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REVIEW

Molecular switches under TGFβ signalling during progression from cardiac hypertrophy to heart failure

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Cardiac hypertrophy is a mechanism to compensate for increased cardiac work load, that is, after myocardial infarction or upon pressure overload. However, in the long run cardiac hypertrophy is a prevailing risk factor for the development of heart failure. During pathological remodelling processes leading to heart failure, decompensated hypertrophy, death of cardiomyocytes by apoptosis or necroptosis and fibrosis as well as a progressive dysfunction of cardiomyocytes are apparent. Interestingly, the induction of hypertrophy, cell death or fibrosis is mediated by similar signalling pathways. Therefore, tiny changes in the signalling cascade are able to switch physiological cardiac remodelling to the development of heart failure. In the present review, we will describe examples of these molecular switches that change compensated hypertrophy to the development of heart failure and will focus on the importance of the signalling cascades of the TGFβ superfamily in this process. In this context, potential therapeutic targets for pharmacological interventions that could attenuate the progression of heart failure will be discussed.

Abbreviations

ALK, activin receptor-like kinase; AMPK, AMP kinase; ANT1, adenine nucleotide translocator 1; AP-1, activator protein 1; Hsp, heat shock protein; IGF2R, insulin-like growth factor receptor II; JDP2, jun dimerization protein 2; LNA, locked nucleic acid; LV, left ventricle; miRNA, microRNA; MPTP, mitochondrial permeability transition pore; NLRP3, nucleotide-binding domain and leucine-rich repeat containing PYD-3; PAH, pulmonary hypertension; RIP, receptor interacting protein; RV, right ventricle; siRNA, silencing RNA; SIRT1, sirtuin 1; SMAD, small mothers against decapentaplegic; TAC, transverse aortic constriction; TAK1, TGFβ activated kinase 1; TGFBR1, TGFβ receptor I; TGFBR2, TGFβ receptor II; TOM, translocase of the mitochondrial outer membrane; UPS, ubiquitin proteasome system; VDAC1, voltage-dependent anion channel-1

Tables of Links

TARGETS		
GPCRs[a]	**Enzymes**[d]	
α-adrenoceptors	AMPK	PDK (PDHK)
β-adrenoceptors	Caspase 8	RIP1 (RIPK1)
AT_1 receptor	ERK	RIP3 (RIPK3)
Catalytic receptors[b]	GRK2	Sirtuin 1
NLRP3	JNK	TAK1
Transporters[c]	p38	TGFBR1 (ALK5)
ANT1		TGFBR2
GLUT1-4		
SERCA2		

LIGANDS	
Angiotensin II	Losartan
Bcl-2	Myostatin
Captopril	Nitric oxide (NO)
Isoprenaline (ISO)	Noradrenaline (NA)
L-NAME	TGFβ1

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 ([a,b,c,d]Alexander *et al.*, 2013a,b,c,d).

Introduction

The main causes of heart failure are on the one hand chronic pressure overload of the left ventricle (LV) resulting in hypertension and on the other the impairment of myocardial perfusion resulting in acute myocardial infarction or chronic hypoperfusion (Hoppe and Erdmann, 2009). While pressure overload creates hypertension and results in cardiac hypertrophy, myocardial infarction primarily results in a loss of cardiomyocytes that is compensated for by hypertrophy of the remaining cells, the generation of fibrosis and ventricular dilatation. Thus, the remodelling processes, caused by pressure overload or ischaemia are different, but both eventually result in heart failure. This has to be considered when using different animal models, which are induced either by chronic pressure overload by aortic banding or by direct damage after myocardial infarction.

In spite of these differences, in both situations, the organism reacts by activation of the sympathetic nervous system and the release of local mediators (cytokines and natriuretic peptides) to ensure a sufficient blood supply under these conditions, thereby resulting in intra- and intercellular remodelling processes. Temporarily, this leads to compensated hypertrophy and preserved heart function. However, in the long run, progressive myocardial dysfunction develops (Narula *et al.*, 1996), either resulting in around 50% of the patients having an impaired diastolic function with preserved ejection fraction, while the other 50% of patients develop systolic dysfunction with reduced ejection fraction (Abbate *et al.*, 2015). With regard to the reduced ejection fraction, apoptotic and necroptotic loss of cardiomyocytes, contractile dysfunction of cardiomyocytes and massive fibrosis are significant factors for the transition from compensated hypertrophy to decompensation and deterioration of systolic heart function, which will be the main focus of the current review.

Enhanced levels of TGFβ are found in patients with heart failure (Khan *et al.*, 2014), in various animal models of cardiac remodelling and during the transition from compensated hypertrophy to heart failure (Boluyt *et al.*, 1994; Lijnen *et al.*, 2000; Rosenkranz, 2004). Therefore, there is a huge drive to clarify the role of TGFβ in heart failure progression. Interestingly, TGFβ modulates nearly all processes that are engaged in heart failure development, that is, cardiac hypertrophy, fibrosis, apoptosis, inflammation and differentiation of cardiac progenitor cells. In spite of this, broad inhibition of TGFβ signalling does not only have positive effects on heart failure progression. Administration of the TGFβ receptor I (TGFBR1) inhibitor (SM16) after aortic banding prevented cardiac fibrosis and attenuated cardiac dysfunction. However, mortality rates increased due to enhanced left ventricular dilatation and inflammation (Engebretsen *et al.*, 2014). Similar results have been found when soluble TGFBR2 was applied after myocardial infarction. In this case, the increase in mortality rates was probably due to reduced inflammatory responses (Ikeuchi *et al.*, 2004). Therefore, a more target-orientated approach needs to be used to inhibit the detrimental TGFβ pathways.

TGFβ signals through binding at a heterotetrameric receptor complex of type II and type I receptor serine/threonine kinases. Upon TGFβ binding, TGFBR2 phosphorylates and thereby activates type I receptor serine/threonine kinases that in turn phosphorylates and activates SMAD transcription factors. Depending on the subtype of type I receptor serine/threonine kinases, also known as activin receptors or activin receptor-like kinases (ALKs), different receptor-activated SMADs (R-SMADs) become activated. In cardiomyocytes and also many other cell types, $TGF\beta_1$ signalling is attributed to TGFBR1, also called ALK5, which then results in SMAD2/3 activation. In endothelial cells TGFβ has also been shown to signal via ALK1 and SMAD1/5/8 (Goumans *et al.*, 2002). However, this appears to be no longer exclusive for endothelial cells; In other cells, TGFbeta1 stimulation has been found to activate SMAD1/5 and SMAD2/3 as well. (Wharton and Derynck, 2009). We also identified both responses in cardiomyocytes. After stimulation of ventricular cardiomyocytes, from adult rats, with 1 $ng \cdot ml^{-1}$ TGFβ1 for 2 h, enhanced phosphorylation of SMAD2/3, SMAD1/3 and SMAD1/5 was detected in Western blots ($n = 5$, $P < 0.05$ vs. unstimulated controls) (Figure 1), thereby indicating that TGFβ signalling is even more complicated than originally thought. Activated R-SMADs form a complex with SMAD4 that translocates into the nucleus and acts as a transcription factor. The binding specificity of SMADs to promoters can be influenced by their association with other transcription factors like activator protein 1 (AP-1). In addition to this canonical SMAD pathway, another prominent signalling molecule of TGFβ is TGFβ-activated kinase 1 (TAK1). TAK1 activation is also mediated by TGFBR2. Downstream targets of TAK1 are c-Jun, N-terminal kinase (JNK) and p38. Furthermore, via binding to its receptor, TGFβ can activate other kinases like ERK, phosphoinositide 3-kinase (PI3K) or small GTPases like Rho (reviewed by Zhang, 2009). This huge variety of TGFβ signalling pathways already implies that the effects of TGFβ in tissues will be complex. In this review, we highlight the signalling molecules that are induced by TGFβ and modulate adverse cardiac remodelling by interfering with adrenoceptor-mediated signalling, mitochondrial proteins, cell death, microRNAs (miRNAs), contractile function or fibrosis. (Figure 2).

