Molecular Signals Elicited by GPCR Agonists in Hypertension, Cardiovascular Remodeling: Are MMPs and ADAMs Elusive Therapeutic Targets?

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Abstract

Hypertension, the condition characterized by sustained elevated blood pressure, affects over 25% of adults in developed countries and is accompanied by pathological cardiac remodeling (*i.e.*, hypertrophy and fibrosis), thus being a major risk factor for cardiac failure. Life style, the environment, genetic factors, diabetes or obesity can all promote development and progression of hypertension associated cardiovascular disease in part because these conditions induce an excess production of pro-hypertensive, pro-hypertrophic and pro-fibrotic agonists.

Here we review signaling pathways shared by major agonists including angiotensin II, catecholamines and endothelins. At the cellular level, these agonists initiate disease signaling by activating cognate G protein-coupled receptors (GPCRs). Early events in agonist-signaling include Ca^{2+} release from intracellular stores, Ca^{2+} uptake from extracellular milieu into cells and reactive oxygen species (ROS) generation by NADPH oxidase. ROS production in turn contributes to activation of matrix metalloproteinases (MMPs) and ‘a disintegrin and metalloproteinases’ (ADAMs). Activated MMPs and ADAMs cleave growth factors, cytokines as well as cell surface receptors, including GPCRs. Excessive activation of MMPs and ADAMs links agonist receptors with transcription and translation of disease-associated genes, including those of MMPs and ADAMs. Recent research indicates a complex and dynamic regulation of MMPs and ADAMs activity and expression by agonists, which poses a significant challenge to strategies aiming at targeting specific MMPs or ADAMs in cardiovascular disease.

Keywords

G protein-coupled receptors; hypertension; cardiovascular hypertrophy; cardiovascular fibrosis; signaling pathways; metalloproteinases

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Conflict of Interest

None.

1. Introduction

Hypertension, the condition of sustained elevated blood pressure (Table 1), has a prevalence worldwide estimated at 1 billion individuals and contributes to approximately 7.1 million deaths per year. While blood pressure <120/80 mmHg is considered as normal, the World Health Organization reports that systolic blood pressure of >115 mmHg is suboptimal and responsible for 62% of cerebrovascular disease and 49% of ischemic heart disease(1, 2).

Morbidity and mortality in hypertension are strongly associated with pathological remodeling of the cardiovascular system. The remodeling process is characterized by development of hypertrophy and fibrosis, leading to target organ dysfunction and failure.

In hypertensive cardiac disease, hypertrophy of the heart is revealed as an increase of cardiac mass and thickening of left ventricular walls due to the enlargement of individual cardiomyocytes. This hypertrophy of cardiomyocytes results from the expression of fetal and developmental genes (*e.g.*, β -myosin heavy chain, α -skeletal actin) that are normally not expressed in the fully-differentiated, quiescent adult cardiomyocyte(3). Pathological cardiac hypertrophy is typically accompanied by an excessive accumulation of extracellular matrix (ECM) proteins (*e.g.*, collagen type I, fibronectin) in the interstitial and perivascular regions of the heart, a process termed cardiac fibrosis. The accumulation of ECM proteins, which are mainly expressed and secreted by cardiac fibroblasts, causes mechanical stiffness and diastolic dysfunction. Excessive ECM protein deposition between layers of cardiomyocytes further disrupts cardiac electric coupling, impairing contraction. Fibrosis of perivascular areas decreases oxygen and nutrient flow from blood to myocardium, contributing to pathological remodeling of the heart(4).

