Three fingers on the brake: Kruppel-like factor 15, a repressor of cardiac gene expression
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Tapping the Brake on Cardiac Growth

endogenous repressors of hypertrophic signaling

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SUMMARY

Cardiac hypertrophy is considered an early hallmark during the clinical course of heart failure and an important risk factor for cardiac morbidity and mortality. Although hypertrophy of individual cardiomyocytes in response to pathological stimuli has traditionally been considered as an adaptive response required to sustain cardiac output, accumulating evidence from studies in patients and animal models suggests that in most instances hypertrophy of the heart also harbors maladaptive aspects. Major strides have been made in our understanding of the pathways that convey pro-hypertrophic signals from the outside of the cell to the nucleus. In recent years it also has become increasingly evident that the heart possesses a variety of endogenous feedback mechanisms to counterbalance this growth response. These repressive mechanisms are of particular interest since they may provide valuable therapeutic options. In this review we summarize currently known endogenous repressors of pathological cardiac growth as they have been studied by gene targeting in mice. Many of the repressors that function in signal transduction appear to regulate calcineurin (e.g. PICOT, calsarcin, RCAN) and JNK signalling (e.g. CDC42, MKP1) and some will be described in greater detail in this review. In addition, we will focus on factors such as Kruppel-like factors (KLF4, KLF15 and KLF10) and histone deacetylases (HDACs), which constitute a relevant group of nuclear proteins that repress transcription of the hypertrophic gene program in cardiomyocytes.
INTRODUCTION

Over the past two decades there has been significant progress in our understanding of the molecular mechanisms that mediate cardiac hypertrophy\(^1\). Cardiac hypertrophy is accompanied by the reprogramming of cardiac gene expression and the activation of “fetal” cardiac genes, which encode proteins involved in contraction, calcium handling, and metabolism\(^1,2\). The reactivation of fetal cardiac genes in response to stress signals suggests that the same transcriptional program is employed to control cardiac gene expression during development and to regulate hypertrophic cardiac growth. Indeed, the same transcription factors that are important for cardiac development, such as MEF2, SRF, GATA4, NFAT and myocardin have also been implicated as mediators of the hypertrophic transcriptional program\(^2,3\).

While the signaling pathways that promote cardiac hypertrophy are increasingly understood, negative regulators of these pathways have received less attention\(^4\). Nevertheless, it is becoming increasingly clear that cardiomyocytes possess a number of repressive mechanisms to prevent hypertrophy in the healthy heart or to limit pro-hypertrophic mechanisms. In this review, we highlight recent insights and discoveries on repressive mechanisms of cardiac hypertrophy, and how these mechanisms are regulated in cardiac disease. Some of these repressive mechanisms are constitutively active and repress the hypertrophic machinery in the healthy heart. Their activity is often decreased upon hypertrophic signaling (e.g. due to decreased expression, translocation to the cytosol or inactivation), which then allows hypertrophy to occur. Other repressors are ‘silent’ at baseline, but their activity or expression increases during hypertrophy. We refer to these classes of repressors as class I (reduced upon stress) or class II (induced upon stress). This classification of repressors has first been applied in 2004 by Hardt et al.\(^4\) and seems useful since it is not only important to understand how a repressor regulates the hypertrophic response, but it is also important to know how the expression of a particular repressor is regulated during hypertrophy. In this review we provide an overview of repressors of cardiac hypertrophy identified thus far. In table 1 and 2 we have summarized these known repressors and categorized them by inducibility (i.e. increased or decreased upon stress). In table 1 we summarize all identified repressors known to act on signal transduction and in table 2 we provide a list of repressors identified thus far that act in the nucleus to inhibit transcription of the hypertrophic gene program. From both categories a number of negative regulators are selected which have been firmly shown to repress cardiac hypertrophy in genetically altered mouse models. These will be described in detail, while others are briefly mentioned in table 1 and 2. Although miRNAs have also been shown to repress pivotal signaling pathways in hypertrophy\(^5-7\), they fall out of the scope of this review. Readers interested in the control of cardiac hypertrophy
Chapter 2

by miRNAs should refer to recent publications 8, 9.

REPRESSORS OF SIGNAL TRANSDUCTION

Cytosolic signaling molecules are key players in the transduction of external signals to the nucleus. One of the best studied signaling pathways in the heart is the mitogen-activated protein kinases (MAPK) pathway, which is activated by a variety of stress-sensing receptors present on the cell membrane and which importantly controls hypertrophic growth of cardiomyocytes 10. This family has four major branches in the heart: the extracellular signal-regulated kinases (ERK1 and ERK2), the Jun N-terminal kinases (JNK1 and JNK2), p38 MAPK (p38α, β, γ and δ) and the extracellular signal-regulated kinase ERK5 11 (See Figure 1). Whereas the ERK1/2 pathway is mainly activated upon stimulation by growth factors, p38 and JNK are induced by physical, chemical or physiological stressors. All four pathways are activated in hypertrophic and failing hearts, and this has been extensively reviewed Rose et al 10. Numerous studies have been performed to elucidate the precise roles of the individual MAP kinases in the heart. Despite many efforts it is still difficult to ascribe clear pro- or antihypertrophic properties to this group of proteins. For example, the ERK1/2 pathway is generally regarded as prohypertrophic, since several in vivo models in which upstream kinases of ERK1/2 are activated induce hypertrophy 12–14. In contrast, deletion of ERK (ERK1–/– and ERK2 +/–) did not diminish the hypertrophic response to various forms of hypertrophic stimuli 15. This indicates that the ERK1/2 pathway acts prohypertrophic, however it does not function as a necessary signaling component in cardiomyocyte hypertrophy 10.

Activation of JNK signaling mainly appears antihypertrophic, however this pathway is also not free from contradictions. Whereas overexpression of various upstream kinases of JNK in the heart mostly results in antihypertrophic effects, inhibition of JNK (dominant negative JNK1/2) significantly enhances hypertrophy following pressure overload 16. In contrast, specific knockout models for JNK1, JNK2 and JNK3 do not show a difference in hypertrophic response when compared to wild-type littermates. This illustrates the complexity of MAPK signaling and it also underlines that there is not always a clear distinction between repressors and activators of hypertrophy. Overall, the majority of in vivo studies support the conclusion that ERK1/2 and ERK5 activation generally results in prohypertrophic signaling, whereas activation of the JNK pathways mainly appears to be anti-hypertrophic 10, 17–19. The role of p38 in hypertrophy is also controversial. In vitro studies indicate that p38 plays a prohypertrophic role in cultured cardiomyocytes whereas in vivo mouse models show that p38 lacks a role in hypertrophy, but rather plays a role in pathological remodeling and regulates apoptosis,
fibrosis and LV dilation. In this section we describe four negative regulators of hypertrophy that all impinge on MAPK and calcineurin signaling: cell division cycle 42 (Cdc42), MAPK phosphatase 1 (MKP1-), Calsarcin-1 and PKC-interacting cousin of thioredoxin (PICOT). The signaling cascades of these antihypertrophic proteins are illustrated in Figure 1.

