from cytokines that activate JAK, which in turn phosphorylate (activate) STAT members, which stimulate an endogenous inhibitory loop consisting of SOCS and a repressive member of the STAT family (STAT3β).

pressure stimulus [32]. More evidence to support a cyto-protective function for JAK/STAT signaling consists in the fact that selective activation of STAT3α causes a mild form of hypertrophy with marked resistance against doxorubicin-induced cardiomyopathy, which could be the result of STAT3α's potential to directly activate protective genes [33]. A recent landmark study by Jacoby et al. [34] reports on mice with cardiomyocyte-restricted deletion of the *stat3* gene, which spontaneously develop heart dysfunction, accompanied by exaggerated fibrosis and myocyte dropout upon a lipopolysaccheride challenge or advancing age, suggesting that signaling through gp130 not only facilitates myocyte hypertrophy but can also protect the heart from stress-induced injury.

Interestingly, a distinct STAT3 isoform, STAT3β, was reported to be vastly activated in tissue biopts of human heart failure [35]. The α and β STAT3 isoforms are splice-forms of one single STAT3 gene, where STAT3β can bind to STAT consensus binding sites, but, unlike the α counterpart, fails to activate transcription, hence STAT3β embodies a potential dominant negative function towards other STAT members [35]. It is tempting to speculate that during the progression of hypertrophy-heart failure, STAT3β gradually becomes abundantly present and provides an endogenous negative feedback loop to this forward signaling cascade which requires transcriptionally competent STAT3α to exert myocyte survival. To further underscore this premise, it remains to be established whether forced expression of STAT3β can indeed antagonize the pro-hypertrophic and pro-survival effects of gp130 signaling in vivo and what transcriptional cues underly regulation of *stat3* gene splicing.

The suppressor of cytokine signaling 3 (SOCS3) has been studied in more detail to act as an intrinsic inhibitor of JAK [36]. SOCS shows biphasic induction in response to TAC within 1 h of aortic banding and peaks after a couple of hours, and is closely correlated with STAT3α phosphorylation, as well as the activation of an embryonic gene program, suggesting that cardiac gp130–JAK signaling is precisely controlled by this endogenous suppressor. Adenovirus-mediated gene transfer of SOCS3 to ventricular cardiomyocytes completely suppressed both the pro-hypertrophy and pro-survival phenotypes induced by LIF and CT-1. Collectively, the delicate balance between the forward activation of gp130–JAK–STAT signaling and the induction of its negative feedback regulator SOCS3 obviously play a delicate balance in the control of the transition between cardiac hypertrophy and failure, via attenuation of myocyte survival signals.

3. The PI3K–Akt axis

A wealth of information implicates phosphoinositide 3-kinase (PI3K)–Akt signaling in such seemingly disparate biological responses as regulating body/organ size, growth

and apoptosis [37]. Insulin-like growth factor-1 (IGF-1) and their downstream effectors, such as insulin receptor substrate (IRS-1) and p70 S6 kinase ($P70^{S6K}$), play an important role in body size determination in mammals [38–42]. PI3K lies downstream of many receptor tyrosine kinases including insulin and IGF-1 receptors and have emerged as major players in pleiotropic biological responses as membrane trafficking, cytoskeletal organization, cell growth and apoptosis [43,44]. PI3Ks phosphorylate the 3-position of the inositol ring of phosphatidylinositol (PtdIns), PtdIns 4-phosphate and PtdIns 3,4-diphosphate to form, among others, PtdIns 3,4,5-triphosphate. A serine/threonine kinase Akt, also known as protein kinase B, is the most well-characterized target of PI3K [45,46]. Akt is known to mediate cell survival by regulating several effectors, including Bad or procaspase-9 [47,48]. Another substrate for PI3K/Akt is $P70^{S6K}$, which is known to be a physiological kinase for the ribosomal S6 protein whose phosphorylation increase the rate of initiation and translation of mRNA by ribosomes [49,50].

