Adaptive and maladaptive hypertrophic pathways: points of convergence and divergence

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Abstract

Myocardial hypertrophy is a response of cardiac muscle to altered conditions of haemodynamic overload caused by a large number of physiological and pathological conditions. Traditionally, it has been considered a beneficial mechanism. However, sustained hypertrophy has been associated with a significant increase in the risk of cardiovascular disease and mortality. Actually, many researchers are trying to understand whether left ventricular hypertrophy is a ‘good’ mechanism to stimulate or a ‘bad’ process to prevent. In this review we investigate the most common biochemical signaling pathways involved in the hypertrophic response to identify the precise role, either ‘adaptive’ or ‘maladaptive’, of each molecular pathway. Delinealing intracellular signaling pathways involved in the different aspects of cardiac hypertrophy will permit future improvements in the signaling that controls beneficial growth.

Keywords: Left ventricular hypertrophy; Signaling; Transgenic mice; Pressure overload

1. Introduction

A large number of physiological and pathological conditions are able to generate a cardiac hypertrophic response [1]. The heart modifies its shape as well as its volume in response to a need for altered force production [2], but, since the heart is a terminally differentiated organ, its adaptations to increased workload are accomplished mainly by increasing muscle mass through hypertrophic remodeling [3]. Recently, it has been proposed that also a small subpopulation of cycling cardiomyocytes coming from the differentiation of cardiac stem-like cells could marginally participate in the cardiac adaptation of the overloaded heart [4]. However, it is widely accepted that cardiac hypertrophy rather than regeneration is responsible for the large part of adaptation to increased demands for cardiac work. In particular, the cardiomyocytes expand in size and increase their protein synthesis. Indeed, although cardiac hypertrophy represents the central phenotype through which the cardiac compensation to a large variety of mechanical, hemodynamic, hormonal, and pathological stimuli is realized, it seems that in this cardiac adaptive response lie the first steps of the subsequent deterioration and failure of the overloaded heart [5]. Thus, the concept that hypertrophy simply compensates for increased wall stress seems outdated.

Cardiac hypertrophy has, at the beginning, beneficial effects in terms of muscular economy, normalizing wall stress [6]. However, several epidemiological studies have demonstrated that chronic hypertrophy is associated with a significant increase in the risk of heart failure, dilated cardiomyopathy, ischemic heart disease, and sudden death, leading to increased cardiovascular mortality [7–11]. Moreover, other studies have demonstrated that the reduction of cardiac mass represents a favorable prognostic marker independent of the treatment used [12,13]. However, it is still unclear whether the improvement in prognosis is a direct consequence of cardiac mass reduction or depends on other favorable intrinsic effects exerted by the various treatments that act concomitantly with mass reduction. This clinical evidence suggests that the hypertrophic process
might not be entirely beneficial. Indeed, several research laboratories are trying to understand whether left ventricular hypertrophy is a ‘good’ mechanism to stimulate or a ‘bad’ process to prevent [1,5,14,15].

Muscle growth and adaptation is a complex, integrative process. In the cell, several regulatory steps can be activated in response to growth signals. A great number of intracellular signaling pathways involved in the hypertrophic response have been characterized in the last decade [1,5,14,15]. All of these molecules do not operate in isolation, but participate in a more orchestrated response that generates interdependent and cross-talking networks. This explains the fact that the mechanisms involved in cardiac hypertrophy are still not completely clear. The generation of transgenic mice over-expressing or lacking specific genes and the possibility of carrying out sophisticated measures of cardiac function in these mice are helping in understanding the precise role of each signaling molecule involved in cardiac hypertrophy. The results of this analysis have led to the concept that the hypertrophy response can be maladaptive or compensatory, depending on the specific signaling pathways involved. This hypothesis could have significant implications for drug development to control the hypertrophic response and direct its to more favorable outcomes. In this review, we will examine different pathways known to be involved in cardiac hypertrophy and their role in maladaptive or adaptive remodeling.