**Cell division cycle 42 (CDC42)**

The small G protein superfamily comprises five main subfamilies with functional similarities: Ras, Rho, Rab, Arf and Ran. Especially members of the Ras and Rho subfamilies have been shown to play a role in hypertrophic signaling, initiated by G protein-coupled receptors such as the adrenergic, angiotensin and endothelin receptors. Cdc42 is a ubiquitously expressed member of the Rho family of small G proteins (also known as small GTPases) and can act as a molecular switch that cycles between an active GTP-bound state and an inactive GDP-bound state. In the active state, GTPases interact with a range of effector molecules to activate signaling cascades.

Maillet and colleagues recently identified Cdc42 as an antihypertrophic switch in the mouse heart by regulating the activity of JNK. Anthypertrophic properties of JNK in the heart have been demonstrated in mice in which JNK1/2 was deleted. These mice developed spontaneous cardiac hypertrophy with aging and aortic banding resulted in a greater hypertrophic response. Mice lacking Cdc42 in cardiomyocytes do not have a cardiac phenotype at baseline, but when these mice are subjected to pressure overload by means of TAC surgery, they show a stronger hypertrophic response compared to controls. These mice also display increased fibrosis and apoptosis which is accompanied by depressed cardiac function. When evaluating the activity of several MAP kinases in these knockout hearts it was noted that JNK activity was attenuated after TAC. The inability to activate JNK in the absence of Cdc42 resulted in enhanced cardiac NFAT activity and it was shown that the Cdc42 - JNK pathway directly interferes with the calcineurin-NFAT pathway. Along these lines, in vitro studies have shown that activated JNK phosphorylates nuclear NFAT, thereby promoting its nuclear export and preventing NFAT mediated cardiac gene expression. Restoration of JNK signaling by overexpressing its upstream kinase, MKK7 in a heart-specific manner in these Cdc42 knockouts reversed the enhanced growth effect. Cardiac specific deletion of MKK4, the other upstream kinase of JNK1/2, aggravates TAC-induced hypertrophy indicating that MKK4 also acts as a repressor of hypertrophy. In conclusion, Cdc42-dependent JNK activation provides an important intrinsic inhibitory feedback pathway to antagonize calcineurin-NFAT activity and cardiac hypertrophy.
Chapter 2

MAPK phosphatase 1 (MKP-1)

MAP kinase activities depend on phosphorylation of specific tyrosine/threonine residues. The dual-specificity phosphatase MKP-1 can specifically dephosphorylate these residues in p38, JNK and ERK to render MAP kinases inactive. As an important regulator of MAP kinase activities, MKP-1 was hypothesized to regulate cardiomyocyte hypertrophy. However, due to the opposing functions of JNK, p38 and ERK in hypertrophy, it was unclear what to expect from interference in MKP1 expression. Interestingly, hearts

Figure 1. Major signal transduction pathways in the heart that control cardiomyocyte hypertrophy. Repressors that control the activity of the MAP kinases and calcineurin pathways are depicted in red. The GTP-binding protein CDC42 binds and activates the MAP kinase kinase MEKK1 that subsequently phosphorylates and activates MKK4 and MKK7, which in turn phosphorylates JNK1/2. Active JNK1/2 eventually phosphorylates NFAT and this leads to nuclear export of the transcription factor NFAT and repression of NFAT-mediated gene expression. The dual specificity phosphatase MKP-1 dephosphorylates MAP kinases (with a preference for JNK) thereby inhibiting MAP kinase mediated hypertrophy. Calsarcin-1 is a calcineurin-interacting protein and is localized at the Z-disc of sarcomeres where it represses calcineurin-mediated hypertrophy. Also at the Z-disc, PICOT can bind muscle-LIM protein (MLP), which is known to anchor calcineurin to the Z-disc. PICOT interferes with the interaction of MLP and calcineurin which results in the displacement of calcineurin from the Z-disc and prevention of nuclear translocation of NFATc4.
of transgenic mice overexpressing MKP-1 were significantly smaller than hearts of WT littermates, and this was associated by a smaller cross sectional area of cardiomyocytes, indicating that MKP-1 functions as a repressor of hypertrophy. Furthermore, hearts of αMHC-MKP-1 transgenic mice failed to undergo a significant hypertrophic response when stressed by aortic banding or isoproterenol infusion and this was accompanied by a profound reduction in phosphorylated JNK1/2 and to a lesser extent of p38 and ERK1/2. A possible explanation for the blunted hypertrophic response in MKP-1 transgenic hearts may relate to the observed reduction in phosphorylated p38 and ERK1/2.

MKP-1 is upregulated in the heart during hypertrophy and failure. Lim et al. demonstrated the existence of cross-talk between calcineurin and MAPK pathways by showing that the promoter of MKP-1 is responsive to calcineurin signaling. Calcineurin is not the only regulator of MKP-1 expression. Oliver and colleagues showed that atrial natriuretic factor (ANF), besides being a well-known marker of hypertrophy also influences hypertrophic growth of the heart. Stimulation of cultured cardiomyocytes with ANF resulted in increased MKP-1 expression and decreased levels of ERK1/2, suggesting that ANF represses hypertrophy, at least partly by activating MKP-1. Despite a clear role for MKP-1 in repressing hypertrophy, there is need for further research since the mechanistic aspects are still incompletely understood.

Calsarcin-1

Calsarcins were originally identified by a yeast two-hybrid screen for calcineurin-binding proteins. Calsarcin-1 is the only member of the calsarcin-1 family that is expressed in the heart, where it is localized to the Z-disk of sarcomeres. Mice lacking the calsarcin-1 gene Myoz2 have macroscopically normal hearts but they have increased NFAT activity, an activated fetal gene program and a remodeled Z-disk. To study the effect of absence of calsarcin-1 on the hypertrophic response of the heart, Myoz2−/− mice were crossed with mice overexpressing calcineurin in their hearts. The hypertrophic response that is usually seen in these calcineurin-overexpressing mice was strongly enhanced in mice lacking calsarcin-1. Also aortic banding induced an exaggerated hypertrophic response in calsarcin-1 null mice. If loss of calsarcin-1 enhances hypertrophy, then forced overexpression of hypertrophy may be protective. Indeed, in vitro studies demonstrated that overexpression of calsarcin-1 prevented PE-, AngII- and ET1-induced hypertrophy, as was measured by cell size and ANF expression. Mice overexpressing calsarcin-1 do not have a phenotype at baseline, but when hypertrophy is induced by AngII, they fail to develop hypertrophy. Interestingly, it has been shown that calsarcin-1 is being shuttled to the nucleus and not uniquely present at the Z-disk, as described earlier. Calsarcin-1 is also phosphorylated, however the consequence and the responsible kinase remain elusive. Taken together, these studies convincingly show that calsarcin-1 is a negative
regulator of calcineurin signaling in the heart and suggest a specific role for calsarcins in the complex interplay between sarcomere function and hypertrophic signaling. Many questions remain unanswered on the function of calsarcin-1 and future research is needed to determine whether there is therapeutic potential in overexpressing calsarcins.