Overexpression of IGF-1 suffices to induce cardiac hypertrophy, a phenotype that gradually leads to reduced cardiac performance [51]. Moreover, IGF-1 deficiency in humans is associated with cardiac atrophy and reduced function [51–53]. Transgenic constructs harboring either a constitutively active or dominant negative mutant of PI3K in the heart resulted in mice with larger or smaller hearts, respectively, convincingly demonstrating that PI3K activation is both necessary and sufficient to control organ growth [54]. Similarly, targeted overexpression of a constitutively active Akt mutant, the direct downstream target of PI3K, to the heart muscle in mice produced a highly similar hypertrophic phenotype, which was characterized by increased contractility and resistance towards noxious signals [55].

The anti-apoptotic capacity of IGF-1 has been described in detail, and, most notably, activation of PI3K appears to form a important component downstream of this pathway [56–58]. Adenoviral overexpression of a constitutively active form of PI3K in neonatal cardiomyocytes, which concomitantly activates Akt, inhibits cardiac apoptosis upon a doxorubicin challenge, suggesting that Akt forms a crucial link between PI3K signaling and inhibition of caspase-3 activation [59]. A mechanistic explanation for the anti-apoptotic effects of PI3K–Akt rely in the potential of latter serine/threonine kinase to directly phosphorylate Bad and caspase-9, which suppresses their pro-apoptotic function, and were shown to account, at least in part, for the potent survival effects of Akt in the heart [47,48,60]. Bad is a pro-apoptotic protein of the Bcl-2 family. The Bcl-2 family proteins function primarily to protect the integrity of the mitochondrial membrane and control the release of pro-apoptotic proteins like cytochrome *c* [61]. Bcl-2 proteins

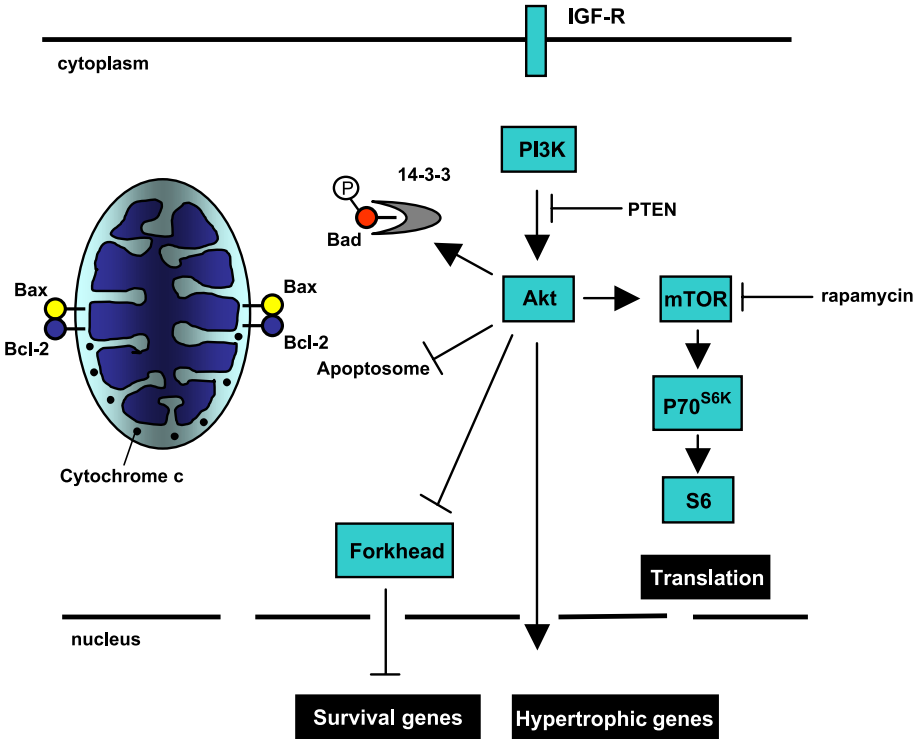

Fig. 4. Signaling through PI3K and Akt have pro-hypertrophic and pro-survival effects on myocytes. Pro-hypertrophic effects are partly due to the ability to stimulate mTOR-$P70^{S6K}$ activation, which stimulates ribosomal translational efficiency. Akt is a potent anti-apoptotic effector that is able to (1) phosphorylate the pro-apoptotic Bcl-2 member Bad, which results in its inhibitory association with 14-3-3 proteins, (2) inactivate caspase-9 (part of the apoptosome), and (3) stimulate a subset of survival genes through activation of the forkhead transcription factor family.