The TAK1 pathway is pro-hypertrophic and prevents cell death while SMADs promote apoptotic signalling in the heart

TGFβ itself is known to be a pro-hypertrophic, pro-apoptotic and pro-fibrotic factor in the heart. TAK1 and not SMADs seems to be the main mediator of TGFβ-induced hypertrophic growth effects. TAK1 is found to be up-regulated *in vivo* after aortic banding, and TAK1 overexpression promotes cardiac hypertrophy in transgenic mice (Zhang *et al.*, 2000) (Figure 3). Furthermore, in neonatal cardiomyocytes, angiotensin II-induced hypertrophic growth could be prevented by knockdown of TAK1 with silencing RNA (siRNA), but not with siRNA against SMAD2/3 (Watkins *et al.*, 2012). This indicates that SMAD signalling is not involved in angiotensin II – $TGF\beta_1$-induced hypertrophic growth. In addition to its pro-hypertrophic effects, TAK1 antagonizes the apoptosis and

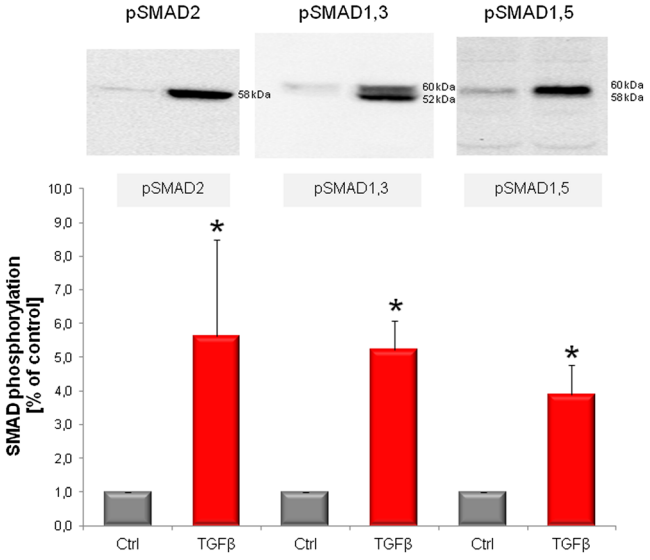

Figure 1

TGFβ signals via the SMAD2/3 and SMAD1/5 pathway in cardiomyocytes. Ventricular cardiomyocytes of adult rat were stimulated with 1 ng·ml^{-1} TGFβ1 for 2 h. Protein extracts of these cells were analysed by Western blots with antibodies specific against phosphoSMAD2, phosphoSMAD1/3 or phosphoSMAD1/5. Phosphorylation, which is indicative of SMAD activation, was detected for all these SMADs. *$P < 0.05$ versus unstimulated controls, $n = 5$ independent culture preparations.

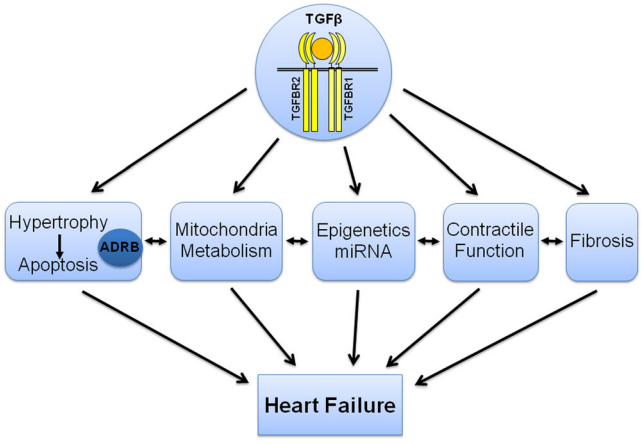

Figure 2

Overview about TGFβ influence on components of cardiac remodelling in left ventricular systolic heart failure. TGFβ has been shown to promote the transition from cardiac hypertrophy to apoptosis and to regulate mitochondrial signalling molecules, miRNA expression and contractile function and fibrosis. All these processes are involved in heart failure progression.

necroptosis induced by TNFα stimulation and prevents adverse cardiac remodelling (Li *et al.*, 2014a). Necroptosis is a form of cell death, which combines features of necrotic and apoptotic cell death, it is a death receptor-mediated process which is executed via receptor activating protein (RIP) complexes (Zhang *et al.*, 2009). During TNFα stimulation, TAK1 associates with RIP1 thereby preventing RIP1 interaction with other death signalling proteins, that is, with caspase 8 or RIP3. This results in a reduction in apoptosis and necroptosis (Li *et al.*, 2014a). As TAK1 is not only induced by TGFβ and TNFα but also by other cytokines (Besse *et al.*, 2007), strong TAK1 activation may act as a pro-survival factor in the heart.

In contrast to TAK1, SMAD signalling seems to counteract hypertrophy, because hypertrophic growth of cardiomyocytes induced by stimulation of α-adrenoceptors was hampered by simultaneous overexpression of SMADs (Heger *et al.*, 2009). Hypertrophic growth of cardiomyocytes induced by stimulation of α-adrenoceptors is mediated via the transcription factor AP-1 (Taimor *et al.*, 2004). Under simultaneous SMAD4 overexpression, AP-1/SMAD complexes are formed, which may detract AP-1 from its hypertrophy-promoting target genes. Indeed, a shift from hypertrophy to the induction of apoptosis is found in α-adrenoceptor-stimulated and SMAD4 overexpressing cardiomyocytes (Heger *et al.*, 2009). Furthermore, cardiac-specific SMAD4 knock-out mice displayed cardiac hypertrophy (Wang *et al.*, 2005). This indicates that SMAD4 acts as a molecular switch for transition from hypertrophy to apoptosis. In addition, TGFβ induces apoptosis in adult cardiomyocytes via enhancement of SMAD and AP-1 activity (Schneiders *et al.*, 2005). Similar to these findings, inhibition of SMAD signalling *in vivo* may preserve the compensating character of hypertrophic growth in cardiac remodelling while preventing the transition to apoptosis (Figure 3).

That AP-1 is a mediator of hypertrophy and apoptosis in β-adrenoceptor stimulated cardiomyocytes has been shown by use of transgenic mice overexpressing the AP-1 inhibitor jun dimerization protein 2 (JDP2). JDP2 overexpression prevented isoprenaline (ISO)-induced hypertrophy as well as TGFβ-induced apoptosis in cardiomyocytes (Hill *et al.*, 2013). But AP-1 is also required to preserve the contractile function of cardiomyocytes because AP-1 inhibition by JDP2 overexpression attenuated contractile responses induced by β-adrenoceptor stimulation (Hill *et al.*, 2013). Therefore, to prevent adverse remodelling, inhibition of SMAD signalling seems to be the better choice than inhibition of AP-1, because this would negatively influence the contractile function of the heart.

Modulation of β-adrenoceptor responses in the presence of TGFβ

During heart failure, progressive desensitization of β-adrenoceptors occurs. β-adrenoceptors are members of the GPCR superfamily whose stimulation results in activation of PKA via AC and cAMP, which regulate different intracellular, sarcolemmal and myofibrillar substrates. Thus, cAMP exerts the cellular effects on cardiac contractile function induced by activation of β-adrenoceptors. However, stimulation of β-adrenoceptors also results in agonist-dependent desensitization of these receptors, a phenomenon found during the development of heart failure. This process is mediated by the receptor adapter protein β-arrestin that binds to β-adrenoceptors. This binding either results in direct

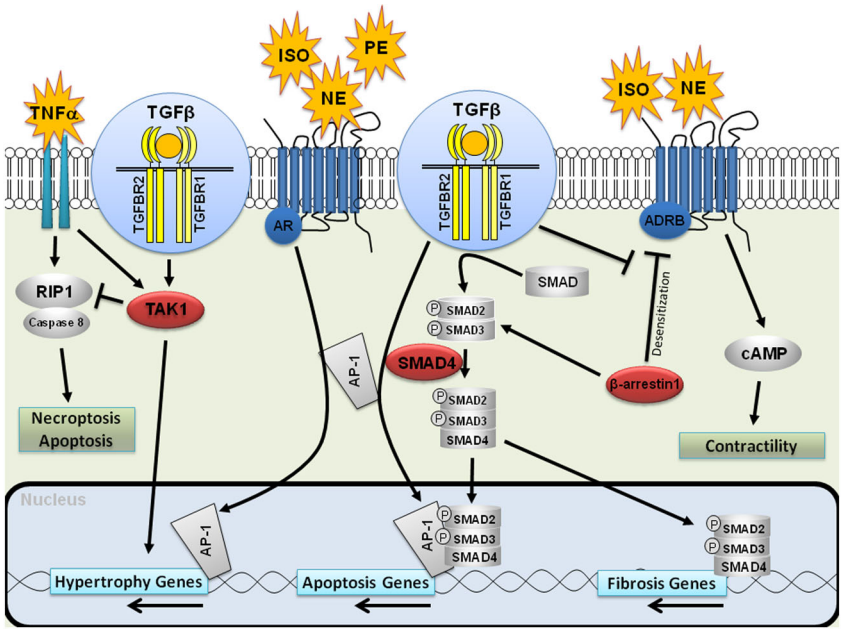

Figure 3

Influence of TGFβ-SMAD and TGFβ-TAK1 signalling on adrenoceptor-mediated pathways in LV heart failure progression. Adrenoceptors (AR) stimulate the expression of genes promoting hypertrophic growth via the transcription factor AP-1. Under simultaneous presence of SMAD4, the pro-hypertrophic response to adrenoceptor stimulation is shifted to a pro-apoptotic gene transcription via AP-1/SMAD complexes. Also, under TGFβ stimulation of cardiomyocytes, AP-1 and SMADs mediate apoptosis. In addition to these effects on cardiomyocytes, activation of the TGFβ/SMAD pathway or induction of SMADs via β-arrestins induces the transcription of fibrotic genes. In contrast to the SMAD pathway, TAK1 activation stimulates hypertrophic growth while inhibiting cardiac necroptosis and apoptosis by interacting with RIP1. Strong β-adrenoceptor (ADRB) activation results in β-adrenoceptor desensitization via β-adrenoceptor /β-arrestin complexes. This process can be inhibited by TGFβ. Depicted in red are switch molecules that can modulate the response of the cell to receptor stimulation and thereby influence the outcome of this stimulation on the remodelling process.