A hallmark of prolonged hypertension is the remodeling of vasculature. Vascular remodeling is characterized by an increase in media to lumen ratio in arteries that elevates systemic resistance to blood flow. Hypertensive vascular remodeling increases resistance by decreasing vasodilatation and enhancing vasoconstriction as well as by increasing arterial stiffness. As a result, arterial blood pressure is elevated, which is strongly linked to increased risk for other complications such as atherosclerosis and multiple organ damage(5–8). There are two kinds of vascular remodeling: inward eutrophic remodeling (the outer and luminal diameters are decreased, media/lumen ratio is increased, and cross-sectional area of the media is unaltered) and hypertrophic remodeling (the media thickens toward the lumen, which increases media cross-sectional area and media/lumen ratio). Both are associated with vascular fibrosis (*i.e.*, the accumulation of ECM protein synthesized by fibroblasts and promoted by growth factors and cytokines, primarily transforming growth factor (TGF)- β), hypertrophic growth (*i.e.*, enlargement of individual vascular smooth muscle cells (VSMCs) resulting from the expression of fetal and developmental genes) and apoptosis (*i.e.*, programmed cell death with minimal inflammation response)(8, 9).

Hypertension in the general population can have a complex, often unknown etiology. Vasoconstrictive agonists such as angiotensin II, catecholamines, and endothelins have all been implicated in causation of hypertension. Diverse conditions such as life style, stress, environmental and genetic factors, diabetes and obesity all predispose to hypertension. This

can be explained, at least in part, by the excessive production in these conditions of vasoconstrictive agonists(10–15). It is remarkable that all vasoconstrictive agonists have cognate receptors coupled to heterotrimeric G proteins(16). These G protein-coupled receptors (GPCRs) connect with many intracellular pathways to initiate and maintain contraction as well as hypertrophic and fibrotic processes in arteries and the heart(17–19) (Figure 1).

From a clinical translation point of view, preventing GPCR activation (by blocking either agonist generation or receptor-binding) has been widely exploited to ameliorate agonist-induced hypertension and cardiovascular remodeling(10, 11, 20). However, when the etiology of the hypertensive cardiac disease is complex (*e.g.*, many agonists are involved) or unknown, tailoring treatment to specific causative agonists is ineffective. In the 21st century physicians still treat hypertension empirically by choosing a drug or drug combination until adequate blood pressure reduction and regression of cardiac remodeling are achieved. Over half of patients receiving treatment do not meet current treatment goals (of <140/90 mmHg for adults with hypertension and <130/80 mmHg for hypertensives with diabetes or chronic renal disease)(1, 21). There is a need for alternative treatment strategies targeting common downstream mediators of multiple agonists. Here we review the major signaling pathways linking GPCRs to contraction, hypertrophy and fibrosis in vasculature and the heart with a focus on major and common mediators of multiple agonists. We further develop the notion that metalloproteinases including matrix metalloproteinases (MMPs) and ‘a disintegrin and metalloproteinases’ (ADAMs) are important mediators in many cardiovascular disease processes (Figure 1).

2. Mechanisms of GPCR agonist-induced contraction, hypertrophy and fibrosis in the myocardium and vascular smooth muscle

GPCRs are transmembrane proteins that are characterized by 7 transmembrane domains. They are linked to heterotrimeric G proteins consisting of 3 subunits: G_α (active when bound to GTP and inactive when bound to GDP), G_β and G_γ (22). Among the receptors of vasoconstrictors, the type I angiotensin II receptor (AT1R), the α_1 adrenoceptor (the prominent catecholamine receptor in the vascular system) and the endothelin receptors type A (ET_A) and type B (ET_B) are mainly coupled to Gq proteins which activate phospholipase C (PLC)- β . The β_1 -adrenoceptor (prominent cardiac catecholamine receptor) is coupled to Gs protein that activates adenylyl cyclase.