form heterodimers and the balance between the pro-apoptotic Bcl-proteins (such as Bad, Bax and Bak) and anti-apoptotic Bcl-proteins (such as Bcl-2, Bcl-xL) is one of the mechanisms that determine the permeability of the mitochondrial membrane (Figs. 1 and 4). However, whether Akt influences Bad phosphorylation in cardiomyocytes remains debated. Negoro et al. [62] examined the effect of LIF on Bad phosphorylation in cultured cardiac myocytes and demonstrated that LIF induces Bad phosphorylation in a PI3K dependent manner. In contrast, Wu et al. [59] revealed that neither IGF-1 nor constitutively active PI3K leads to phosphorylation of Bad in cardiac myocytes, suggesting that the pro-survival functions of PI3K in the heart are not uniquely restrained to Bad phosphorylation, but may encompass several additional targets.

Another potential survival effect of Akt signaling encompasses its ability to phosphorylate members of the Forkhead transcription factor family, upon which Forkhead is retained in the cytoplasm and unable to activate pro-apoptotic genes [63]. Whether this mechanism is functional in cardiomyocytes remains to be explored, although forkhead exists in this cell type and is readily phosphorylated by Akt [64]. Nevertheless, the combined data clearly support a model in which signaling through PI3K–Akt–$p70^{S6K}$ on the one hand is intimately involved in producing the hypertrophy response of the heart muscle cell, but also plays a crucial role in cellular survival of this particular cell type (Fig. 4).

4. Calcium-dependent signaling

One pathway that has received considerable attention with regard to myocyte hypertrophy encompasses by the calcium/calmodulin-activated protein phosphatase, calcineurin (PP2B). Calcineurin is activated by sustained elevations in intracellular calcium, which facilitates binding to its primary downstream transcriptional effector, nuclear factor of activated T cells (NFAT) [65]. NFAT transcription factors are normally hyperphosphorylated and sequestered in the cytoplasm, but rapidly translocate to the nucleus after calcineurin-mediated dephosphorylation [65]. Cardiac-specific activation of calcineurin or its downstream effector NFAT suffices to induce a robust hypertrophic response in transgenic mice [66], while genetic inhibition strategies of calcineurin or NFAT have convincingly shown the pathway to be necessary for a full hypertrophy response in a number of rodent models [67]. Interestingly, recent evidence even supports the notion that calcineurin may be uniquely activated in pathological forms of hypertrophy, and not during more physiological hypertrophic