2. Maladaptive mechanisms

Calcineurin has been implicated as a crucial regulator of the hypertrophic response to different stimuli [16,17]. In particular, most studies ascribe this factor to a ‘detrimental’ hypertrophic pathway. Calcineurin is a calcium/calmodulin-regulated, serine/threonine phosphatase that dephosphorylates members of the NFAT transcription factor family, causing their nuclear translocation and the activation of immune response genes such as interleukin-2 [18]. It was initially discovered in T-cells and then found in cardiomyocytes in higher concentrations. Molkentin et al. have demonstrated the role of calcineurin in cardiac hypertrophy, generating transgenic mice that overexpressed activated forms of calcineurin or NF-AT3 in the heart [19]. These mice developed cardiac hypertrophy and heart failure within 2 months, suggesting the involvement of calcineurin in a ‘maladaptive’ type of hypertrophy because of the accelerated transition toward heart failure. Moreover, elevated levels of calcineurin A have been found in failed human hearts [20]. Further studies have confirmed this evidence. In particular, the overexpression of MCIP1, an endogenous inhibitor of calcineurin, was able to blunt cardiac hypertrophy, the progression to dilated cardiomyopathy, and the reduction of ejection fraction that otherwise occurred in mice over-expressing a constitutively active form of calcineurin [21]. MCIP1 overexpression was able to blunt and preserve systolic function in mice subjected to chronic pressure overload by thoracic aortic banding [22]. Similarly, treatment of hypertensive rats with FK506, another inhibitor of calcineurin, attenuates left ventricular hypertrophy and prevents heart failure [23]. On the other hand, the studies using cyclosporine A, an inhibitor of calcineurin, show conflicting results. In particular, mice treated with cyclosporine displayed a blunted left ventricular hypertrophy in response to pressure overload accompanied by a reduced ejection fraction and a higher susceptibility to decompensation and heart failure [24]. Moreover, rats treated with cyclosporine A showed a reduced cardiac hypertrophy with enhanced contractile dysfunction in response to myocardial infarction, suggesting that calcineurin signaling could be adaptive under these experimental conditions [25]. However, this pharmacological approach has several limitations because of the difficulties of in vivo studies with cyclosporine A. In fact, at high doses, cyclosporine A not only inhibits calcineurin, but also has toxic consequences for other cells and nonspecific effects on other regulatory factors that participate in the hypertrophic response, thus confounding the interpretation of results. On the other hand, it is unclear whether calcineurin plays a role in the so-called ‘physiological’ hypertrophy that is induced by exercise and causes reversible cardiac hypertrophy that does not progress to decompensation [1,26].

Some studies suggest its involvement in this physiological hypertrophy, thus contradicting its role in maladaptive left ventricular hypertrophy. Actually, Eto et al. have found an activation of calcineurin in exercised rats [27], and, more interestingly, Rothermel et al. have observed in transgenic mice overexpressing MCIP1 attenuation of an exercise-induced increase in cardiac mass [21]. More recently, Wilkins et al. have shown that physiological hypertrophy produced in models of exercise training failed to recruit significant calcineurin-NFAT coupling, thus suggesting a more specialized role of calcineurin in the hypertrophic response to pathological stimuli [28]. Other studies need to clarify the precise role of calcineurin in ‘adaptive’ or ‘maladaptive’ hypertrophic growth in myocardium.

The role of G-protein signaling in cardiac hypertrophy has been extensively investigated. Three GTP-binding proteins that transduce different growth stimuli, Gq, Gs, and Gi, are mainly involved. Gq is the target of angiotensin II, endothelin-1, and α-adrenergic stimulation that have been demonstrated to determine cardiac hypertrophy [29–31]. Several studies in transgenic mice overexpressing either Gq-coupled receptors or the Gq protein have emphasized the role of Gq signaling in cardiac hypertrophy leading to heart failure [32–34]. In particular, myocardial overexpression of a constitutively active α1B adrenergic receptor induced cardiac hypertrophy [35]. In agreement, mice lacking this receptor failed to develop cardiac hypertrophy in response to chronic infusion of adrenergic agonists but still displayed a hypertrophic response to pressure overload [36]. Interestingly, transgenic mice with cardiac-directed overexpression of the wild-type α1B adrenergic receptor showed a dilated
cardiomyopathy, thus suggesting that chronic α1AR activity could be deleterious for cardiac function [37]. Accordingly, Gq-overexpressing transgenic mice showed a typical, eccentric, hypertrophic remodeling coupled to left-ventricular contractile dysfunction in response to aortic banding [38], further suggesting a role of Gq signaling in ‘maladaptive’ hypertrophy. On the other hand, the inhibition of Gq-mediated signaling, or the lack of dopamine β-hydroxylase, the key enzyme for the synthesis of norepinephrine, the main sympathetic neurotransmitter, showed a reduced hypertrophic response to chronic pressure overload associated with a preserved cardiac contractility, unlike wild-type mice that developed a significant increase in chamber dimensions and progressive deterioration in cardiac function [39]. Thus, cardiac hypertrophy must not be considered any longer an adaptive response only. There are some signaling events that, like Gq signaling, can mediate negative aspects of the hypertrophic process and must be inhibited to prevent the transition toward heart failure. To explain the maladaptive role of Gq signaling in cardiac hypertrophy and the ensuing progression to heart failure, it is important to emphasize the involvement of Gq signaling in enhancing susceptibility to apoptosis [40,41]. In particular, Sabri et al. have pointed out that Gq(q) stimulation induces not only cardiac hypertrophy but also cardiomyocyte apoptosis [41]. Moreover, using recombinant Pasteurella multocida toxin (rPMT) as a Gq(q) agonist, they have also observed an impaired AKT phosphorylation, both basally and stimulated by EGF or IGF-1. Since AKT, as pointed out later, has a central role in cell survival, this mechanism could be crucial in the transition from hypertrophy to cardiac failure observed in response to activation of the Gq signaling pathway.