Agonists typically bind in the pocket formed by the transmembrane domains of GPCRs, leading to conformational changes of these receptors. These GPCRs then serve as guanine nucleotide exchange factors (GEFs) to trigger the activation of their coupled G proteins by promoting the exchange of GDP to GTP on G_α . The activated G_α of Gq ($G_{q\alpha}$) protein and Gs ($G_{s\alpha}$) protein then dissociate from the $\beta\gamma$ subunits ($G_{\beta\gamma}$). Free $G_{q\alpha}$ and $G_{s\alpha}$ interact with and activate membrane-bound PLC- β and adenylyl cyclase respectively to trigger downstream signaling. In addition, PLC- β and adenylyl cyclase have GTPase-activating protein (GAP) activity which increases the intrinsic GTPase activity of G_α , facilitating hydrolysis of bound GTP and subsequent deactivation and reassociation with $G_{\beta\gamma}$ (23). Free

$G_{\beta\gamma}$ released from the active G protein can also act as signal molecules triggering the phosphatidylinositol 3 kinase (PI3K) pathway and the mitogen-activated protein kinase (MAPK) pathway as well as regulating Ca^{2+} homeostasis(24–28).

2.1. Regulation of contraction

The elevation of systemic blood pressure by GPCR agonists is in part due to an increase in cardiac output (associated with augmented myocardial contraction) and peripheral resistance (associated with enhanced vascular tone). The contraction of VSMCs and subsequent vasoconstriction in response to GPCR agonists rely on the contractile proteins myosin (in the thick filament in the sarcomere) and actin (in the thin filament of the sarcomere). Phosphorylation of myosin light chain (MLC) in the myosin globular head initiates the interaction between myosin and actin. This interaction leads to the activation of myosin ATPase (also in the myosin globular head) and hydrolysis of ATP, resulting in the cycling of the myosin-actin cross-bridge, subsequent VSMC contraction and vasoconstriction(29). The phosphorylation state of MLC is primarily regulated by MLC kinases (which are targets of signaling proteins including Ca^{2+} -activated calmodulin (CaM) and protein kinase C (PKC)) and MLC phosphatases. VSMC contraction is also controlled by other regulatory proteins, such as caldesmon which inhibits myosin ATPase activity and blocks binding between myosin and actin to prohibit VSMC contraction(30).

In similarity to smooth muscle cells, the contraction of cardiomyocytes is also mainly regulated by the change in the intracellular concentration of Ca^{2+} , $[Ca^{2+}]_i$. The increase in $[Ca^{2+}]_i$ triggers the association of Ca^{2+} with troponin, a protein complex composed of three subunits: TnI (which binds actin in the thin filament), TnC (which binds Ca^{2+} to induce a conformational change of TnI) and TnT (which binds tropomyosin). Binding of Ca^{2+} to troponin triggers a conformational change in tropomyosin, exposing the myosin-binding sites on actin, which can then bind to the myosin heads of thick filaments, leading to the process of cross-bridge cycling (which requires the energy released by myosin ATPase in the myosin head) and cardiomyocyte contraction(31). In addition, cardiomyocyte contraction is modulated by kinases and phosphatases which respectively phosphorylate and dephosphorylate troponin and tropomyosin, although the exact effects of their phosphorylation are not fully understood(32, 33) (Figure 2).

2.2. Regulation of hypertrophic growth

The development of hypertrophy, or cell enlargement, depends on an increase in protein synthesis. Although a potential decrease in protein degradation has been suggested(34), most studies on development of cardiac hypertrophy have focused on the increase in protein synthesis, at the levels of both transcription and translation (Figure 3).

One of the effects triggered by GPCR agonists so as to promote the development of hypertrophy in cardiomyocytes and VSMCs is the increased activity of transcription factors, such as myocyte enhancer factor-2 (MEF-2), nuclear factor of activated T-cell (NFAT) and activation protein-1 (AP-1). This activation is mediated by signal molecules including MAPKs, Ca^{2+} and calcineurin. GPCR agonists also induce nuclear export of histone deacetylases (HDACs), facilitating chromosomal de-condensation to promote

transcription(35, 36). Among the targets of these transcription factors are the genes coding for α -skeletal actin and β -MHC, whose increased expression contributes to the changes in contractility of vascular smooth muscle and myocardium(37–39). These genes, which are usually only expressed during fetal development, are induced in hypertrophic cells and are often considered markers of hypertrophy.