**PKC-interacting cousin of thioredoxin (PICOT)**

Protein kinase C (PKC) is a ubiquitously expressed serine/threonine kinase that has been implicated in the development of hypertrophy. PICOT (PKC-interacting cousin of thioredoxin) is a PKC inhibitor that is strongly induced in the stressed myocardium, in response to TAC-induced pressure overload. That PICOT acts as a repressor of cardiac hypertrophy was first demonstrated by Jeong et al., who showed that cardiomyocyte-specific overexpression of PICOT abrogated TAC-induced hypertrophy, as evidenced by reduced heart weight/body weight ratio, a reduction in ANF and alpha skeletal actin (α-SKA) levels and improved cardiac function. Homozygous PICOT knockout mice die between embryonic day 12.5 and 14.5 due to non-cardiac causes. Heterozygous PICOT mice are viable and display normal heart weight at baseline, but upon TAC-induced pressure overload the hypertrophic response is larger than in wild-types. Whereas cardiac function was improved in PICOT overexpression mice, loss of PICOT attenuates cardiomyocyte contractility.

Mechanistically, PICOT impinges on multiple signaling cascades that can explain the repression on cardiomyocyte growth. Firstly, forced overexpression of PICOT in cultured cardiomyocytes nearly completely suppresses PKC activity. Because MAPK signaling pathways appear to be a merging point for signaling pathways involving PKC, the effect of PICOT on JNK, p38 and ERK1/2 was further investigated in neonatal rat cardiomyocytes. In these in studies, PICOT abrogated the PE and ET-1 induced activation of ERK1/2 and JNK, but not p38. These results suggest that PICOT counteracts hypertrophy by inhibiting PKC and the downstream ERK1/2 and JNK signaling pathways, however it is currently unknown whether this also occurs in vivo.

Secondly, the inhibitory effect of PICOT on cardiac hypertrophy may also relate to its interaction with muscle LIM protein (MLP) at the Z-disc of cardiomyocytes. MLP is known to play a role in anchoring calcineurin to the Z-disc of the sarcomere, which is critical for calcineurin-NFAT signalling. PICOT appears to interfere with the interaction of MLP and calcineurin by competitive binding to MLP and displacing calcineurin from the Z-disc (see figure 1). PICOT-induced displacement of calcineurin from the Z-disc prevented dephosphorylation and nuclear translocation of NFATc4 in cultured cardiomyocytes as well as in the overloaded myocardium of PICOT transgenic mice. Taken together, PICOT is a potent negative regulator of cardiomyocyte hypertrophy and probably functions through inhibition of calcineurin and MAPK-dependent signaling pathways. Particularly
interesting with regard to possible therapy is the observation that PICOT overexpression improves cardiac contractility. As an underlying mechanism it has been suggested that 1) PICOT increases calcium sensitivity of contractile filaments, probably via altering the phosphorylation status of contractile proteins such as troponin I and 2) PICOT increases SERCA activity via regulating phosphorylation of phospholamban.

REPRESSORS OF TRANSCRIPTION

Every hypertrophic signaling pathway, regardless the way it is activated (via neurohormones, growth factors or stretch) eventually results in the activation or repression of transcription factors. Cardiac gene expression is regulated by a group of transcription factors of which MEF2, NFAT, GATA4 and SRF are the best studied examples. These transcription factors directly bind to DNA in a sequence specific manner and essentially mark a gene for activation or repression through recruitment of coactivators or corepressors. Coactivators often contain enzymatic activities necessary for alteration in chromatin structure from a quiescent state to one allowing active gene transcription. One class of coactivators modifies histones in ways that allow greater access of transcription factors to DNA: the histone acetyltransferases (HATs), of which p300 and CBP are well known examples. Corepressors have an opposite effect on chromatin structure; they render chromatin inaccessible to the binding of transcription factors. They are often associated with histone deacetylase (HDAC) activity, but also other mechanisms of gene silencing exist. Since transcription factors are the end-point of signaling cascades, therapies aimed at modulating certain transcription factors could have a more direct and specific effect. This section will focus on the repressive mode of action HDACs, the antioxidant thioredoxin-1 (TRX1) the three kruppel-like transcription factors: KLF15, KLF4 and KLF10.

Histone deacetylases (HDACs)

The HDAC superfamily can be grouped in three families (class I, II and III), which differ in structure, enzymatic activity and expression pattern. Although mostly class II HDACs have been shown to repress growth of myocytes, there is increasing evidence that also certain members of class I and III HDACs repress cardiomyocyte growth. One of the best characterized group of repressors of cardiac gene expression is the family of Class II HDACs (HDAC4,5,7 and 9), which are highly expressed in the heart, skeletal muscle, brain and T-cells. An important target of class II HDACs is the muscle specific transcription factor MEF2, which is known to activate many fetal cardiac genes and may serve as a nuclear end-point for stress signals in the adult myocardium.
Class II HDACs can directly bind to the DNA binding domain of MEF2 thereby inhibiting its function. In the healthy adult myocardium, in the absence of stress signals, class II HDACs interact with MEF2 to repress the fetal gene program and counteract cardiac growth. Upon stress signals, these HDACs are phosphorylated by kinases such as calcium/calmodulin-dependent protein kinase (CaMK) and protein kinase D (PKD), and bind the chaperone protein 14-3-3. As a result, the HDAC/14-3-3 complex is shuttled from the nucleus to the cytoplasm (see Figure 2). Dissociation of class II HDACs from MEF2 allows p300 to associate with MEF2, thereby relieving the repressive signals from MEF2 allowing it to act as a transcriptional activator. The signal-responsiveness of class II HDACs provides a mechanism for linking stress signals with transcription, which appears an important mechanism in the control of cardiac growth. In a more recent study it was shown that phosphorylation is not the only mechanism by which class II HDACs can be exported from the nucleus. Ago and colleagues showed that the redox status of HDACs and associated proteins is also important for nuclear localization of HDACs. Interestingly, the oxidation of HDACs is an extraordinary fast process and it precedes phosphorylation of HDACs but this will discussed below, in the paragraph about thioredoxin-1 (TRX1).

The importance of class II HDACs in LVH has been demonstrated in HDAC5 and HDAC9 knockout mice. Mutant HDAC9 or HDAC5 mice in which the MEF2 binding site is deleted develop age-related hypertrophy and are extremely sensitive to calcineurin- and TAC-induced hypertrophy. This shows that these HDACs are constitutively active repressors of hypertrophy and that removal is sufficient to induce hypertrophy. It must be noted that isoproterenol-induced hypertrophy is not enhanced in both HDAC5 and HDAC9 mutant mice, suggesting that β-adrenergic induced hypertrophy does not employ class II HDACs. Besides inhibition of MEF2 activity, class II HDACs modulate the activities of numerous other transcription factors complexes, such as SRF/myocardin, Camta2/Nkx2.5, Mre/NFAT and the estrogen receptor.