Gs protein stimulates the production of cAMP, and this signaling effect is apparently involved more in cardiac contractility than in cardiac growth. However, some studies have shown that transgenic overexpression of β1-adrenergic receptors or of the α subunit of the Gs heterotrimeric G protein can determine increased myocardial contractility, but also a progressive cardiomyopathy initially consisting of deteriorated cardiac performance, later followed by cardiomyocyte apoptosis and fibrosis [42–44]. Active Gs is known to trigger the adenyl cyclase-dependent production of cAMP and the subsequent cAMP-mediated activation of cAMP-dependent protein kinase (PKA), which phosphorylates numerous target proteins involved in Ca2+ mobility and eventually in the control of contractility [1]. Interestingly, maladaptive Gs signaling seems to act through PKA. In fact, transgenic mice overexpressing PKA develop cardiac fibrosis and, finally, dilated cardiomyopathy [45]. Consistent with this view, therapeutic intervention aimed at sustaining cardiac contractility by raising cAMP production through phosphodiesterase inhibitors failed to show long-term, beneficial effects and rather increased mortality [46]. On the other hand, failing hearts show an enhanced signaling by Gi as a possible endogenous counter-regulatory mechanism to blunt the catecholaminergic intracellular drive [47–50]. Thus, the use of β-adrenergic antagonists, which apparently restore a correct β-adrenergic signaling, significantly protects hypertrophic hearts from degeneration into dilated cardiomyopathy [51].

Another important type of intracellular signaling involved in left ventricular hypertrophy is that converging on protein kinase C (PKC). The PKC family consists of several serine/threonine kinases that are activated not only by calcium but by almost all membrane-activated signal transduction pathways [52]. PKCα is the most representative isoform in the heart. Recently, it has been demonstrated that hearts of PKCα-deficient mice are hypercontractile, while hearts mice overexpressing PKCα are hypocontractile and more sensitive to transition toward heart failure [53]. More interestingly, it has been demonstrated that PKCα deletion protects against both heart failure induced by chronic pressure overload and dilated cardiomyopathy typical of LIM protein-deficient mice, thus suggesting that PKCα activation represents a maladaptive signaling participating in cardiac hypertrophic remodeling of the over-loaded left ventricles. This negative impact of PKCα on the transition of cardiac hypertrophic remodeling toward heart failure is newly thought to be due to an abnormal Ca2+ recycling on the sarcoplasmic reticulum [53].

Mitogen-activated protein kinase (MAPK) pathways represent another complex system involved in hypertrophic growth. They consist of three major subfamilies: JNKs, p38, and ERKs [54]. These are interesting molecules since they are activated by several mitogenic stimuli and, therefore, are involved in several forms of cardiac hypertrophy. ERK 1/2 MAPKs are likely adaptive, since transgenic mice expressing activated MEK1, an upstream activator of ERK1/2, showed a compensated, concentric, cardiac hypertrophy with enhanced contractile performance [55]. Based on these findings, it will be important to determine whether the ERK1/2 pathway can be protective under conditions of increased workload or in genetic models of dilated cardiomyopathy. On the other hand, the cardiac-specific overexpression of constitutively activated MEK5-ERK5 leads to an eccentric cardiac hypertrophy that progresses to dilated cardiomyopathy and sudden death [56].

Conflicting evidence exists in the literature about the role of JNK in cardiac hypertrophy. In fact, JNK is essential for cardiac hypertrophy and dysfunction induced by Gq, since mice overexpressing Gq but deficient for MEK1, an upstream regulator of JNK, did not show hypertrophy and retained good systolic function [57]. On the other hand, MEK1 knockout mice showed cardiac dilation, ejection fraction reduction, and higher mortality as compared to controls, suggesting that the MEKK1-JNK pathway protects against heart failure and sudden death in response to pressure overload [58].