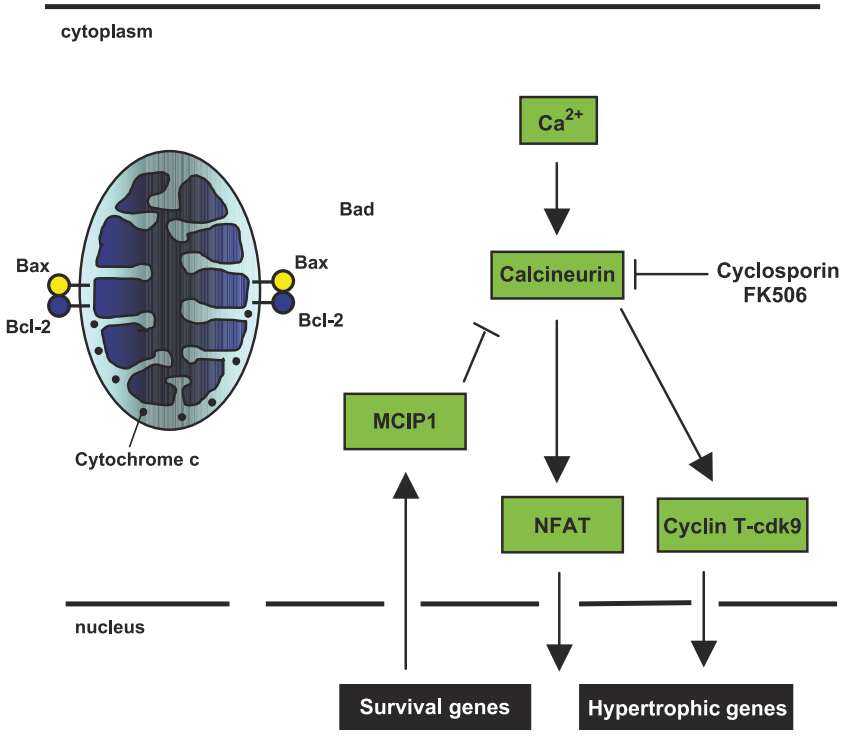

Fig. 5. Calcium signalling through calcineurin and NFAT activates a potent hypertrophic phenotype by direct transcriptional activation of a hypertrophic gene program and stimulation of a cyclin T-cdk9 complex to enhance transcript initiation. NFAT further activates a transcriptional program, the identity of which remains obscure, that enhances the survival of myocytes. A negative feedback loop is provided by the NFAT-responsive gene modulatory calcineurin interacting protein-1 (MCIP1).

phenotypes, associated with endurance training of IGF-GH infusion [68].

The role of calcineurin as an effector of cell death is more controversial. Studies conducted in neurons, lymphocytes, and tumor cell lines have shown both pro- or anti-apoptotic effects of calcineurin activation [69–73]. The exact decision of cytoprotection versus apoptosis is likely regulated by the activation status of other co-stimulated signaling pathways or depends on cell-type specific calcineurin effector/docking proteins. Indeed, calcineurin activation was shown to either induce apoptosis or to antagonize apoptosis depending on the status of p38 activation [74]. More recently, calcineurin was shown to localize to the mitochondria in fibroblasts through docking with the inhibitory protein FKBP38, resulting in Bcl-2 and Bcl-xL redistribution [75]. Calcineurin has also been implicated as a direct inducer of apoptosis in hippocampal neurons through dephosphorylating the pro-apoptotic factor Bad [69], but this effect appears to be non-functional or absent in heart muscle cells [76].

Recently, it was demonstrated that genetic disruption of the *CnAβ* gene in the mouse enhanced cardiac damage induced by ischemia–reperfusion injury [77]. Consistent with this notion, transgenic mice expressing a constitutively active mutant of calcineurin in the heart are significantly protected from ischemia–reperfusion-induced cell death [76]. In cultured cardiomyocytes, adenoviral-mediated gene transfer of activated calcineurin reduced oxidative stress induced cell death, whereas calcineurin antagonism increased TUNEL positivity of myocyte nuclei [76]. These findings indicates that calcineurin signaling imparts a degree of protection against cell death in the heart. By comparison, Kakita et al. [78]c recently identified an anti-apoptotic role for calcineurin activation in cardiomyocytes after endothelin-1 stimulation. Specifically, endothelin-1 stimulation protected cardiac myocytes in culture from H_2O_2-induced TUNEL reactivity, DNA laddering, caspase-3 cleavage, and loss of mitochondrial membrane potential.

The mechanistic explanation of these unexpected phenotypic particulars of calcineurin activation in the heart may be dominated by the transcriptional activation of survival genes by NFAT. This interpretation is consistent with the known role of NFAT transcription factors as important, if not sole, effectors of calcineurin-regulated gene expression in most cell types [65]. Indeed, the full potency of calcineurin-induced hypertrophy in the heart was shown to require *NFATc3* using gene-targeted mice [79], while overexpression of activated *NFATc4* in cultured neonatal cardiomyocytes partially antagonized 2-deoxyglucose-induced apoptosis [76]. Additionally, endothelin-1-mediated protection from H_2O_2-induced apoptosis promoted NFAT dephosphorylation [78], and Pu et al. [80] demonstrated that NFAT inhibition augmented cardiac myocyte apoptosis after phenylephrine stimulation in culture. Collectively, these results suggest that the relative transcriptional activition status of NFAT factors induces a specific gene expression profile that affords cardiac "health" and resistance to apoptotic stimuli even in the setting of clear pathological hypertrophy. However, the exact array of downstream effectors that are regulated by NFAT factors in providing cardioprotection remains to be determined (Fig. 5).