Class I HDACs (HDAC 1,2,3 and 8) which are expressed more ubiquitously than class II HDACs have also been shown to participate in the hypertrophic response of the heart. Administration of class I HDAC inhibitors has been shown to attenuate cardiac hypertrophy, indicating that this class of HDACs functions prohypertrophic. HDAC1 and 2 appear to be functionally redundant, as removal of one of both isoforms in the heart does lead to a phenotype, while homozygous loss of both genes results in dilated cardiomyopathy. As HDAC1 and 2 function as activators of hypertrophy, knockout studies for HDAC3 have shown that this HDAC acts as an endogenous inhibitor of hypertrophic growth in the heart, with an underlying mechanism presumably involving modulation of PPARα regulated metabolic genes. The relative contribution of individual class I HDACs isoforms in the control or propagation of cardiac hypertrophy remains
to be determined by characterization of individual knockouts and transgenic models of HDAC1, 2, 3 and 8.

The sirtuins are the third group of deacetylases (class III HDACs), and at least two members have been shown to repress cardiac hypertrophy. Rather than the other HDAC families, sirtuins are NAD-dependent deacetylases and therefore their activity is directly linked to the metabolic state of the cell. Sirtuins have been reported to play an important role in mitochondrial metabolism and longevity. Alcendor et al. showed that mild overexpression of Sirt1 protects against age-related hypertrophy and fibrosis, most likely due to increased expression of antioxidants like catalase and activation of the FoxO dependent mechanisms. Sirt3-deficient mice show signs of hypertrophy and fibrosis at 8 weeks of age. Administration of hypertrophic stimuli to these mice produced a severe cardiac hypertrophic response, whereas Sirt3 overexpressing transgenic mice were protected from these stimuli. As an underlying mechanism it has been shown that Sirt3 blocked cardiac hypertrophy by activation of FoxO2 and antioxidant encoding genes (MnSOD and catalase). Reduced ROS levels subsequently suppress Ras activation and downstream signaling through the MAPK and Akt pathways.

**Thioredoxin-1 (TRX1)**

Reactive oxygen species (ROS) are produced from various sources in the cell, such as mitochondrial electron transport leakage, NAD(P)H oxidases and uncoupled nitric oxide synthases. Cells protect themselves against oxidative stress through an ingenious system of antioxidants. Since oxidative stress is an important cause of cardiac hypertrophy it was hypothesized that antioxidants may suppress this process. In the heart, one of the most important antioxidants are components of the thioredoxin (TRX) system. The TRX system consists of several thioredoxins (TRX1 and TRX2), thioredoxin reductases (TRXR1 and TRXR2), and peroxiredoxins. Using transgenic mouse models with cardiac-specific overexpression of Trx1 or its dominant negative (DN) form it was demonstrated that Trx1 represses cardiac hypertrophy. At an age of 3 months Tg-DN-Trx1 mice spontaneously develop hypertrophy and when these mice were treated with an antioxidant, spontaneous hypertrophy was prevented. TAC-induced pressure overload in Tg-DN-Trx1 mice resulted in an enhanced hypertrophic response compared to the response that was seen in WT littermates. Analysis of several MAPK pathways in the hearts of Tg-DN-Trx1 mice revealed that activation of the ERK pathway was enhanced compared to WT littermates. No differences were seen in JNK and p38 phosphorylation. These data convincingly show that Trx1 is an endogenous repressor of cardiac hypertrophy, possibly via inhibition of the Ras/Raf1/Erk pathway.

Trx1 not only functions as an antioxidant but also controls oxidative modifications of important signaling molecules and transcription factors. In this context...
regard, it was elegantly shown by Ago et al. that Trx1 controls the nuclear shuttling of class II HDACs through a redox-dependent mechanism (see figure 2). By forming a multiprotein complex with heat shock protein 40 (DnaJb5) and a Trx1-binding protein (TBP-2), Trx1 reduces HDAC4 at Cys-667 and Cys-669. These cysteine residues in HDAC4 are known to be oxidized in response to hypertrophic stimuli, such as PE. The redox state of HDAC4 critically affects its nuclear localization, in such a way that reduction of HDAC4 by Trx1 inhibits its nuclear export. Importantly, this process is independent of the phosphorylation status of HDAC4. Initially it was believed that nuclear export of class II HDACs solely depends on the phosphorylation status of HDACs. As described

Figure 2. Transcriptional repression by histone deacetylases (HDACs). Class II HDACs act as transcriptional co-repressors by deacetylating histones in the vicinity of target promoters. Their repressive activity relies on interactions with transcription factors complexes, such as MEF2, SRF/Myocardin, NKX2.5/CAMTA, NFAT/Mrj. During hypertrophy, class II HDACs become phosphorylated by kinases such as CamKII or PKD, which leads to nuclear export of HDACs by the chaperone molecule 14-3-3. This relieves repression of gene expression and activates the hypertrophic gene program. Besides phosphorylation, also the redox-state of HDACs critically affects its nuclear localization. Two cysteine residues in HDAC4 are known to be oxidized by reactive oxygen species (ROS) in response to hypertrophic stimuli (e.g. phenylephrine) or mechanical strain and this results in nuclear export of HDAC4. The antioxidant thioredoxin-1 (TRX1) reduces HDAC thereby promoting the return of HDAC4 in the nucleus and inhibiting hypertrophic gene expression.
in our previous section, several hypertrophic pathways lead to phosphorylation of serine residues in class II HDACs. This results in binding to 14-3-3 and export from the nucleus, relieving its repression on its target genes. The study of Ago and colleagues demonstrated that, in addition to phosphorylation also oxidation of class II HDACs takes place during hypertrophic responses. Interestingly, the oxidation processes of HDAC4 occurs more rapidly than phosphorylation. These findings imply that nuclear export of HDAC4 may be biphasic, with redox regulation mediating the early phase of nuclear export and phosphorylation would later maintain HDACs in the cytosol.

Trx1 expression is enhanced under numerous stress conditions, such as pressure-overload, ischemia and cardiac failure, indicating that Trx1 functions as a negative feedback regulator of stress responses. The promoter of the Trx1 gene contains a series of stress-responsive elements, antioxidant responsive elements and a heat-shock responsive element.

Summarized, the antioxidant Trx1 is activated in response to oxidative stress as is seen during hypertrophy. This increase in Trx1 counteracts the ROS-mediated activation of the pro-hypertrophic Ras-Raf1-ERK pathway and inhibits the nuclear export of class II HDACs by reducing specific residues.