Finally, p38 signaling has been involved in different stimuli leading to cardiac hypertrophic remodeling [59,60]. The maladaptive role of p38 has been suggested by the evidence that the overexpression of Tak1, an up-
stream activator of p38, causes cardiac hypertrophy that leads to severe myocardial dysfunction and early lethality [61]. This evidence is further supported by a study where a pharmacological inhibitor of p38 enhanced survival by reducing left ventricular hypertrophy and dysfunction in hypertensive rats [62].

The signaling pathways described above participate in cardiac hypertrophic remodeling favoring the transition toward heart failure, and should thus be considered maladaptive. These results suggest pharmacological approaches might be used to prevent cardiac hypertrophy and heart failure through the inhibition of these maladaptive pathways.

3. Adaptive mechanisms

Among the signaling events mediating an adaptive hypertrophic mechanism, of emerging importance is the pathway that causes the activation of the PI3K (phosphatidylinositol 3-kinase)/Akt/GSK3β (glycogen synthase kinase 3β) cascade [63]. This pathway is regulated through several classes of transmembrane receptor, including receptor protein tyrosine kinases, such as the IGF-I receptor, and G protein-coupled receptors, such as α- and β-adrenergic receptors [64]. It has been demonstrated that the overexpression of constitutively active PI3K or Akt in the hearts of transgenic mice induces an increase in cardiomyocyte size and concentric hypertrophy in the absence of fibrosis, with preserved systolic function [65–67]. In further support of this notion, deletion of PTEN, which physiologically counteracts PI3K activity by dephosphorylating phosphatidylinositol (3,4,5)-trisphosphate, results in compensated cardiac hypertrophy in the absence of cardiac dilatation or decompensation [68]. On this issue, relevant is the finding that the PI3K/Akt pathway acts as an inhibitor of apoptosis [63]. In particular, Akt promotes cell survival by inhibiting apoptosis at multiple points through transcription-independent and -dependent mechanisms. In fact, Akt phosphorylates directly or indirectly several molecules, such as Bad, caspase 9, and Forkhead box transcription factors of the class O subfamily (FOXOs), inhibiting their pro-apoptotic role [69,70]. Moreover, Akt regulates other apoptotic pathways including NF-κB, CREB, p53 [71–73]. According to this ‘adaptive’ role of the PI3K pathway in hypertrophic growth, McMullen et al. have recently discovered the role of p110α isoform of PI3K in the transduction of a biochemical pathway of ‘physiological’ exercise-induced hypertrophy by using transgenic mice expressing a dominant negative PI3K(p110α) [74]. These mice developed hypertrophy as a consequence of pressure overload but not in exercise training, suggesting the specific role of this molecule in the ‘physiological’ hypertrophy. This is very interesting because, as mentioned above, all authors agree on the compensatory role of the cardiac hypertrophy in athletes that permits their hearts to develop enhanced force. Moreover, PI3K and Akt signals converge on phosphorylation of GSK3β by inhibiting its kinase activity [75]. Activated GSK3β is able to phosphorylate and inhibit several molecules involved in hypertrophic signaling, such as the members of the NFAT transcription factor family that are activated by another hypertrophic factor, calcineurin [76]. Thus, GSK3β can be considered a point of convergence of different hypertrophic signaling pathways. Recent studies have shown that inactivation of GSK3β via phosphorylation by the upstream kinase Akt is both necessary and sufficient for cardiomyocyte hypertrophy in vitro [77,78], while expression of a non-phosphorylatable form of GSK3β prevents cardiac hypertrophy in response to pressure overload as well as to other hypertrophic stimuli [79]. Studies on transgenic mice lacking a functional Fas receptor further support the role of GSK3β [80]. These mice subjected to pressure overload by aortic banding failed to inactivate GSK3β and developed rapid left ventricular dilatation, heart failure, and an increased mortality. Moreover, Fas ligand-induced cardiomyocyte hypertrophy requires inactivation of GSK3β via the PI3K and Akt pathway [80]. Therefore, this study suggests the important role of GSK3β phosphorylation and inhibition in the development of compensatory hypertrophy.

Akt and GSK3β are also phosphorylated in response to mechanical stimuli via the muscle-specific protein melusin [81,82]. Melusin binds to the β1-integrin cytoplasmic region and behaves as a stretch-activated protein crucial in the hypertrophic response to mechanical overload [82]. In particular, melusin-null mice show normal cardiac structure and function in physiological conditions, but develop an abnormal cardiac remodeling that evolves into dilated cardiomyopathy and contractile dysfunction when subjected to pressure overload. Analysis of intracellular signaling events induced within 10 min from mechanical stimuli indicated that phosphorylation of Akt and GSK3β was specifically blunted in melusin-null hearts, suggesting that melusin controls this very early response in the hypertrophic signaling cascade. Thus, this genetic mouse model indicates that melusin participates in a beneficial hypertrophic molecular pathway involving Akt GSK3β signaling.