5. TNFα–NF-κB

Cytokines are intimately involved in inflammatory processes such as wound healing after infarction [81]. Consistent with this view, myocardial accumulation of tumor necrosis factor alpha (TNFα), IL1β and IL-6 has been observed following a myocardial infarction [82–84]. Previously, the cardiac source of cytokines was attributed to infiltrating macrophages or leucocytes [85,86], but now it is accepted that these cytokines are also expressed in cardiac myocytes following ischemic stress or hypertrophy suggesting myocyte-autonomous effects for this class of cytokines [87–89]. Indeed, TNFα can induce both myocytes hypertrophy and apoptosis in culture, while transgenice mice engineered to overexpress a secreted form of TNFα develop concentric hypertrophy that transitions to a dilated cardiomyopathy over time [90,91]. From various studies in non-cardiac cell types, it is known that binding of TNFα to its cognate receptor (TNFR) directly provokes caspase and NFκB activation, with each phenomenon having opposite phenotypic effects on cell survival [92].

TNFα signaling involves the binding of the TNF trimer to the extracellular domain of TNF-receptor-1 (TNFR1 or TNFR55), recruitment of several intracellular adaptor proteins and, finally, caspase-8, whose prodomains can bind to adaptor proteins interacting with these receptors [93] (see Fig. 1). Caspase-8 subsequently becomes activated, presumably by self-cleavage, and initiates a protease cascade that leads to apoptosis [94–96]. Transgenic mice (TG) with cardiomyocyte-restricted overexpression of TNFα develop myocardial inflammation, pronounced myocyte hypertrophy, and multiple signs of heart failure [90,91, 97]. These mice display activation of pro-apoptotic pathways in cardiac myocytes, as evidenced by the upregulated expression of several death-domain-related proteins, including TNFR1, Fas, FADD, TRADD, and RIP, and caspase-8 [98].

The other major arm of TNFα signaling involves recruitment of the multiprotein IκB kinase (IKK) complex to the TNFR1 in a TNFα-dependent fashion that mediates phosphorylation and degradation of inhibitor of κB (IκB) proteins, which normally retain NF-κB within the cytoplasm of unstimulated cells [99,100]. In most resting cells, NF-κB is bound to its cytoplasmic inhibitory proteins, IκB (α, β, and γ), and remains in the cytoplasm as a latent form transcription factor [101]. Upon stimulation, the IκB kinase (IKK) complex [102–108], which is composed of two catalytic subunits IKKα and IKKβ and a regulatory subunit IKKγ [108–110], is activated and it in turn

phosphorylates IκB proteins on specific Ser residues (Ser-32 and -36 on IκBα and Ser-19 and -23 on IκBβ) [111–113]. The phosphorylation triggers ubiquitination-dependent degradation of IκB proteins by the 26S proteosome, resulting in the release of NF-κB [114–116]. Subsequently, NF-κB translocates into the nucleus, where it stimulates transcription of specific target genes [114].

NF-κB plays a role in regulating cell growth. Genetic disruption of members of the NF-κB family, such as p65, p50, or c-Rel, impairs proliferation of lymphocytes [117–119]. Furthermore, NF-κB can be activated by oncogenic Ras and Raf and is involved in Ras-induced transformation of NIH 3T3 or liver epithelial cells [120,121]. Recent studies also show that NF-κB regulates expression of cyclin D1 and its activation is required for the G1-S transition [122,123]. The role of NF-κB in hypertrophic growth of terminally differentiated cells has remained, until very recently, uncertain. Purcell et al. [126] demonstrated that viral mediated transfer of a "superrepressor" IκBα protein, a dominant negative NF-κB approach, prevented several features of cardiomyocyte hypertrophy in response to GPCR agonists as phenylephrine, endothelin-1 and angiotensin II. Later, Gupta et al. [124] and Hirota et al. [125] indicated a crucial role for NF-κB activation in myotrophin-induced and GPCR-related cardiac hypertrophy in vivo in their respective mouse models. Taken together, sufficient evidence now exists in support of the contention that NF-κB plays a necessary role for myocyte hypertrophy in vitro and in vivo, at least downstream of GPCR agonist stimulation.