inhibition of β-adrenoceptors, known as functional desensitization, or in internalization of β-adrenoceptors that reduces their density (reviewed by Lymperopoulos and Negussie, 2013). Studies in β-arrestin1 knock-out mice demonstrated a major role for β-arrestin1 in cardiac dysfunction, because contractile responses to β-adrenoceptor stimulation were enhanced in these knock-out animals (Conner *et al.*, 1997). Furthermore, knock-down of β-arrestin1 prevented adverse cardiac remodelling after myocardial infarction by inhibiting apoptosis and preserving cardiac function (Bathgate-Siryk *et al.*, 2014). Interestingly, an increase in myocardial β-adrenoceptor density and a reduction in negative regulators such as $G_{i\alpha}$ and β-adrenoceptor-kinase-1 were demonstrated in transgenic TGFβ-overexpressing mice (Rosenkranz *et al.*, 2002). And in isolated cardiomyocytes of adult rat, TGFβ enhanced the hypertrophic response to β-adrenoceptor stimulation (Schlüter *et al.*, 1995). These findings indicate that TGFβ can prevent β-adrenoceptor desensitization in cardiomyocytes and thereby promote pro-hypertrophic signalling. Whether this response is mediated by the down-regulation of β-arrestin1 by TGFβ has not yet been clarified. But TGFβ may be a plausible target in order to prevent β-adrenoceptor desensitization.

So far, a connection between β-arrrestin expression and TGFβ signalling has been shown in cardiac fibroblasts. β-Arrestins were found to be up-regulated in cardiac fibroblasts during heart failure. Overexpression of β-arrestin in cardiac fibroblasts results in the uncoupling of β-adrenoceptors and activation of SMAD2/3, thereby promoting a pro-fibrotic phenotype. This may cause enhanced stiffness of the ventricular wall and contribute to the development of heart failure.

Although TGFβ stimulation prevents the uncoupling of β-adrenoceptors and enhances the pro-hypertrophic signalling, the inotropic β-adrenoceptor-mediated response was diminished in TGFβ-overexpressing mice. This is due to an up-regulation of mitochondrial uncoupling proteins during β-adrenoceptor stimulation, which results in a decreased mitochondrial energy production. Thus, TGFβ-overexpressing mice resemble a phenotype occurring at the transition to heart failure, namely, displaying cardiomyocytes hypertrophy and promoting apoptosis as well as mitochondrial and contractile dysfunction (Schneiders *et al.*, 2005; Huntgeburth *et al.*, 2011).

That these interacting pathways of ADRB-TGFβ signalling are even more complex was indicated by the findings that GPCRs not only activate TK receptors but also also transactivate the serine/threonine kinase TGFBR1 in different cell types (Burch *et al.*, 2012). The proposed mechanism for this transactivation is activation of integrin by GPCRs. Subsequently, integrin binding to the large latent TGFβ complex causes a conformational change and allows TGFβ to bind and activate TGFBR2/TGFBR1, thereby resulting in SMAD

activation (Munger *et al.*, 1999). Whether this β-adrenoceptor-induced SMAD signalling holds true in cardiomyocytes has yet to be analysed.

The ubiquitin system in the context of β-adrenoceptor and TGFβ stimulation

Another focus for identification of the triggers contributing to heart failure development or progression relies on the analysis of the proteasome, as degradation of proteins is changed in cardiac hypertrophy. The primary cellular response to β-adrenoceptor stimulation in the heart is an increased pool of 20S subunits with catalytic activity, while chronic β-adrenoceptor stimulation enhanced the 26S proteasome but decreased 20S proteasomal activity, accompanied by a decrease in ubiquitinated proteins (Drews *et al.*, 2010). Elevated 26S proteasome activities were also observed in a pressure overload model stimulating ventricular hypertrophy (Depre *et al.*, 2006). The switch in proteasome subpopulations, which is facilitated by different β-subunits of the proteasome, is decisive for the development of hypertrophy and depends again on the strength of β-adrenoceptor activation. Proteins involved in cardiac hypertrophy are targeted by muscle-specific ubiquitin ligase atrogin-1 for degradation (Zaglia *et al.*, 2014). Atrogin-1 KO hearts revealed increased apoptosis and hypertrophy. The effects were mediated by the up-regulation of an autophagy controlling protein, the endosomal sorting complex required for transport III (ESCRT-III) family protein charged multivesicular body protein 2B (CHMP2B). Thus, Zaglia *et al.* (2014) demonstrated the interplay between the ubiquitin proteasome system (UPS) and autophagy and the importance of controlled degradation of proteins for the control of cardiac hypertrophy and apoptosis.

UPS regulates important signalling pathways in the heart, including MAPK, JNK and calcineurin (Portbury *et al.*, 2012). Huang *et al.* (2014) suggested a proteasome-dependent mechanism for angiotensin II–induced apoptosis in hearts that is accompanied by activation of insulin-like growth factor receptor II (IGF2R) signalling. Heat shock transcription factor 1 (HSF1) acts as a repressor of IGF2R gene expression only if deacetylated by sirtuin 1. However, angiotensin II and subsequently JNK activation mediates sirtuin 1 degradation via the proteasome. This results in an increase in the acetylation of HSF1 that is then not able to bind to the IGF2R promoter So, sirtuin is a negative regulator of IGF2R, thereby protecting cardiomyocytes from apoptosis. In this context, it is remarkable that the IGF2R is required for the activation of latent TGFβ (Dennis and Rifkin, 1991). In human umbilical-vein endothelial cells, the association of IGF2R and the urokinase receptor –converts plasminogen (uPAR) to active plasmin – is essential for the activation of latent TGFβ, the release of TGFβ and induction of apoptosis (Leksa *et al.*, 2005). Whether this also holds true for cardiomyocytes remains to be evaluated, but we have already shown that angiotensin II induces the release of TGFβ and SMAD-dependent apoptosis in cardiomyocytes (Schröder *et al.*, 2006). Not only is the intracellular activity of TGFβ controlled by UPS but also the stability and levels of TGFβ receptor complexes are determined by ubiquitination (Xu *et al.*, 2012).

Influence of TGFβ on mitochondria, energy metabolism and heart failure

Mitochondria are the power houses of the cell, generating ATP via oxidative phosphorylation. On average, 30% of the cardiomyocytes volume is filled with mitochondria (Barth *et al.*, 1992). One side product of the major respiratory enzyme complexes is the generation of reactive oxygen species (ROS) that modifies the redox potential of the cell and is essential for numerous signalling pathways (Chen and Zweier, 2014). Mitochondrial dysfunction occurs under pathophysiological conditions and involves malfunction of complexes of oxidative phosphorylation, and an increase in ROS production that leads to cell death contributing to the development of heart failure. The enzymes of the respiratory chain seem to be the main site of ROS formation, but many other enzymes contribute to ROS production in failing hearts, including monoamine oxidases and the cytosolic adaptor protein $p66^{Shc}$ (Di Lisa *et al.*, 2009). Cellular stress signals lead to translocation of $p66^{Shc}$ into the mitochondrial intermembrane space, where it oxidizes cytochrome c and generates ROS (Heusch, 2015). Factors that influence ROS production, therefore, critically determine the cell's fate.

A newly identified signalling molecule in the control of mitochondrial ROS production that is under the control of TGFβ signalling is nucleotide-binding domain and leucine-rich repeat containing PYD-3 (NLRP3), a pattern recognition receptor that is involved in the pathogenesis of chronic diseases and inflammation. NLRP3 is expressed in the heart, localized in mitochondria, and interacts with components of the redox system (Figure 4). Upon TGFβ stimulation of cardiac fibroblasts, NLRP3 increases mitochondrial ROS production, which supports SMAD2 phosphorylation and results in the differentiation of cardiac fibroblasts into myofibroblasts, an important process in adverse cardiac remodelling (Bracey *et al.*, 2014). The involvement of NLRP3 in cardiac fibrosis has been confirmed in an *in vivo* model of hypertension: angiotensin II infusion for 28 days resulted in TGFβ-mediated fibrosis in wild-type mice, but NLRP3-deficient mice were protected against this angiotensin II-induced fibrosis. NLRP3, therefore, is a newly identified mitochondrial signalling factor in TGFβ-induced cardiac remodelling that may promote the transition to heart failure as it facilitates ROS-mediated fibrosis.