Kruppel-like factors (KLFs)

Kruppel-like factor 15 (KLF15)

Kruppel-like factors form a large family of transcription factors that share common structures of a transcriptional activation or repression domain, a nuclear localization signal and three Kruppel-like zinc fingers. KLF15 was identified in 2000 by screening clones of a kidney cDNA library. The first known function of KLF15 was transcriptional repression of CLC-K1, a kidney specific chloride channel. Gray et al. subsequently showed that the expression of KLF15, although highly expressed in the kidney, is not restricted to this organ but is also abundantly expressed in the liver, adipose tissue, skeletal muscle and the heart. KLF15 null mice, generated by Fish et al. revealed that KLF15 acts as an endogenous repressor of cardiac hypertrophy. At twelve weeks of age KLF15 null mice have normal cardiac function without signs of spontaneous hypertrophy. However, when the hearts of these mice are stressed by TAC- or AngII-induced pressure overload they develop an exaggerated hypertrophic response and cardiac function deteriorates to a greater extent than their wild-type littermates. Mechanistically, KLF15 acts as a repressor of the cardiac transcription factors MEF2 and GATA4 and the transcriptional coactivator myocardin (Chapter 4). Association of KLF15 with myocardin prevents binding of myocardin to SRF resulting in repressed expression of CArG box dependent...
Chapter 2

genes like ANF and αSKA \(^\dagger\) (Figure 3). Recently, it was also shown that KLF15 inhibits the p300-mediated activation of p53, a transcription factor that regulates cell cycle progression, cellular senescence, and apoptosis. Elevation of p53 levels has been shown to cause the development of pressure overload-induced heart failure \(^\dagger\). Interestingly, both genetic ablation of p53 or pharmacological inhibition of p300 rescued the cardiac phenotype of the KLF15 knockout mice. The observation that p300 acetyltransferase activity was increased in KLF15 null hearts and the recognition that p300 also acetylates GATA, MEF2 and myocardin, suggests that the exaggerated hypertrophic response in the KLF15 null mice is partly the consequence of unbridled activity of these cardiac transcription factors \(^\dagger\).

The expression of KLF15 is consistently down-regulated during pathological hypertrophy and heart failure, as was shown in numerous animal models \(^\dagger, \dagger\dagger, \dagger\ddagger\) and patients with aortic stenosis and non-ischemic cardiomyopathy \(^\dagger\dagger, \dagger\dagger\dagger\). This indicates that loss of KLF15 during hypertrophy removes transcriptional repressive mechanisms and enable cardiac growth. In this regard, it is also striking that KLF15 is not (or at very low levels) expressed during cardiac development, but that its expression gradually increase after birth to reach adult levels around day 20 \(^\dagger\ddagger\). Interestingly, loss of KLF15 seems to be unique for pathological hypertrophy since KLF15 levels were not changed in exercised-induced hypertrophy. Down-regulation of KLF15 by pathological stress signals was shown to be mediated by the TGFβ-p38-MAPK signaling pathway, however it is currently unknown whether this is a direct effect on the promoter regions of KLF15 or whether this is indirect (Chapter 4).

In conclusion, KLF15 provides an example of a transcription factor that counteracts cardiomyocyte growth. The fact that KLF15 represses hypertrophy and is significantly downregulated in pathological LV hypertrophy suggests that therapeutic interventions aimed at preventing the decrease of KLF15 levels could be beneficial in the prevention of heart failure.

**Kruppel-like factor 4 (KLF4)**

Besides KLF15, also other KLFs repress cardiac hypertrophy. Two studies recently reported on the in vivo role of KLF4 in this process \(^\dagger\dagger, \dagger\dagger\dagger\). Cardiomyocyte-specific deletion of KLF4 in mice results in a slightly increased heart weight and increased ANF levels compared to controls. When subjected to pressure overload, these mice develop more hypertrophy, and this is accompanied by an impaired cardiac function, an increase in cardiac fibrosis and apoptosis, and an increased mortality, when compared to the αMHC-Cre control banded mice \(^\dagger\dagger\). In vitro studies confirmed that KLF4 acts as a repressor of cardiomyocyte hypertrophy. Overexpression of KLF4 in cultured cardiomyocytes represses serum-induced protein synthesis as measured by \(^3\)H leucine incorporation and
Chapter 2

it completely abolished the PE-induced increase in cell size. Conversely, knockdown of KLF4 in H9c2 cells by antisense technologies enhanced the serum-induced hypertrophic response, as evidenced by increased ANF expression and enhanced stress fiber formation. Furthermore, luciferase assays have shown that KLF4 represses the minimal (-638)ANF promoter. To start exploring the underlying mechanisms of repression by KLF4, Yoshida et al. performed in vivo ChIP assays and demonstrated that KLF4 binds to the GATA4 promoter in wild-type but not in KLF4 knockout hearts. These results demonstrate that KLF4 regulates GATA4 expression, a cardiac transcription factor that plays an essential role in promoting cardiac development and differentiation of the myocardium, as well as in regulating hypertrophic growth of the adult heart.

Pressure overload-induced hypertrophy is just like KLF15, also associated with a loss of KLF4 expression. As an underlying mechanisms it was proposed that an increase in HDAC activity as occurs both in vitro and in vivo models of hypertrophy is responsible for this loss of KLF4 expression. Collectively, these studies have implicated KLF4 as a negative regulator of cardiac hypertrophy. The facts that KLF4 is substantially downregulated shows that transcriptional repressive mechanisms are lost during the process of hypertrophy. Loss of KLF4 in cardiomyocytes may therefore play a causal role in the development of pathological hypertrophy and heart failure.

Kruppel-like factor 10 (KLF10 or TIEG1)

KLF10 was first identified in osteoblasts, where it was found to be induced following TGFβ treatment and it was named after its TGFβ-responsiveness: TGFβ Inducible Early Gene-1 (TIEG1). The function of KLF10 in the heart was studied in knockout mice. Interestingly, the male but not the female KLF10 null mice develop features of cardiac hypertrophy at 16 month of age. The gender-specific effects of KLF10 may suggests that this Kruppel factor acts downstream of the estrogen pathway in the heart. Indeed cross-talk between estrogen-TGFβ signaling has been described for numerous cell types, including bone cells, T cells, mesenchymal cells and adipocytes, but not yet for cardiomyocytes. The gender differences may also occur independent of KLF10, since it is found more frequently that females are protected against cardiac diseases; in fact that is the reason that female mice are generally not included in cardiac studies. Insights into the molecular mechanism by which KLF10 regulates cardiac hypertrophy were gained after performing microarray analysis, which revealed a 14-fold increase in pituitary tumor-transforming gene-1 (Pttg1). Pttg1 is known to be regulated by FGF secretion and regulates chromatid separation during mitosis and has been implicated in hypertrophy of tumor cells. Whether the upregulation of Pttg1 can explain the observed hypertrophic phenotype in the KLF10 knockouts is currently unknown and warrants further investigations.
CONCLUSIONS AND PERSPECTIVES

It is becoming evident that signals that promote hypertrophy are balanced by mechanisms that repress hypertrophy. These negative regulators of hypertrophy provide interesting targets for therapy. In this review we aimed to discuss currently known intracellular repressors of pathological cardiac growth and categorized them by their function (i.e. effect on signal transduction or transcription) and by their inducibility (i.e. induced or reduced expression during hypertrophy). Some repressors are active in the healthy heart, but their activity is lost during hypertrophy, due to either downregulated expression, translocation to the cytosol or inactivation. Removal of these repressors in the stressed myocardium (e.g. KLF4, KLF15, HDAC5, HDAC9 and GSK3α/β) may therefore be crucial to allow the development of hypertrophy and possibly heart failure. The second group of repressors has low activity in the healthy heart, but their activity or presence increases upon induction of hypertrophy. These repressors (e.g. JNK, Cdc42, PICOT, MKP-1 and TRX1) seem to function as a counterbalancing mechanism to limit the induced hypertrophy.