Increased translational activity also contributes to the development of hypertrophy. GPCR agonists promote protein translation by upregulating the synthesis and assembly of ribosomes as well as increasing the activity of eukaryotic translation initiation factors (*e.g.*, eukaryotic translation initiation factor 4E (eIF4E)) and elongation factors (*e.g.*, eukaryotic elongation factor 2 (eEF-2)) in cardiomyocytes and VSMCs(40, 41).

2.3. Regulation of fibrosis

In the development of cardiac and vascular fibrosis, the excessive ECM protein synthesis depends on growth factors and cytokines (primarily TGF- β) which initiate signaling pathways involving Smad transcription factors (Figure 3). The expression and secretion of TGF- β are induced by stimulation with GPCR agonists both *in vitro* and *in vivo*(42–44). In addition, GPCRs facilitate TGF- β release by activating metalloproteinases, including MMP-2 and MMP-9, to cleave and release active TGF- β (45). MMP-2, MMP-3 and MMP-7 can also cleave decorin, a TGF- β binding proteoglycan. Once TGF- β is free from the extracellular milieu, it can bind to its membrane receptors and trigger downstream signaling(46). Experimentally, blocking or deleting TGF- β inhibits GPCR agonist-induced development of cardiac and vascular fibrosis(17, 43).

Incubation with TGF- β induces the transition of cardiac and vascular fibroblasts into myofibroblasts, which are characterized by the expression of α -smooth muscle actin and the increased release of ECM proteins, such as type I and type III collagens and fibronectin. Specifically, TGF- β binds to the type-II TGF- β receptor (T β RII), which recruits the type-I TGF- β receptor (T β RI) and leads to T β RI phosphorylation and activation to initiate downstream signaling. Active T β RI, with the aid of the Smad anchor for receptor activation (SARA), recruits and phosphorylates the regulatory Smads (R-Smads) Smad-2 and Smad-3. Phosphorylated R-Smads subsequently bind with the cooperative Smad (C-Smad) Smad-4, and translocate to the nucleus to initiate transcription of target genes including those encoding ECM proteins. In addition, this process is inhibited by inhibitory Smads (I-Smads) Smad-6 and Smad-7, which compete with R-Smads for interaction with receptors and Smad-4, or facilitate the ubiquitination and degradation of R-Smad and TGF- β receptors(47) (Figure 3).

In addition to fibroblasts, studies also suggest that angiotensin II can induce Smad signaling and ECM protein expression in VSMCs to mediate the development of fibrosis, which is dependent on extracellular signal-regulated kinases-1/2 (ERK-1/2) or p38 MAPK activity(48, 49).

2.4. Molecular signals downstream of GPCRs

Stimulation of GPCRs by agonists (such as angiotensin II) leads to activation of their coupled Gq or Gs proteins, triggering multiple signaling pathways, including PLC-PKC,

protein kinase A (PKA), MAPKs, PI3K-Akt and calcineurin-NFAT, to mediate VSMC and cardiomyocyte contraction and hypertrophic growth. As in many other systems, these signaling pathways do not act independently, but cross-talk to mediate contraction (Figure 2), hypertrophy and fibrosis (Figure 3). As Figures 2 and 3 show, there is substantial overlap among pathways that signal contraction and those that signal remodeling.

2.4.1. The role of PLC- β and downstream pathways in GPCR regulated contraction and hypertrophy—The members of the Gq α protein family (including Gq α , G $_{11\alpha}$, G $_{14\alpha}$ and G $_{15/16\alpha}$) are characterized by their ability to activate PLC- β (50). In addition to Gq α , G $\beta\gamma$ released from active heterotrimeric G proteins can also activate PLC- β (51, 52). PLC- β leads to the activation of PKC and the elevation of $[Ca^{2+}]_i$, which are major regulators of VSMC and cardiomyocyte contraction. Inhibition of PLC- β activity blocks GPCR agonist-induced contraction in VSMCs and cardiomyocytes (53, 54). In addition, the significance of PLC- β in the development of cardiac hypertrophy has been proven by the hypertrophic growth of neonatal cardiomyocytes overexpressing PLC- β 1b, one of the isoforms of PLC- β (55).