Increased ROS production induces the opening of the mitochondrial permeability transition pore (MPTP) (Figure 4) that changes the permeability of the inner mitochondrial membrane, leading to mitophagy, fusion/fission events and biogenesis (Brenner and Moulin, 2012). Opening of the MPTP facilitates the release of pro-apoptotic factors from the mitochondria that stimulates the activation of caspases and finally leads to cell death (Kinnally *et al.*, 2011). The addition of noradrenaline induced a concentration-dependent decrease in mitochondrial membrane potential that was associated with a switch from compensated hypertrophy to apoptosis, thereby indicating that MPTP opening is involved in

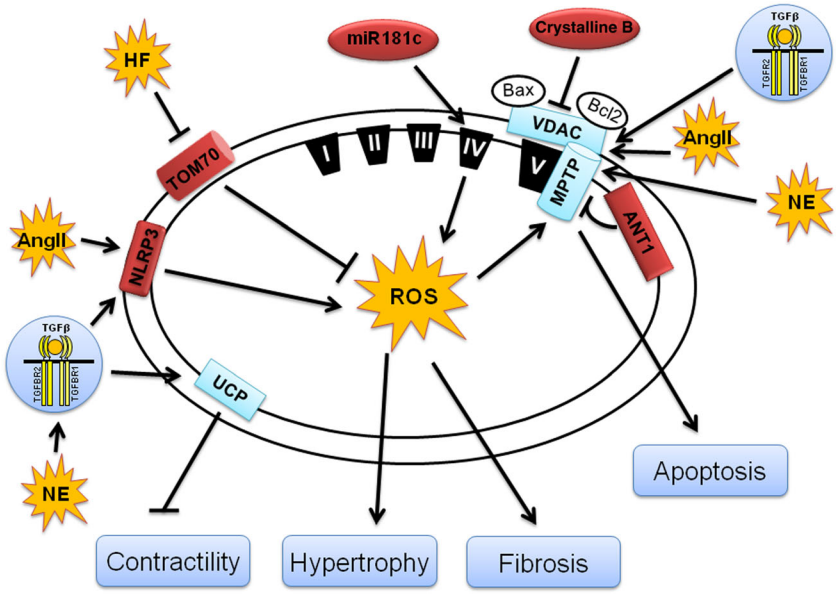

Figure 4

The central role of mitochondria in LV heart failure can be modulated by TGFβ. Hypertrophy, fibrosis and apoptosis can be controlled by mitochondria via generation of ROS. NLRP3 is a newly identified molecule that enhances mitochondrial ROS production and that is controlled by TGFβ or angiotensin II (AngII). miR181c enhances ROS production via modulation of complex IV of the respiratory chain. TOM70, acting as a repressor of mitochondrial ROS production, is found to be reduced in heart failure. This reduction then provokes enhancement of ROS. Enhancement of mitochondrial uncoupling protein during stimulation of β-adrenoceptors by noradrenaline (NA) and TGFβ-receptor activation results in reduced energy production and impaired contractile function. Opening of the MPTP plays a central role in the induction of apoptosis. Opening of this pore can be modulated by the accessory proteins VDAC and ANT1. Their expression is regulated by crystalline B, TGFβ, AngII, ROS and β-adrenoceptors (ADRB). Central molecules that modulate mitochondrial processes in heart failure are depicted in red.

adverse remodelling (Jain *et al.*, 2015). Inhibiting MPTP opening by overexpression of adenine nucleotide translocase 1 (ANT1) prevented $TGF\beta_1$-induced apoptosis in ventricular cardiomyocytes (Heger *et al.*, 2012) and improved cardiac function in rats with an activated renin–angiotensin system (Walther *et al.*, 2007). These findings highlight the contribution MPTP opening has to the adverse cardiac remodelling induced by TGFβ stimulation and indicate that ANT1 is a critical component at the inner mitochondria membrane for regulating MPTP opening (Figure 4).

Besides modulation of MPTP opening, the B-cell lymphoma 2 (Bcl-2) family is a well-known gate keeper in mitochondria-mediated apoptosis. TGFβ can stimulate or inhibit the expression of pro-apoptotic and anti-apoptotic Bcl-2 family members (Grünenfelder *et al.*, 2002). After renal artery ligation, a model for angiotensinII/TGFβ-mediated cardiac hypertrophy, up-regulation of the pro-apoptotic family member Bax and the voltage-dependent anion channel-1 (VDAC1) occurred (Figure 4). Together, they lead to permeabilization of the outer mitochondrial membrane, release of cytochrome c from the intermembrane space into the cytosol, formation of the apoptosome, activation of caspases and finally the induction of apoptosis (Mitra *et al.*, 2013). The small heat shock protein, crystalline B, is able to block the pro-apoptotic action of VDAC1, and thereby acts as a molecular key that guides VDAC1 to be anti-apoptotic. Therefore, crystalline B may become an interesting therapeutic target for the prevention of the transition from compensated hypertrophy to heart failure. Another heat shock protein (Hsp) with anti-apoptotic action on the mitochondrial level is Hsp22 (Qiu *et al.*, 2011). Overexpression of Hsp22 results in physiological hypertrophy via up-regulation of NFκB, and binding of Hsp22 to signal transducer and activator of transcription 3 (STAT3), which is a marker of cardiac stress responses. Down-regulation of Hsp22 leads to an increased remodelling of the heart and death in knock-out mice after transverse aortic constriction (TAC) by modulating the nuclear and mitochondrial function of STAT3 and STAT3-dependent genes.

A further mitochondria-associated candidate, mediating a switch to pathophysiological hypertrophy is TOM70, a translocase of the mitochondrial outer membrane (TOM) complex that mediates the import of mitochondrial preproteins (Figure 4). Li *et al.* (2014b) nicely showed a down-regulation of TOM70 in pathophysiological hypertrophy in humans as well as animal models. This results in the reduced import of optical atrophy-1 (OPA1) – a protein important for mitochondrial fusion –, a reduction in complex I activity and finally in ROS production. As a consequence, changes in the outer mitochondrial membrane and/or inner mitochondrial membrane occurred, followed by apoptotic events as discussed above. In addition, increased TOM70 levels made cardiomyocytes completely resistant to the effects of various pro-hypertrophic stimuli.

These findings explain the significance of the modulation of mitochondrial pores by, for example, VDAC1, crystalline B or TOM70 for cardiac hypertrophy to progress to heart failure.

At the onset of the development of heart failure, a metabolic shift from fatty acid to glucose metabolism has been

described. This shift is due to the down-regulation of enzymes for fatty acid oxidation, whereas glycolytic enzymes are up-regulated (Sack *et al.*, 1996). This enables the heart to increase its metabolic substrate efficiency in relation to O_2 consumption. However, the metabolic shift seems to be related to adverse cardiac remodelling. A key regulator of energy homeostasis induced by stimulation of glycolysis and glycogen accumulation is AMP-activated kinase (AMPK), which is activated during cardiac remodelling (Dolinsky and Dyck, 2006; Kolwicz and Tian, 2011). Just recently, myostatin, a member of the TGFβ superfamily, was identified as a repressor of AMPK (Biesemann *et al.*, 2014). Myostatin reduces muscle growth (skeletal or cardiac), and thus protects the heart against hypertrophy and failure, and this function of myostatin is, in part, mediated via repression of AMPK and the prevention of a metabolic switch towards glycolysis.

In addition to the metabolic shift, a down-regulation of transporters for glucose (GLUT1/4) and fatty acid (CD36) uptake into cardiomyocytes as well as a reduction of transporters for pyruvate (PDH) or the carnitine shuttle (CPT1/2) in mitochondria contribute to heart failure development, as deletions of these transporters provoked cardiac remodelling and or dysfunction (Bersin *et al.*, 1994; Liao *et al.*, 2002; Domenighetti *et al.*, 2010; Lai *et al.*, 2014).

Thus, enhancing the uptake mechanisms for glucose and fatty acids into cardiomyocytes, as well as for metabolized substrates into mitochondria can attenuate heart failure progression. Furthermore, prevention of the metabolic switch, probably via AMPK, is a promising target for therapeutic approaches against heart failure development.

MicroRNAs in heart failure

An increased ability to regulate the processes involved in cardiac remodelling is attributed to miRNAs. miRNAs are small non-coding RNAs that target the 3´-untranslated region or 5'-untranslated region of mRNA transcripts. This results in the destabilization or translational repression of mRNAs (Bartel, 2004). Furthermore, miRNAs can regulate gene transcription by inducing histone modifications or DNA methylations (Hawkins and Morris, 2008). In fact, one single miRNA can affect many target genes generating a broad network of miRNA-controlled gene expression that has a huge effect on different biological processes including cardiac remodelling. Analysing the role of miRNAs in heart failure development has already identified some promising new therapeutic targets.

The RNase III endonuclease Dicer is essential for the processing of pre-miRNA into its mature form. In the adult myocardium, a loss of Dicer-induced biventricular enlargement is accompanied by hypertrophic growth of cardiomyocytes, ventricular fibrosis and functional defects (da Costa Martins *et al.*, 2008). A similar study by Chen *et al.* (2008) revealed signs of dilated cardiomyopathy and heart failure after cardiac-specific deletion of Dicer. Furthermore, they found that the level of Dicer protein was significantly reduced in in human patients with dilated cardiomyopathy and failing hearts. These findings indicate that miRNAs have a major function in the control of heart failure development and progression.