By categorizing all hypertrophic repressors we noted that two specific pathways were primarily involved: the calcineurin and the JNK pathway (see Figure 1). Many repressors have been shown to either blunt the activity of calcineurin (i.e. Calsarcin-1, PICOT, RCAN1) or to inhibit nuclear export of NFATs (i.e. JNK, Dyrk1A) thereby antagonizing calcineurin signaling. The antihypertrophic JNK pathway on the other hand has been shown to be stimulated by CDC42 and MKK4/7. Intriguingly, the JNK and calcineurin pathways are intertwined. Firstly, JNK phosphorylates NFAT directly to antagonize the calcineurin-mediated NFAT nuclear translocation (see Figure 1). Secondly, calcineurin has also been shown to regulate the phosphorylation (activation) of JNK indirectly, by controlling the expression levels of MKP1.

Interestingly, it was recently found that calcineurin activity displayed large circadian oscillations in the healthy mouse heart. Calcineurin-dependent transcript levels and substrates fluctuated as much as 20-fold over the course of a day. Calcineurin activity fluctuates out of phase with phosphorylation of proteins such as phospholamban and inhibitor I, which regulate cardiac contractility. One of the direct transcriptional targets of calcineurin/NFAT, the RCAN1.4 gene also showed a circadian pattern of mRNA expression. RCAN1.4 represses cardiomyocyte hypertrophy (Table I) by repressing calcineurin activity, suggesting that the circadian expression of RCAN1 counteracts the dynamics of calcineurin-dependent signaling. This also indicates that the identification of upregulated or downregulated proteins in heart disease should be interpreted with caution, as the circadian rhythm or time of sacrifice may interfere.
When categorizing the negative regulators of transcription, it became evident that the mechanisms by which these repressors inhibit cardiac hypertrophy generally do not require direct interaction with DNA. Rather, these repressors exert their function by binding to transcription factors or transcriptional activators. For example, the transcription factor KLF15 represses gene expression by binding to the transcription factor GATA4 or the coactivator myocardin. Other examples of non-DNA binding transcriptional regulators are HDAC5 and 9 (bind to and repress the activity of MEF2 transcription factors and regulate acetylation of histones), NAB1 (binds and represses EGR-1), CHF1/HEY2 (binds and represses GATA4), LXRα (binds and represses NF-xB). Only for KLF4 is it has been shown that it can directly interact with the promoter region of GATA4.

For other transcriptional repressors like KLF10 and BACH1 more studies are required to elucidate the molecular mechanisms.

**Therapeutic possibilities**

Negative repressors of hypertrophy may serve as attractive candidates for therapeutic intervention, since it is already evident that they are capable of inhibiting cardiac growth, at least in animal models. *In vivo* studies to explore the function of these repressors are mainly performed using knockout models. On other hand, overexpression models demonstrate whether activation of these repressors suffices to prevent the development of hypertrophy and more importantly, to improve cardiac function. We searched the literature for available mouse models where increased activity of the repressor blunted the hypertrophic response, and this is summarized in Table III. This revealed fifteen genes whose cardiac-specific upregulation or activation blunted the hypertrophic response. Of these fifteen genes, overexpression of six (RALT1, CHF1-HEY2, SIRT3, PICOT, MCIP, HDAC5/9) also appeared to improved cardiac function in mice. Activation of four other systems (Calsarcin-1, RSG4, S100β and c-FLIP) reduced hypertrophy, but did not improve function. In the remaining overexpression models, cardiac function was not studied. These results indicate that increasing activity of repressors of cardiac hypertrophy may have attractive therapeutic potential.

Efficient and long-term delivery of potential therapeutic genes to the heart remains a major challenge for clinical implementation. Safe virus based methods are recently emerging as a way to introduce genes in a target organ. In recent years there has been much focus on adeno-associated viruses (AAVs) for the *in vivo* delivery of transgenes. The major advantage of AAVs is that they do not evoke an immune response. Recently it has been shown that the serotypes AAV6, AAV9 and AAVM41 have a preference for the heart. *In vivo* studies in mice show that these AAVs specifically and efficiently target the myocardium. This has been elegantly shown by Swinnen et al.
who revealed that AAV9-mediated overexpression of trombospondin-2 (TSP-2) rescued age-induced cardiomyopathy in TSP-2 null mice. In 2009, Jaski et al. performed a human phase 1/2 clinical trial using rAAV1 to overexpress SERCA2a in 9 patients with advanced heart failure. This study did not raise any safety concerns. Even though the study cohort was too small to conduct statistical analysis, some patients showed improvements in LV function (ejection fraction and end systolic volume), biomarkers (NT-proBNP) and function (walking test and VO₂max). The above described findings emphasize that AAV mediated gene therapy is a potentially efficient and successful way to overexpress cardiac transcriptional repressors to the heart, however further clinical evaluation is warranted.
Another approach to inhibit cardiac growth therapeutically is to control the activity of the described repressors, by chemical or organic compounds. In this respect, small molecule HDAC inhibitors are intensively studied, not only in relation to prevention and treatment of hypertrophy and failure but also in other diseases \cite{63,102,103}. Surprisingly, not the activation but the inhibition of HDACs, by for instance trichostatin A (TSA) has been shown to prevent pressure overload induced hypertrophy \cite{66,104}. One reason for the anti-hypertrophic effect of broad spectrum HDAC inhibitors is that these HDAC inhibitors also target the pro-hypertrophic Class I HDACs. The Class I HDACs repress anti-hypertrophic signals and their role is probably dominant over the roles of the Class II HDACs \cite{105}. Another explanation for this could be that many proteins are acetylated and that inhibiting deacetylase enzymes increases acetylation of a subset of proteins which results in repression of hypertrophy \cite{104}. Large scale studies to assess the \textit{in vivo} effect of HDAC inhibitors are essential to establish whether they are able to repress pathological cardiac remodeling \cite{102}. These studies are currently lacking. Phase I clinical trials have been performed to study the effect of HDAC inhibitors on specific types of cancer, but the cardiac effects were not examined \cite{105,106}. Future studies are also warranted to define the therapeutic window of HDAC inhibitors for the treatment of pathological hypertrophy and heart failure.

With the identification of an increasing number of negative regulators of hypertrophy and the recent developments in chemical and viral based methods to manipulate the activity of those repressors, the future should be able to bring us new therapies for cardiac hypertrophy and heart failure.
REFERENCES


43. Jeong D, Kim JM, Oh JG, Park CS, Park J, Yun SH, Ju EJ, Leong EJ, Hajjar RJ, Park WJ. PICOT attenuates


56. Santorelli V, Huang J, Hamamoni Y, Kedes L. Molecular mechanisms of myogenic coactivation by p300: direct interaction with the activation domain of MyoD and with the MADS box of MEF2C.