Moreover, apart from the role of tyrosine kinase and G protein signaling, the myocardial growth and survival is also modulated by signals derived from gp130, the receptor for the interleukin 6 family of cytokines [83,84]. In particular, transgenic mice, overexpressing selectively in the heart a dominant negative mutant of gp130 develop a strongly impaired left ventricular hypertrophic response to pressure-induced overload [85]. More interestingly, mice lacking the gp130 gene rapidly developed dilated cardiomyopathy with dramatic cardiomyocyte apoptosis in response to chronic pressure overload, thus suggesting a crucial adaptive role of gp130 in cardiac hypertrophy and in the prevention of its transition toward heart failure [86].
4. Conclusion

As described above, some biochemical pathways are involved in the development of an adaptive form of cardiac hypertrophy that does not show decompensation in the long run. In contrast, other biochemical pathways determine a maladaptive cardiac hypertrophy that rapidly leads to reduction of cardiac contractility and heart failure (Fig. 1). Accordingly, it appears that therapeutic strategies should be aimed not simply at the reduction of cardiac mass, but at the control of adaptive and maladaptive signaling pathways.

One approach would be to counteract cardiac fibrosis: the degree of fibrosis of a hypertrophied heart contributes to determining the prognosis in human patients and it is well understood that a higher degree of fibrosis is associated with increased cardiovascular morbidity and mortality [87]. In fact, accumulation of collagen in workload-hypertrophied hearts can be considered an initial step leading to cardiac failure. It is initially responsible for increased myocardial stiffness and diastolic dysfunction until it causes a deleterious systolic dysfunction [88–90]. Remarkably, athletes with hypertrophic hearts, a condition representing the classical ‘physiological’ compensated hypertrophy, do not show collagen accumulation in the myocardium [1,26,74]. However, it is presently known that diverse pathways involved in cardiac hypertrophy determine increasing extracellular matrix deposition [81,91]. It will thus be important to identify the molecular factors involved in this process in order to develop drugs that, by inhibiting cardiac fibrosis, will improve the functional aspects of the hypertrophic process. As fibrosis might be triggered by apoptotic loss of cardiomyocytes, it could be important to inhibit some signals, such as those induced by Gq and Gs that cause apoptotic loss of cardiomyocyte both in vitro and in vivo [40,41], and sustain others that determine compensatory hypertrophy, such as PI3K and AKT promoting cell survival [63,69–73].

Finally, the changes in intracellular calcium handling, concomitant with structural remodeling, could have a crucial role in maintaining the hypertrophic response toward heart failure. In fact, depressed sarcoplasmic reticulum calcium cycling is a common feature of the reduced contractility observed in hypertrophied and failing myocardium. The sarcoplasmic reticulum Ca\(^{2+}\)-ATPase pump (SERCA2a) is responsible for most of cytoplasmic calcium removal, the size of calcium store, and its release in the subsequent beat, thus affecting cardiac contractility. The SERCA2a pump is kept inactive by its interaction with phospholamban. However, upon PKA-mediated phosphorylation of phospholamban, this complex dissociates and the SERCA2a pump becomes active [92]. Accordingly, phospholamban ablation increases the activity of SERCA2a, causes improved myocyte contractility, and rescues heart failure induced by different genetic alterations in mice such as the inactivation of muscle LIM protein or the over-

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Fig. 1. Adaptive and maladaptive intracellular signaling molecules activated during cardiac hypertrophic remodeling. The green signaling molecules, when activated, exert a protective role in the progression towards heart failure; on the contrary, the activation of the red molecules favors the transition towards heart failure.
expression of β-adrenergic receptors, calsequestrin, or a mutant myosin heavy chain [93–96]. Moreover, PKCα signaling determines a hypertrophy leading to heart failure by inhibiting phospholamban phosphorylation, while PKC α-ablation protects from decompensated hypertrophy in response to pressure overload, thus suggesting that regulation of calcium cycling by targeting phospholamban and/or PKC might be used to promote an adaptive remodeling [53]. In conclusion, delineating the impact of intracellular cardiac signaling pathways involved in the different aspects of cardiac hypertrophy and remodeling will have significant implications for drug development to prevent the transition to heart failure.

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