Either an up-regulation or down-regulation of miRNAs under pressure overload can mediate cardiac remodelling, for example, when miR25 is increased the activity of the sarcoplasmic reticulum (SR) Ca^{2+} ATPase (SERCA2A) is reduced

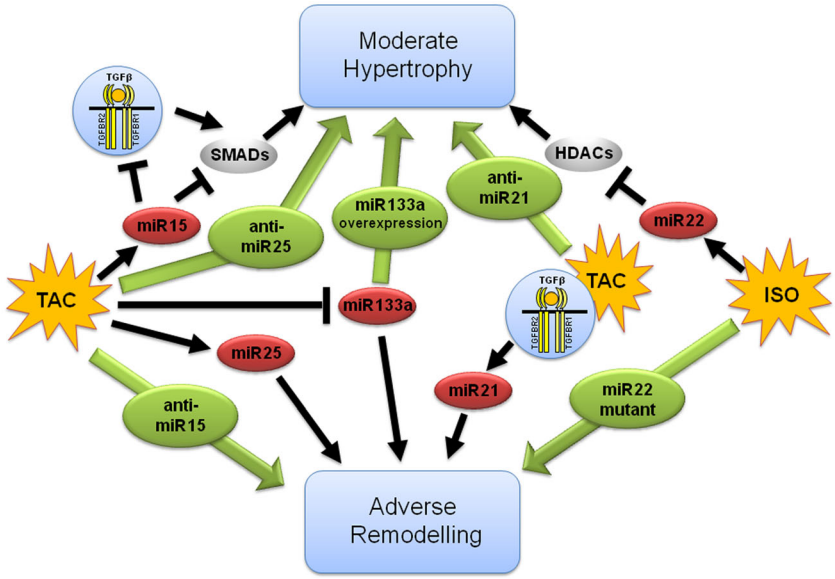

Figure 5

Influence of miRNAs on LV adverse remodelling can be modulated by β-adrenoceptors (ADRBs) or TGFβ. miRNAs that have been demonstrated to reverse or promote adverse cardiac remodelling are depicted. Up-regulation of miR15 or miR22 prevents the induction of fibrosis or apoptosis under pressure overload (TAC) or β-adrenoceptor stimulation (ISO) while preserving effects on moderate, compensatory hypertrophy, as when these miRs are inhibited adverse remodelling develops. In contrast, up-regulation of miR25 or miR21 under TAC enhances adverse cardiac remodelling, and down-regulation of miR133a under TAC preserves cardiac function, whereas the overexpression of miR133a results in the development of adverse remodelling. Black arrows indicate the responses of the cell to TAC or ISO. Switch molecules in the process of adverse remodelling are depicted in red. Green arrows and symbols indicate interference of miR expression by anti-miRs or transgenic overexpression.

and Ca-handling is impaired. Anti-miR25 reverses hypertrophy, fibrosis and heart failure progression after TAC (Wahlquist *et al.*, 2014) (Figure 5). miR133a is down-regulated under pressure overload and when this down-regulation is prevented in transgenic mice TAC-induced fibrosis and apoptosis are attenuated, whereas hypertrophy is not affected (Matkovich *et al.*, 2010); hence, these diverse processes of cardiac remodelling are differentiated (Figure 5). This indicates that by altering the levels of miR133a, it may be possible to stop the adverse remodelling processes while maintaining the compensatory effects of hypertrophy. The development of moderate hypertrophy and physiological cardiac remodelling induced by an infusion of isoprenaline is converted to adverse remodelling in miR22 knock-out mice with a marked enhancement of fibrosis and apoptosis that finally leads to dilated cardiomyopathy (Huang *et al.*, 2013). The effects of miR22 seem to be mediated by the inhibition of histone deacetylases, which indicates that miR22 has a role in the epigenetic regulation of gene expression during cardiac hypertrophy. These findings indicate that during β-adrenoceptor stimulation miR22 prevents the transition from compensated hypertrophy to heart failure progression (Figure 5).

A new aspect of miRNA-controlled signalling relates to the translocation of nuclear-encoded miR181c to mitochondria (Das *et al.*, 2014). This results in the remodelling of mitochondrial complex IV and an enhanced production of ROS (Figure 4). The overexpression of miR181c induces ventricular dysfunction indicating that miRNAs can modulate exercise capacity directly at the mitochondrial level. Interestingly, this miR181c-induced dysfunction was not accompanied by cardiac hypertrophy. Unfortunately, these observations were only carried out *in vitro* with a simulated overexpression of miR181c and its role in cardiovascular disease *in vivo* has still to be proven.

With regard to TGFβ signalling, the miR15 family needs to be mentioned. This family comprises six highly conserved family members that are up-regulated in human heart failure and can inhibit numerous components of the TGFβ signalling pathway (Tijsen *et al.*, 2014). Inhibition of one family member by anti-miR15b in mice also resulted in the down-regulation of other family members and predominantly enhanced SMAD-signalling and cardiac fibrosis, especially after TAC (Figure 5). Therefore, an up-regulation of miR15 members in heart failure acts as negative feedback mechanism on TGFβ signalling in order to restrict adverse remodelling. However, due to the ubiquitous expression of miR15 and because miR15 is also involved in inducing apoptosis after acute myocardial infarction (Hullinger *et al.*, 2012), the therapeutic application of miR15 against heart failure progression should be treated with caution as a substantial amount of additional work needs to be done to exclude negative side effects. In addition to miRs modifiying TGFβ signalling pathways, TGFβ itself is also known as a regulator of miRs. In the context of heart failure progression, miR21 should be highlighted; miR21 is selectively up-regulated in fibroblasts upon TGFβ stimulation, and in the failing myocardium (Thum *et al.*, 2008; Topkara and Mann, 2011). It has been shown to induce cardiac fibrosis by enhancing the proliferation of fibroblasts and to stimulate endothelial mesenchymal transition during TGFβ stimulation (Kumarswamy *et al.*, 2012) and also to act as a mediator of adverse cardiac remodelling after TAC (Thum *et al.*, 2011) (Figure 5). Just recently, cardiac fibroblasts were shown to secrete miRNA-enriched exosomes (including miR21). Fibroblast-derived miR21 acts as a potent paracrine RNA molecule that induces cardiomyocyte hypertrophy (Bang *et al.*, 2014). miR155, secreted by macrophages, also has paracrine effects on the heart, because miR155 knockout in macrophages prevented angiotensin II-induced or TAC-induced cardiac hypertrophy and dysfunction although fibrosis was still present.

These findings showing that miRNAs can exert paracrine effects implies that systemic pharmacological interference of miRNA signalling by the use of anti-miRNAs might be useful for preventing heart failure progression. Such a systemic therapeutic intervention would be easy to apply clinically. However, in cardiac pathophysiology, the systemic application of anti-miRNAs is still restricted to basic science studies. A promising approach in this direction has been shown by Montgomery *et al.* (2011) using locked nucleic acid (LNA)-modified miR208a-antisense oligonucleotides. Systemic delivery of these oligos silenced miR208a-expression in the heart, thereby preventing hypertension-induced heart failure in Dahl hypertensive rats by reducing cardiomyocyte hypertrophy, cardiac fibrosis and improving cardiac function. Because cardiac miR208 overexpression in transgenic mice induced cardiac hypertrophy, the reduction of miR208a in the heart by LNA-antisense oligos is at least in part responsible for cardioprotection in hypertensive Dahl rats. However, reductions in the levels of circulating miR499 and miR208b were also found during the treatment with LNA-modified 208a antisense oligos. Therefore, a combination of local and systemic effects may contribute to the protective effects.

All these studies demonstrate the enormity of the miRNA network and its influence on heart failure progression. Fine tuning of specific miRNAs is essential for physiological hypertrophy or decompensation, and thus has great therapeutic potential for the treatment of heart failure patients.

Right heart failure

For many years, analysis of left ventricular systolic dysfunction was at the centre of heart failure research, which is also the focus of our review. However, in recent years, some promising advances in the analysis of right heart failure have been made that should be discussed here.

One major cause for right ventricular dysfunction is pulmonary hypertension (PAH). Due to an increase in pulmonary vascular resistance, afterload on the right ventricle (RV) increases, leading to right heart failure which determines the prognosis of patients with PAH. Therefore, it is of utmost importance to define new therapeutic strategies to reduce RV remodelling in order to improve patient prognosis (Ryan *et al.*, 2015).

The RV, similar to the LV, compensates an increased work load due to hypertension by hypertrophic growth processes. However, compensatory remodelling is limited and over time, the RV decompensates, finally leading to heart failure. There seem to be chamber-specific responses, and thus, a simple extension of LV findings to the RV is not possible. An interesting new finding in this respect comes from Schreckenberg *et al.* (2015) who analysed the effect of chronic NO deprivation on the remodelling processes in the LV and RV. Treatment of rats with the NOS inhibitor L-NAME resulted in moderate ventricular hypertrophy without signs of dysfunction. However, the

RV responded with dilatation and dysfunction. A massive increase in oxygen radicals due to a down-regulation of the anti-oxidative protein, SOD, was only found in the RV, and this was the cause of RV remodelling, whereas in the LV, anti-oxidative enzymes were even increased under the L-NAME treatment. Thus, oxidative stress is a much greater risk factor in the RV compared with the LV. The reduction in SOD and the development of RV dysfunction could be attenuated by captopril, an angiotensin-converting enzyme inhibitor with high anti-oxidative capacity. Interestingly, enhanced ROS production in the RV is responsible for HIF1α inhibition and suppression of angiogenesis, thereby resulting in capillary rarefication of the RV and chronic ischaemia (Bogaard *et al.*, 2009).