Chapter 2


Chapter 2


### Chapter 2

**GENE** | **FUNCTION** | **COMMENTS** | **REF**
---|---|---|---
**CLASS I - reduced**

<table>
<thead>
<tr>
<th><strong>GENE</strong></th>
<th><strong>FUNCTION</strong></th>
<th><strong>COMMENTS</strong></th>
<th><strong>REF</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>PPARγ</td>
<td>Nuclear receptor</td>
<td>PPARγ−/− mice are more susceptible to PA-induced hypertrophy</td>
<td>90</td>
</tr>
<tr>
<td>PLA2G4A</td>
<td>Phospholipase</td>
<td>Phospholipase A2, ER stress (loss of PLA2G4A results in cardiomyopathy, cardiac growth)</td>
<td>91</td>
</tr>
<tr>
<td>OR51A</td>
<td>Kinase</td>
<td>Null mice develop pacemakers dependent on Gαq, hypertrophy and death due to stimulation</td>
<td>102</td>
</tr>
<tr>
<td>GNAI1</td>
<td>Gαi</td>
<td>Overexpression represses calcium-mediated hypertrophy</td>
<td>110, 111</td>
</tr>
<tr>
<td>MCNP2</td>
<td>Ca2+ binding</td>
<td>in vivo gene transfer of MCNP2 represses cardiomyocyte hypertrophy</td>
<td>112, 113</td>
</tr>
</tbody>
</table>

**CLASS II - induced on stress**

<table>
<thead>
<tr>
<th><strong>GENE</strong></th>
<th><strong>FUNCTION</strong></th>
<th><strong>COMMENTS</strong></th>
<th><strong>REF</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sirt3</td>
<td>Histone deacetylase</td>
<td>Prevents mitochondrial ROS accumulation. Sirt3 represses ROS-induced activation of pro-hypertrophic MAPK and Akt signaling.</td>
<td>74, 114</td>
</tr>
<tr>
<td>G6PD</td>
<td>GTPase</td>
<td>Absence of G6PD enhances TAC, PE and exercise-induced hypertrophy, via attenuation of JNK activation. G6PD and INS-2 antagonize calcineurin-NFAT activity.</td>
<td>26</td>
</tr>
<tr>
<td>MXR</td>
<td>MAP-kinase</td>
<td>Kinase specifically activating JNK. Mice lacking MXR have exaggerated hypertrophy in response to TAC. Exercise induced hypertrophy is not affected.</td>
<td>17</td>
</tr>
<tr>
<td>Jnk</td>
<td>MAP-kinase</td>
<td>Jnk represses hypertrophy by antagonizing calcineurin-mediated NFAT dephosphorylation.</td>
<td>16</td>
</tr>
<tr>
<td>Cdc42</td>
<td>GTPase</td>
<td>Absence of Cdc42 enhances TAC-, PE- and exercise-induced hypertrophy, via attenuation of JNK activation. Cdc42 and JNK antagonize calcineurin-NFAT activity.</td>
<td>26</td>
</tr>
<tr>
<td>MKK4</td>
<td>MAP-kinase</td>
<td>MAP-kinase specifically activating JNK. Mice lacking MKK4 have exaggerated hypertrophy in response to TAC. Exercise induced hypertrophy is not affected.</td>
<td>27</td>
</tr>
<tr>
<td>JNK</td>
<td>MAP-kinase</td>
<td>Jnk represses hypertrophy by antagonizing calcineurin-mediated NFAT dephosphorylation.</td>
<td>16</td>
</tr>
<tr>
<td>Calsarcin-1</td>
<td>Calcineurin anchoring protein</td>
<td>Mutant mice display an enhanced calcineurin and TAC mediated hypertrophic response. β-Adrenergic or exercise induced hypertrophy is not enhanced. Overexpression is protective.</td>
<td>36, 37, 115</td>
</tr>
<tr>
<td>PICOV/SOX3</td>
<td>PAC and calcium binding protein</td>
<td>PACO/βC and calcium binding protein repress pro-hypertrophic signaling, presumably by repressing ERK1/2 signaling.</td>
<td>41-43</td>
</tr>
<tr>
<td>MCIP1/RCAN1</td>
<td>Calcium-binding protein</td>
<td>Overexpression protects against calcineurin, isoproterenol and exercise induced hypertrophy. Null mice are more susceptible to LVH.</td>
<td>116-118</td>
</tr>
<tr>
<td>RALT</td>
<td>GTPase</td>
<td>Overexpression protects against Ang II-induced hypertrophy, fibrosis, presumably by repression of ERK1/2 and Akt signaling.</td>
<td>119</td>
</tr>
<tr>
<td>CAV3</td>
<td>Scaffolding protein</td>
<td>CAV3 null mice develop spontaneous hypertrophy, presumably by repression ERK1/2 signaling.</td>
<td>120, 121</td>
</tr>
<tr>
<td>ANF</td>
<td>c-Fos</td>
<td>Forset overexpression of ANF protects against hypertrophy by repressing pro-hypertrophic TAC signaling.</td>
<td>122, 123</td>
</tr>
<tr>
<td>ROSA</td>
<td>GTPase</td>
<td>ROSA represses pro-hypertrophic signaling, presumably by repression of G protein-coupled receptor signaling.</td>
<td>124</td>
</tr>
<tr>
<td>ROSA</td>
<td>GTPase activating protein</td>
<td>Transgenic mice overexpressing ROSA are protected against hypertrophy and have reduced MAP kinase signaling.</td>
<td>125, 126</td>
</tr>
<tr>
<td>SOC3</td>
<td>Suppressor of cytokine signaling</td>
<td>SOC3 represses pro-hypertrophic signaling.</td>
<td>127</td>
</tr>
<tr>
<td>SOCS3</td>
<td>Suppressor of cytokine signaling</td>
<td>Suppressor of cytokine signaling represses pro-hypertrophic signaling.</td>
<td>127</td>
</tr>
<tr>
<td>A20</td>
<td>Zinc finger protein</td>
<td>Forced overexpression of A20 protects against hypertrophy by repressing calcineurin/NFAT signaling.</td>
<td>128, 129</td>
</tr>
<tr>
<td>RGS4</td>
<td>GTPase activating protein</td>
<td>Overexpression represses cardiac hypertrophy and reduces MAP kinase signaling.</td>
<td>125, 126</td>
</tr>
<tr>
<td>AMPK</td>
<td>AMP-activated protein</td>
<td>AMPK represses cardiac hypertrophy and reduces MAP kinase signaling.</td>
<td>125, 126</td>
</tr>
<tr>
<td>RGS2</td>
<td>GTPase</td>
<td>RGS2 overexpression represses cardiac hypertrophy and reduces MAP kinase signaling.</td>
<td>125, 126</td>
</tr>
<tr>
<td>RGS4</td>
<td>GTPase activating protein</td>
<td>Transgenic mice overexpressing RGS4 are protected against hypertrophy and have reduced MAP kinase signaling.</td>
<td>125, 126</td>
</tr>
<tr>
<td>cFlip</td>
<td>Caspase inhibitor</td>
<td>Overexpression represses cardiac hypertrophy and reduces MAP kinase signaling.</td>
<td>130, 131</td>
</tr>
<tr>
<td>S100β</td>
<td>Calcium-binding protein</td>
<td>Overexpression represses cardiac hypertrophy and reduces MAP kinase signaling.</td>
<td>130, 131</td>
</tr>
<tr>
<td>AMPK</td>
<td>AMP-activated protein</td>
<td>AMPK represses cardiac hypertrophy and reduces MAP kinase signaling.</td>
<td>130, 131</td>
</tr>
</tbody>
</table>