NF-κB has a more ambivalent character in cellular survival as it is involved in the direct regulation of both pro- and anti-apoptotic genes, including anti-apoptotic factors as cIAPs, Bcl-2 family (Bcl xL) and FLICE inhibitory protein (FLIP), and pro-apoptotic factors such as Fas, FasL, caspase-8, caspase-11 and TNF-α [127]. In fact, in vivo transfection of an NF-κB decoy nucleotide into rat hearts reduced NF-κB activity and resulted in a reduction in ischemia reperfusion damage, which would suggest a pro-apoptotic function for NF-κB [128,129]. Nevertheless, transgenic mice harboring cardiac specific expression of the suppressor IκBα mutant protein which negates nuclear relocalization of NF-κB, displayed a significant increase in myocyte apoptosis following an acute ischemia/reperfusion insult, and an enlarged infarct size after myocardial infarction compared to their wildtype counterparts [130]. These findings correlated with decreased expression levels of c-IAP1 and Bcl-2, suggesting that NF-κB in the heart has cytoprotective effects and that these pro-survival signals are mediated, at least in part, by its transcriptional activity. Additional evidence for a pro-survival function of NF-κB stem from inhibition studies which predisposes cultured cardiomyocytes to apoptosis after TNF-α treatment or hypoxia/reoxygenation stimulation [131,132]. Combined, activation of the transcriptional activator NF-kB appears to play a key role in myocyte hypertrophy, while at the same time directing a transcriptional gene expression profile that provides resistance, at least in part, against noxious cell damaging insults.

6. Perspective

Growth of the heart during embryogenesis occurs primarily through proliferation of cardiac myocytes. Soon after birth, cardiac myocytes withdraw largely from the cell cycle and subsequent growth of the heart occurs predominantly through hypertrophy rather than myocyte hyperplasia. Stress signals activate hypertrophic growth at multiple molecular levels: transcription initiation, transcript elongation, and protein translation [129,133,134]. An intricate web of interconnected signaling modules has been implicated in hypertrophy of postnatal cardiomyocytes (for reviews, see Refs. [135,136]). These signaling pathways culminate in the nucleus with the posttranslational activation of a set of transcription factors, all of which had prior roles in embryonic heart development. When activated in the adult myocardium, however, these factors reactivate a "fetal" cardiac gene program. Although elements of this program might be salutary adaptations to stress initially, increasing evidence suggests that the aberrant expression of fetal proteins involved in contractility, calcium handling, and myocardial energetics leads to maladaptive changes in cardiac function [137,138]. Accordingly, myocyte enlargement solely serving to relieve the myocardium from excessive wall stress obedient to LaPlace's laws plays an subordinate role in the organ's response to load [139,140]. The emerging notion that hypertrophic signaling routes also provide strong survival aspects to the cardiac myocyte poses yet another level of complexity to our desire to design rationale drug therapies based upon inhibition of these stress pathways.

Therapies based upon pure inhibition of hypertrophy (signaling) as a mechanism to target its maladaptive particulars [3,139,140] may become complicated by the fact that some signaling paradigms seem to have retained an evolutionary conserved role in enhancing the survival characteristics of the individual cardiac muscle cell. Accordingly, one focus of future research should be to uncover whether the myocyte, actively suppressed in its ability to hypertrophy, now has become more, equal or less sensitive to cell death stimuli. This will prove to be even more important in the setting of the diseased, aged myocardium when ongoing cellular stress prevail in the form of volume or pressure load, intrinsic cues due to mutant sarcomeric or cytoskeletal proteins, or the existence of subregional situations of hypoxic stress causing cardiac stunning or hibernation.