A further positive effect of therapeutics against the renin–angiotensin system was demonstrated by Friedberg *et al.* (2013). Pulmonary artery banding in rabbits induced RV hypertrophy and biventricular up-regulation of the TGFβ pathways, including activation of SMAD3, fibrosis and apoptosis. These responses were blocked by losartan, an inhibitor of the AT_1 receptor. In addition, TGFβ had negative effects on vascular remodelling in PAH (Zaiman *et al.*, 2008). Therefore, blocking this pathway has positive effects on the vasculature as well as on both ventricles and seems to be a promising therapeutic target.

Further interesting targets in RVare (i) the up-regulation of pyruvate dehydrogenase kinase (PDK), as it is responsible for the metabolic shift to inefficient cytosolic glyocolysis and RV dysfunction (Piao *et al.*, 2010) and (ii) the inhibition of G-protein receptor kinase 2 (GRK2) to prevent the down-regulation of β-adrenoceptors and preserve RV function in PAH (Piao *et al.*, 2012).

Conclusion and outlook

Cardiac remodelling is a multifactorial-induced process. The present pharmacological interventions are able to postpone the onset of heart failure but are unable to prevent the ongoing process of cardiac remodelling in hypertrophied hearts. Newly identified signalling molecules might have the potential to serve as therapeutic targets in the treatment of heart failure. Therefore, different and common pathways of left and RVs should be considered. Among the decisive signalling molecules for LV heart failure progression are modulators of mitochondrial pores, such as NLRP3, ANT1, VDAC1 or TOM70 and various miRNAs; for example, miR22 specifically impairs the progression from compensated hypertrophy to left ventricular heart failure and miR21 has significant potential to induce fibrosis. Furthermore, prevention of the down-regulation of mitochondrial transporters might improve the metabolic situation in the remodelled myocardium. Other switch molecules control the adrenoceptor-mediated signalling pathway, like SMAD4, TAK1 or β-arrestin, thereby modulating hypertrophic, apoptotic necroptotic and contractile responses of the cell. In the RV, a higher susceptibility to oxygen radical production has been found, thereby indicating the importance of using therapeutic ROS scavengers. Furthermore, PDK and GRK2 have been identified as new targets in RV.

Many of these targets are regulated by TGFβ, and administration of an ALK5 inhibitor after aortic banding prevented cardiac fibrosis and attenuated cardiac dysfunction. However, mortality rates of animals increased due to enhanced left ventricular dilatation and inflammation (Engebretsen *et al.*, 2014). Therefore, a more target-orientated approach needs to be used to inhibit the detrimental TGFβ pathways but preserve the protective ones. As exemplified in this review, newly identified switch molecules may offer novel options in the therapeutic approach against heart failure development and progression.

Conflict of interest

None.

References

Abbate A, Arena R, Abouzaki N, Van Tassell BW, Canada J, Shah K *et al.* (2015). Heart failure with preserved ejection fraction: refocusing on diastole. Int J Cardiol 179: 430–440. doi:10.1016/j.ijcard.2014.11.106.

Alexander SP, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al.* (2013a). The Concise Guide to PHARMACOLOGY 2013/14: G protein-coupled receptors. Br J Pharmacol 170: 1459–1581. doi:10.1111/bph.12445.

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al.* (2013b). The concise guide to PHARMACOLOGY 2013/14: catalytic receptors. Br J Pharmacol 170: 1676–1075.

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al.* (2013c). The concise guide to PHARMACOLOGY 2013/14: transporters. Br J Pharmacol 170: 1706–1796.

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al.* (2013d). The concise guide to PHARMACOLOGY 2013/14: enzymes. Br J Pharmacol 170: 1797–1867.

Bang C, Batkai S, Dangwal S, Gupta SK, Foinquinos A, Holzmann A *et al.* (2014). Cardiac fibroblast-derived microRNA passenger strand-enriched exosomes mediate cardiomyocyte hypertrophy. J Clin Invest 124: 2136–2146. doi:10.1172/JCI70577.

Bartel DP (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116: 281–297. doi:10.1016/S0092-8674(04)00045-5.

Barth E, Stämmler G, Speiser B, Schaper J (1992). Ultrastructural quantitation of mitochondria and myofilaments in cardiac muscle from 10 different animal species including man. J Mol Cell Cardiol 24: 669–681. doi:10.1016/0022-2828(92)93381-S.

Bathgate-Siryk A, Dabul S, Pandya K, Walklett K, Rengo G, Cannavo A *et al.* (2014). Negative impact of β-arrestin-1 on post-myocardial infarction heart failure via cardiac and adrenal-dependent neurohormonal mechanisms. Hypertension 63: 404–412. doi:10.1161/HYPERTENSIONAHA.113.02043.

Bersin RM, Wolfe C, Kwasman M, Lau D, Klinski C, Tanaka K *et al.* (1994). Improved hemodynamic function and mechanical efficiency in congestive heart failure with sodium dichloroacetate. J Am Coll Cardiol 23: 1617–1624. doi:10.1016/0735-1097(94)90665-3.

Besse A, Lamothe B, Campos AB, Webster WK, Maddineni U, Lin SC *et al.* (2007). TAK1-dependent signaling requires functional interactionwith TAB1/TAB3. J Biol Chem 282: 3918–3928. doi:10.1074/jbc.M608867200.

Biesemann N, Mendler L, Wietelmann A, Hermann S, Schäfers M, Krüger M *et al.* (2014). Myostatin regulates energy homeostasis in the heart and prevents heart failure. Circ Res 115: 296–310. doi:10.1161/CIRCRESAHA.115.304185.

Boluyt MO, O'Neill L, Meredith AL, Bing OH, Brooks WW, Conrad CH *et al.* (1994). Alterations in cardiac gene expression during the transition from stable hypertrophy to heart failure. Circ Res 75: 23–32. doi:10.1161/01.RES.75.1.23.

Bogaard HJ, Natarajan R, Henderson SC, Long CS, Kraskauskas D, Smithson L *et al.* (2009). Chronic pulmonary artery pressure elevation is insufficient to explain right heart failure. Circulation 120: 1951–1960. doi:10.1161/CIRCULATIONAHA.109.883843.

Bracey NA, Gershkovich B, Chun J, Vilaysane A, Meijndert HC, Wright JR Jr *et al.* (2014). Mitochondrial NLRP3 protein induces reactive oxygen species to promote Smad protein signaling and fibrosis independent from the inflammasome. J Biol Chem 289: 19571–19584. doi:10.1074/jbc.M114.550624.

Brenner C, Moulin M (2012). Physiological roles of the permeability transition pore. Circ Res 111: 1237–1247. doi:10.1161/CIRCRESAHA. 112.265942.

Burch ML, Osman N, Getachew R, Al-Aryahi S, Poronnik P, Zheng WG *et al.* (2012). Protein coupled receptor transactivation: extending the paradigm to include serine/threonine kinase receptors. Int J Biochem Cell Biol. 44: 722–727. doi:10.1016/j.biocel.2012.01.018.

Chen JF, Murchison EP, Tang R, Callis TE, Tatsuguchi M, Deng Z *et al.* (2008). Targeted deletion of Dicer in the heart leads to dilated cardiomyopathy and heart failure. Proc Natl Acad Sci U S A 105: 2111–2116. doi:10.1073/pnas.0710228105.

Chen Y-R, Zweier JL (2014). Cardiac mitochondria and reactive oxygen species generation. Circ Res 114: 524–537. doi:10.1161/CIRCRESAHA. 114.300559.

Conner DA, Mathier MA, Mortensen RM, Christe M, Vatner SF, Seidman CE *et al.* (1997). beta-Arrestin1 knockout mice appear normal but demonstrate altered cardiac responses to beta-adrenergic stimulation. Circ Res 81: 1021–1026. doi:10.1161/01.RES81.6.1021.

da Costa Martins PA, Bourajjaj M, Gladka M, Kortland M, van Oort RJ, Pinto YM *et al.* (2008). Conditional dicer gene deletion in the postnatal myocardium provokes spontaneous cardiac remodeling. Circulation 118: 1567–1576. doi:10.1161/CIRCULATIONAHA.108.769984.

Das S, Bedja D, Campbell N, Dunkerly B, Chenna V, Maitra A *et al.* (2014). miR-181c regulates the mitochondrial genome, bioenergetics, and propensity for heart failure in vivo. PLoSOne 9: e96820. doi:10.1371/journal.pone.0096820.

Dennis PA, Rifkin DB (1991). Cellular activation of latent transforming growth factor beta requires binding to the cation-independent mannose 6-phosphate/insulin-like growth factor type II receptor. Proc Natl Acad Sci U S A 88: 580–584, PMID: 1846448.

Depre C, Wang Q, Yan L, Hedhli N, Peter P, Chen L *et al.* (2006). Activation of the cardiac proteasome during pressure overload promotes ventricular hypertrophy. Circulation 14: 1821–1828. doi:10.1161/CIRCULATIONAHA.106.637827.

Di Lisa F, Kaludercic N, Carpi A, Menabò R, Giorgio M (2009). Mitochondria and vascular pathology. Pharmacol Rep 61: 123–130, PMID: 19307700.