**Table 1. Repressors of signal transduction**
Table 2. Repressors of transcription

<table>
<thead>
<tr>
<th>GENE</th>
<th>FUNCTION</th>
<th>COMMENTS</th>
<th>REF</th>
</tr>
</thead>
<tbody>
<tr>
<td>KLF15</td>
<td>Transcription factor</td>
<td>KLF15 null mice are susceptible to TAC and Ang-II induced hypertrophy, presumably by repressing GATA4 and myocardin activity.</td>
<td>17,83,84</td>
</tr>
<tr>
<td>KLF14</td>
<td>Transcription factor</td>
<td>Mice lacking KLF14 specifically in the heart have hypertrophy at baseline and are sensitized to pressure overload-induced hypertrophy and heart failure. This transcription factor represses hypertrophy by inhibition of GATA4 expression.</td>
<td>87</td>
</tr>
<tr>
<td>HSAD3 and 9</td>
<td>Class II histone deacetylase</td>
<td>Knocked out mice develop age-dependent hypertrophy and are sensitive to pressure overload-induced hypertrophy.</td>
<td>57,60</td>
</tr>
<tr>
<td>HDAC3</td>
<td>Class I histone deacetylase</td>
<td>Represses hypertrophy via MEF2 and the regulation of cardiac energy metabolism.</td>
<td>69,135</td>
</tr>
<tr>
<td>NAB1</td>
<td>Transcriptional repressor</td>
<td>Endogenous repressor of early growth response transcription factor 1 (Egr1). Mice with cardiac specific overexpressing of Nab1 are protected against TAC- and ISO/PE- but not exercise-induced hypertrophy.</td>
<td>136</td>
</tr>
<tr>
<td>LKB1a</td>
<td>Transcription factor</td>
<td>LKB1a null mice are resistant to TAC-induced hypertrophy, via suppression of HIF1 signaling.</td>
<td>137</td>
</tr>
<tr>
<td>MNP-1</td>
<td>Phosphatase</td>
<td>MNP-1 overexpressing mice fail to develop pressure overload induced hypertrophy, most likely by repressing pro-hypertrophic MAP kinase signaling.</td>
<td>30,138</td>
</tr>
<tr>
<td>DYNLT4</td>
<td>Kinase</td>
<td>Hypersensitivity of Dyn4 to mitosis-sensitive mice to TAC-induced hypertrophy.</td>
<td>139,140</td>
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<tr>
<td>IER2/IRX-1</td>
<td>Immediate early gene</td>
<td>IER2 is rapidly upregulated and transported to the nucleus during hypertrophy. Represses cardiomyocyte growth in vitro.</td>
<td>143</td>
</tr>
<tr>
<td>SIRT1</td>
<td>NAD dependent deacetylase</td>
<td>SIRT1 represses hypertrophy by protecting the heart against oxidative stress</td>
<td>73</td>
</tr>
<tr>
<td>TRK1</td>
<td>Antioxidant</td>
<td>Reduces oxidized HDAC, thereby promoting nuclear localization. Also represses Ras/ERK signaling. Mice overexpressing Trk are less sensitive to hypertrophy.</td>
<td>99,80</td>
</tr>
<tr>
<td>KLF30</td>
<td>Transcriptional regulator</td>
<td>TGFβ induced transcription factor. KLF30 null mice develop age-related hypertrophy.</td>
<td>91</td>
</tr>
<tr>
<td>BACH1</td>
<td>Transcription factor</td>
<td>Bach1 null mice have an impaired TAC-induced hypertrophic response, possibly via heme oxygenase-1 (HO-1).</td>
<td>142</td>
</tr>
<tr>
<td>CHF1/Hey2</td>
<td>bHLH transcription factor</td>
<td>Overexpression represses cardiac growth via an inhibitory interaction with GATA4. Heterozygous mice have a more pronounced hypertrophic response to aortic banding.</td>
<td>143,144</td>
</tr>
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</table>
### Table 3. Genes that repress the hypertrophic response when overexpressed in the mouse heart

<table>
<thead>
<tr>
<th>Gene</th>
<th>Transgenic Model</th>
<th>Hypertrophy model</th>
<th>Cardiac function improved compared to WT?</th>
<th>REF</th>
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<tbody>
<tr>
<td>RALT1</td>
<td>αMHC-Ralt</td>
<td>AngII and Iso</td>
<td>Yes (%FS)</td>
<td>119</td>
</tr>
<tr>
<td>CHF1-HEY2</td>
<td>αMHC-CHF1</td>
<td>TAC</td>
<td>Yes (%FS)</td>
<td>145</td>
</tr>
<tr>
<td>SIRT3</td>
<td>αMHC-SIRT3</td>
<td>Iso</td>
<td>Yes (%FS)</td>
<td>74</td>
</tr>
<tr>
<td>PLOC1</td>
<td>αMHC-PLOC1</td>
<td>TAC</td>
<td>Yes (%FS)</td>
<td>41</td>
</tr>
<tr>
<td>RCAN/RCNP1</td>
<td>αMHC-RCAN</td>
<td>calcineurin</td>
<td>Yes (%FS)</td>
<td>116</td>
</tr>
<tr>
<td>TRX1</td>
<td>αMHC-Trx1</td>
<td>TAC</td>
<td>N/A</td>
<td>80</td>
</tr>
<tr>
<td>NAB1</td>
<td>αMHC-Nab1</td>
<td>Iso, PE and TAC</td>
<td>N/A</td>
<td>136</td>
</tr>
<tr>
<td>NPP1</td>
<td>αMHC-Npp1</td>
<td>Iso and TAC</td>
<td>N/A</td>
<td>12</td>
</tr>
<tr>
<td>A20</td>
<td>αMHC-A20</td>
<td>TAC</td>
<td>N/A</td>
<td>123</td>
</tr>
<tr>
<td>GSK3B</td>
<td>αMHC-Gsk3B</td>
<td>Iso and calcineurin</td>
<td>N/A</td>
<td>110</td>
</tr>
<tr>
<td>Labinan-1</td>
<td>αMHC-Labinan-1</td>
<td>AngII</td>
<td>N/A</td>
<td>37</td>
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<tr>
<td>S100B</td>
<td>S100B-S100B</td>
<td>NE</td>
<td>No</td>
<td>129</td>
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<tr>
<td>c-FLIP</td>
<td>Stra8-C-Flip</td>
<td>TAC</td>
<td>No</td>
<td>134</td>
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<tr>
<td>RGS4</td>
<td>αMHC-Rgs4</td>
<td>TAC</td>
<td>decreased survival</td>
<td>125</td>
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</table>