The opposite alternative, leaving hypertrophic signals untargeted as a measure to enhance the chances of cellular survival, may result to be even more deleterious, exemplified by the grim survival statistics of heart failure patients. For example, calcineurin activation per se, intimately in-

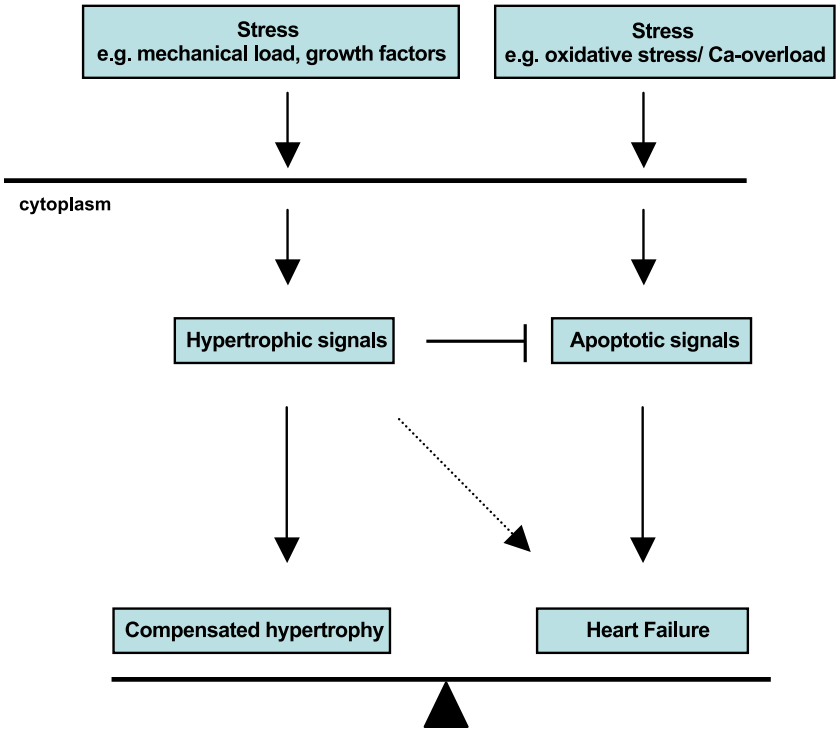

Fig. 6. Hypertrophy and apoptosis are signalling cues both involved in the etiology of heart failure, and traditionally perceived as separate entities. However the hypertrophic and apoptotic pathways entail multiple parallel and converging signals. Hypertrophic signalling influences myocyte apoptosis at several levels, the resultant of which is cell survival promotion. The phenotypic outcome, either maintenance of compensated hypertrophy or, alternatively, rapid transition into heart failure, may very well depend on the balance between the hypertrophic (survival) and apoptotic signals.

volved in pathological forms of hypertrophy, suffices to propel the heart towards severe dilation, loss of contractile force and lethal arrhythmias, even though its activation equips the cell with a more "healthy" genetic environment in terms of cellular survival. And even prosurvival pathways that drive a more benign form of hypertrophy (e.g. due to IGF stimulation) have the propensity to decompensation in the ageing animal Fig. 6.

A third approach could involve a combinatorial approach aimed at suppressing adverse signalling cues and, perhaps, enhancing the salutary ones, while providing "cellular survival support therapy" in the form of pharmacological caspase inhibitors [12]. This ultimate goal would be contingent upon ongoing research into the molecular dissection of the intracellular circuits that couple stress signals to developmental transcription factors and specific gene expression profiles in the normal vs. hypertrophied vs. failing heart that must reveal additional nodal points that could function as targets for drug discovery. Also, the systemic safety of caspase inhibitors or other anti-apoptotic devices should be evaluated in rodent and large animal models in terms of long-term safety, especially with relation to tumorigenesis and metastasis of extra-cardiac organs. Thus, a detailed mechanistic understanding of how the heart responds during disease promises to yield unanticipated therapeutic targets and novel strategies as long as the delicate balance between cellular life and death is respected.

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