Dolinsky VW, Dyck JR (2006). Role of AMP-activated protein kinase in healthy and diseased hearts. Am J Physiol Heart Circ Physiol 291: H2557–H2569. doi:10.1152/ajpheart.00329.2006.

Domenighetti AA, Danes VR, Curl CL, Favaloro JM, Proietto J, Delbridge LM (2010). Targeted GLUT-4 deficiency in the heart induces cardiomyocyte hypertrophy and impaired contractility linked with Ca(2+) and proton flux dysregulation. J Mol Cell Cardiol 48: 663–672. doi:10.1016/j.yjmcc.2009.11.017.

Drews O, Tsukamoto O, Liem D, Streicher J, Wang Y, Ping P (2010). Differential regulation of proteasome function in isoproterenol-induced cardiac hypertrophy. Circ Res 107: 1094–1101. doi:10.1161/CIRCRESAHA.110.222364.

Engebretsen KV, Skårdal K, Bjørnstad S, Marstein HS, Skrbic B, Sjaastad I *et al.* (2014). Attenuated development of cardiac fibrosis in left ventricular pressure overload by SM16, an orally active inhibitor of ALK5. J Mol Cell Cardiol 76: 148–157. doi:10.1016/j.yjmcc.2014.08.008.

Friedberg MK, Cho MY, Li J, Assad RS, Sun M, Rohailla S *et al.* (2013). Adverse biventricular remodeling in isolated right ventricular hypertension is mediated by increased transforming growth factor-β1 signaling and is abrogated by angiotensin receptor blockade. Am J Respir Cell Mol Biol 49: 1019–1028. doi:10.1165/rcmb.2013-0149OC.

Goumans MJ, Valdimarsdottir G, Itoh S, Rosendahl A, Sideras P, ten Dijke P *et al.* (2002). Balancing the activation state of the endothelium via two distinct TGF-beta type I receptors. EMBO J 21: 1743–1753, PMID:11927558.

Grünenfelder J, Miniati DN, Murata S, Falk V, Hoyt EG, Robbins RC (2002). Up-regulation of Bcl-2 through hyperbaric pressure transfection of TGF-beta1 ameliorates ischemia-reperfusion injury in rat cardiac allografts. J Heart Lung Transplant 21: 244–250. doi:10.1016/S1053-2498(01)00377-1.

Hawkins PG, Morris KV (2008). RNA and transcriptional modulation of gene expression. Cell Cycle 7: 602–607. doi:10.4161/cc.7.5.5522.

Heger J, Peters SC, Piper HM, Euler G (2009). SMAD-proteins as a molecular switch from hypertrophy to apoptosis induction in adult ventricular cardiomyocytes. J Cell Physiol 220: 515–523. doi:10.1002/jcp.21805.

Heger J, Abdallah Y, Shahzad T, Klumpe I, Piper HM, Schultheiss HP *et al.* (2012). Transgenic overexpression of the adenine nucleotide translocase 1 protects cardiomyocytes against TGFβ1-induced apoptosis by stabilization of the mitochondrial permeability transition pore. J Mol Cell Cardiol 53: 73–81. doi:10.1016/j.yjmcc.2012.04.013.

Heusch G (2015). Mitochondria at the heart of cardiovascular protection: p66shc-friend or foe? Eur Heart J 36: 469–471. doi:10.1093/eurheartj/ehu409.

Hill C, Würfel A, Heger J, Meyering B, Schlüter KD, Weber M *et al.* (2013). Inhibition of AP-1 signaling by JDP2 overexpression protects cardiomyocytes against hypertrophy and apoptosis induction. Cardiovasc Res 99: 121–128. doi:10.1093/cvr/cvt094.

Hoppe UC, Erdmann E (2009). Kapitel 5 Chronische Herzinsuffizienz, aus Erdmann E. Klinische Kardiologie. Krankheiten des Herzens, des Kreislaufs und herznaher Gefäße. 7. Auflage, Springer Verlag

Huang CY, Kuo WW, Yeh YL, Ho TJ, Lin JY, Lin DY *et al.* (2014). ANG II promotes IGF-IIR expression and cardiomyocyte apoptosis by inhibiting HSF1 via JNK activation and SIRT1 degradation. Cell Death Differ 21: 1262–1274. doi:10.1038/cdd.2014.46.

Huang ZP, Chen J, Seok HY, Zhang Z, Kataoka M, Hu X *et al.* (2013). MicroRNA-22 regulates cardiac hypertrophy and remodeling in response to stress. Circ Res 112: 1234–1243. doi:10.1161/CIRCRESAHA.112.300682.

Hullinger TG, Montgomery RL, Seto AG, Dickinson BA, Semus HM, Lynch JM *et al.* (2012). Inhibition of miR-15 protects against cardiac ischemic injury. Circ Res 110: 71–81. doi:10.1161/CIRCRESAHA.111.244442.

Huntgeburth M, Tiemann K, Shahverdyan R, Schlüter KD, Schreckenberg R, Gross ML *et al.* (2011). Transforming growth factor β_1 oppositely regulates the hypertrophic and contractile response to β-adrenergic stimulation in the heart. PLoS One 6: e26628. doi:10.1371/journal.pone.0026628.

Ikeuchi M, Tsutsui H, Shiomi T, Matsusaka H, Matsushima S, Wen J *et al.* (2004). Inhibition of TGF-beta signaling exacerbates early cardiac dysfunction but prevents late remodeling after infarction. Cardiovasc Res 64: 526–535. doi:10.1016/j.cardiores.2004.07.017.

Jain A, Atale N, Kohli S, Bhattacharya S, Sharma M, Rani V (2015). An assessment of norepinephrine mediated hypertrophy to apoptosis transition in cardiac cells: a signal for cell death. Chem Biol Interact 225: 54–62. doi:10.1016/ j.cbi.2014.11.017.

Khan S, Joyce J, Margulies KB, Tsuda T (2014). Enhanced bioactive myocardial transforming growth factor-β in advanced human heart failure. Circ J 78: 2711–2718. doi:10.1253/circj.CJ-14-0511.

Kinnally KW, Peixoto PM, Ryu SY, Dejean LM (2011). Is mPTP the gatekeeper for necrosis, apoptosis, or both? Biochim Biophys Acta 4: 616–624. doi:10.1016/j.bbamcr.2010.09.013.

Kolwicz SC Jr, Tian R (2011). Glucose metabolism and cardiac hypertrophy. Cardiovasc Res 90: 194–201. doi:10.1093/cvr/cvr071.

Kumarswamy R, Volkmann I, Jazbutyte V, Dangwal S, Park DH, Thum T (2012). Transforming growth factor-β-induced endothelial-to-mesenchymal transition is partly mediated by microRNA-21. Arterioscler Thromb Vasc Biol 32: 361–369. doi:10.1161/ ATVBAHA.111.234286.

Lai L, Leone TC, Keller MP, Martin OJ, Broman AT, Nigro J *et al.* (2014). Energy metabolic reprogramming in the hypertrophied and early stage failing heart: a multisystems approach. Circ Heart Fail 7: 1022–1031. doi:10.1161/CIRCHEARTFAILURE.114.001469.

Leksa V, Godar S, Schiller HB, Fuertbauer E, Muhammad A, Slezakova K (2005). TGF-beta-induced apoptosis in endothelial cells mediated by M6P/IGFII-R and mini-plasminogen. J Cell Sci 118 (Pt 19): 4577–4586. doi:10.1242/ jcs.02587.

Li L, Chen Y, Doan J, Murray J, Molkentin JD, Liu Q (2014a). Transforming growth factor β-activating kinase 1 signaling pathway critically regulated myocardial survival and remodeling. Circulation 130: 2162–2172. doi:10.1161/CIRCULATIONAHA.114.011195.

Li J, Qi M, Li C, Shi D, Zhang D, Xie D *et al.* (2014b). Tom70 serves as a molecular switch to determine pathological cardiac hypertrophy. Cell Res 24: 977–993. doi:10.1038/Cr.2014.9.

Liao R, Jain M, Cui L, D'Agostino J, Aiello F, Luptak I *et al.* (2002). Cardiac-specific overexpression of GLUT1 prevents the development of heart failure attributable to pressure overload in mice. Circulation 106: 2125–2131. doi:10.1161/01.CIR.0000034049.61181.

Lijnen PJ, Petrov VV, Fagard RH (2000). Induction of cardiac fibrosis by transforming growth factor-beta(1). Mol Genet Metab 71: 418–435. PMID: 11001836.

Lymperopoulos A, Negussie S (2013). βArrestins in cardiac G protein-coupled receptor signaling and function: partners in crime or "good cop, bad cop"? Int J Mol Sci 14: 24726–24741. doi:10.3390/ijms141224726.

Matkovich SJ, Wang W, Tu Y, Eschenbacher WH, Dorn LE, Condorelli G *et al.* (2010). MicroRNA-133a protects against myocardial fibrosis and modulates electrical repolarization without affecting hypertrophy in pressure-overloaded adult hearts. Circ Res 106: 166–175. doi:10.1161/CIRCRESAHA.109.202176.

Mitra A, Basak T, Datta K, Naskar S, Sengupta S, Sarkar S (2013). Role of α-crystallin B as a regulatory switch in modulating cardiomyocyte apoptosis by mitochondria or endoplasmic reticulum during cardiac hypertrophy and myocardial infarction. Cell Death Dis 4: e582. doi:10.1038/cddis.2013.114.

Montgomery RL, Hullinger TG, Semus HM, Dickinson BA, Seto AG, Lynch JM *et al.* (2011). Therapeutic inhibition of miR-208a improves cardiac function and survival during heart failure. Circulation 124: 1537–1547. doi:10.1161/CIRCULATIONAHA.111.030932.

Munger JS, Huang X, Kawakatsu H, Griffiths MJ, Dalton SL, Wu J *et al.* (1999). The integrin alpha v beta 6 binds and activates latent TGF beta 1: a mechanism for regulating pulmonary inflammation and fibrosis. Cell 6: 319–328. doi:10.1016/S0092-8674(00)80545-0.

Narula J, Haider N, Virmani R, DiSalvo TG, Kolodgie FD, Hajjar RJ (1996). Apoptosis in myocytes in end-stage heart failure. N Engl J Med 335: 1182–1189. doi:10.1056/NEJM199610173351603.

Pawson AJ, Sharman JL, Benson HE, Faccenda E, Alexander SP, Buneman OP *et al.* (2014). The IUPHAR/BPS Guide to PHARMACOLOGY: an expert-driven knowledgebase of drug targets and their ligands. Nucl Acids Res 42 (Database Issue): D1098–106.

Piao L, Fang YH, Cadete VJ, Wietholt C, Urboniene D, Toth PT *et al.* (2010). The inhibition of pyruvate dehydrogenase kinase improves impaired cardiac function and electrical remodeling in two models of right ventricular hypertrophy: resuscitating the hibernating right ventricle. J Mol Med (Berl) 88: 47–60. doi:10.1007/s00109-009-0524-6.

Piao L, Fang YH, Parikh KS, Ryan JJ, D'Souza KM, Theccanat T *et al.* (2012). GRK2-mediated inhibition of adrenergic and dopaminergic signaling in right ventricular hypertrophy: therapeutic implications in pulmonary hypertension. Circulation 126 (24): 2859–2869. doi:10.1161/CIRCULATIONAHA.112.109868.

Portbury AL, Ronnebaum SM, Zungu M, Patterson C, Willis MS (2012). Back to your heart: ubiquitin proteasome system-regulated signal transduction. J Mol Cell Cardiol 52: 526–537. doi:10.1016/j.yjmcc.2011.10.023.

Qiu H, Lizano P, Laure L, Sui X, Rashed E, Park JY *et al.* (2011). H11 kinase/heat shock protein 22 deletion impairs both nuclear and mitochondrial functions of STAT3 and accelerates the transition into heart failure on cardiac overload. Circulation 24: 406–415. doi:10.1161/CIRCULATIONAHA.110.013847.

Rosenkranz S, Flesch M, Amann K, Haeuseler C, Kilter H, Seeland U *et al.* (2002). Alterations of beta-adrenergic signaling and cardiac hypertrophy in transgenic mice overexpressing TGF-beta(1). Am J Physiol Heart Circ Physiol 283: H1253–H1262. doi:10.1152/ajpheart.00578.2001.

Rosenkranz S (2004). TGF-beta1 and angiotensin networking in cardiac remodeling. Cardiovasc Res 94: 2837–2842. doi:10.1016/j.cardiores.2004.04.030.

Ryan JJ, Huston J, Kutty S, Hatton ND, Bowman L, Tian L *et al.* (2015). Right ventricular adaptation and failure in pulmonary arterial hypertension. Can J Cardiol 31: 391–406. doi:10.1016/j.cjca.2015.01.023.

Sack MN, Rader TA, Park S, Bastin J, McCune SA, Kelly DP (1996). Fatty acid oxidation enzyme gene expression is downregulated in the failing heart. Circulation 94: 2837–2842. doi:10.1161/01.CIR.94.11.2837.

Schlüter KD, Zhou XJ, Piper HM (1995). Induction of hypertrophic responsiveness to isoproterenol by TGF-beta in adult rat cardiomyocytes. Am J Physiol 269 (5 Pt 1): C1311–C1316. PMID: 7491923.

Schneiders D, Heger J, Best P, Piper HM, Taimor G (2005). SMAD proteins are involved in apoptosis induction in ventricular

cardiomyocytes. Cardiovasc Res 67: 87–96. doi:10.1016/j.cardiores.2005.02.021.

Schreckenberg R, Rebelo M, Deten A, Weber M, Rohrbach S, Pipicz M *et al.* (2015). Specific Mechanisms Underlying Right Heart Failure: The Missing Upregulation of Superoxide Dismutase-2 and Its Decisive Role in Antioxidative Defense. Antioxid Redox Signal Epub ahead of print, PMID:25978844 .

Schröder D, Heger J, Piper HM, Euler G (2006). Angiotensin II stimulates apoptosis via TGF-beta1 signaling in ventricular cardiomyocytes of rat. J Mol Med (Berl) 84: 975–983. doi:10.1007/s00109-006-0090-0.

Taimor G, Schlüter KD, Best P, Helmig S, Piper HM (2004). Transcription activator protein 1 mediates alpha- but not beta-adrenergic hypertrophic growth responses in adult cardiomyocytes. Am J Physiol Heart Circ Physiol 286: H2369–H2375. doi:10.1152/ajpheart.00741.2003.

Thum T, Gross C, Fiedler J, Fischer T, Kissler S, Bussen M *et al.* (2008). MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. Nature 456: 980–984. doi:10.1038/nature07511.

Thum T, Chau N, Bhat B, Gupta SK, Linsley PS, Bauersachs J, *et al.* (2011). Comparison of different miR-21 inhibitor chemistries in a cardiac disease model. J Clin Invest 121:461-462: 462–463. doi:10.1172/JCI45938.

Tijsen AJ, van der Made I, van den Hoogenhof MM, Wijnen WJ, van Deel ED, de Groot NE *et al.* (2014). The microRNA-15 family inhibits the TGFβ-pathway in the heart. Cardiovasc Res 104: 61–71. doi:10.1093/cvr/cvu184.

Topkara VK, Mann DL (2011). Role of microRNAs in cardiac remodeling and heart failure. Cardiovasc Drugs Ther 25: 171–182. doi:10.1007/s10557-011-6289-5.

Wahlquist C, Jeong D, Rojas-Muñoz A, Kho C, Lee A, Mitsuyama S *et al.* (2014). Inhibition of miR-25 improves cardiac contractility in the failing heart. Nature 508: 531–535. doi:10.1038/nature13073.

Walther T, Tschope C, Sterner-Kock A, Westermann D, Heringer-Walther S, Riad A *et al.* (2007). Accelerated mitochondrial adenosine diphosphate/adenosine triphosphate transport improves hypertension-induced heart disease. Circulation 115: 333–344. doi:10.1161/CIRCULATIONAHA.106.643296.

Wang J, Xu N, Feng X, Hou N, Zhang J, Cheng X *et al.* (2005). Targeted disruption of Smad4 in cardiomyocytes results in cardiac hypertrophy and heart failure. Circ Res 97: 821–828. doi:10.1161/01.RES.0000185833.42544.06.

Watkins SJ, Borthwick GM, Oakenfull R, Robson A, Arthur HM (2012). Angiotensin II-induced cardiomyocyte hypertrophy in vitro is TAK1-dependent and Smad2/3-independent. Hypertens Res 35: 393–398. doi:10.1038/hr.2011.

Wharton K, Derynck R (2009). TGFbeta family signaling: novel insights in development and disease. Development 136: 3691–3697. doi:10.1242/dev.040584.

Xu P, Liu J, Derynck R (2012). Post-translational regulation of TGF-β receptor and Smad signaling. FEBS Lett 586: 1871–1884. doi:10.1016/j.febslet.2012.05.010.

Zaglia T, Milan G, Ruhs A, Franzoso M, Bertaggia E, Pianca N *et al.* (2014). Atrogin-1 deficiency promotes cardiomyopathy and premature death via impaired autophagy. J Clin Invest 124: 2410–2424. doi:10.1172/JCI66339.

Zhang D, Gaussin V, Taffet GE, Belaguli NS, Yamada M, Schwartz RJ *et al.* (2000). TAK1 is activated in the myocardium after pressure overload and is sufficient to provoke heart failure in transgenic mice. Nat Med 6: 556–563. doi:10.1038/75037.

Zhang YE (2009). Non-Smad pathways in TGF-beta signaling. Cell Res 19: 128–139. doi:10.1038/cr.2008.328.

Zhang DW, Shao J, Lin J, Zhang N, Lu BJ, Lin SC *et al.* (2009). RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis. Science 325: 332–336. doi:10.1126/science.1172308.

Zaiman AL, Podowski M, Medicherla S, Gordy K, Xu F, Zhen L *et al.* (2008). Role of the TGF-beta/Alk5 signaling pathway in monocrotaline-induced pulmonary hypertension. Am J Respir Crit Care Med 177: 896–905. doi:10.1164/rccm.200707-1083OC.