2.4.1.1. Ca^{2+} in GPCR regulated contraction and hypertrophy: Upon the activation of GPCRs, Gq α activates PLC- β , allowing it to hydrolyze the phosphodiester bond of phosphatidylinositol 4,5-bisphosphate (PI(4,5)P $_2$), one of the phospholipids in the plasma membrane. This process results in the formation of hydrophobic 1,2-diacylglycerol (DAG) and water-soluble inositol 1,4,5-trisphosphate (IP $_3$), two potent second messengers. DAG remains in the plasma membrane and serves as an activator of PKC, as discussed later. IP $_3$ diffuses from the plasma membrane to the sarcoplasmic reticulum (SR), where it binds to IP $_3$ receptors (one of the Ca^{2+} channels on SR membrane), leading to the release of Ca^{2+} from the SR and the elevation of $[Ca^{2+}]_i$. GPCR agonists also activate plasma membrane L-type Ca^{2+} channels through G $\beta\gamma$ subunits to trigger extracellular calcium entry. This process is probably mediated by the ability of G $\beta\gamma$ to activate PI3K (56). The increase in $[Ca^{2+}]_i$ (from extracellular space and intracellular stores) can induce supplementary release of Ca^{2+} from the SR by activating ryanodine receptors (Ca^{2+} -dependent Ca^{2+} channels on SR membrane), further elevating $[Ca^{2+}]_i$ (57).

The EF hand Ca^{2+} -binding protein CaM is one of the primary targets of elevated $[Ca^{2+}]_i$. Ca^{2+} binding triggers CaM's conformational change and activation. In VSMCs, activated CaM is able to bind to and activate MLC kinase, which mediates contraction as described above (29). However, in cardiomyocytes, elevated $[Ca^{2+}]_i$ triggers the association of Ca^{2+} with troponin, which allosterically modulates tropomyosin, exposing the myosin binding sites of thin filaments. This permits subsequent cross-bridge cycling and cardiomyocyte contraction (31). In addition to its role in the regulation of contraction, activated CaM also promotes hypertrophic growth in cardiomyocytes and VSMCs through the activation of the calcineurin-NFAT pathway and nuclear export of HDACs (35, 58).

2.4.1.2. PKC in GPCR regulated contraction and hypertrophy: The activation of PLC- β and the subsequent release of DAG and IP $_3$ triggered by Gq protein-coupled receptor agonists also induce the activation of PKCs. The mammalian PKC family has 11 members and can be divided into conventional PKCs (cPKCs; - α , - β I, - β II, - γ , which require both

Ca^{2+} and DAG for activation), novel PKCs (nPKCs; $-\delta$, $-\epsilon$, $-\eta$, $-\theta$, which only require DAG for activation) and atypical PKCs ($-\zeta$, $-\lambda$, $-\mu$, which cannot be activated by DAG).

The elevation of $[\text{Ca}^{2+}]_i$ in response to GPCR activation results in the binding of Ca^{2+} to cPKCs in the cytoplasm, triggering cPKCs' structural changes and translocation from the cytoplasm to the inner face of the plasma membrane. On the other hand, DAG remaining in the plasma membrane cooperates with phosphatidylserine (PS) to interact with and activate membrane-bound PKCs, including both cPKCs and nPKCs(59).

PKCs, which are serine/threonine protein kinases, can activate the small G protein Ras which in turn initiates downstream signaling pathways including the MEK-ERK pathway and the PI3K pathway, signaling the development of cardiovascular hypertrophy(60–64). Experimentally, the overexpression of PKC- α , $-\beta$, $-\delta$, or $-\epsilon$ triggers ERK and/or PI3K activation and hypertrophic growth in cultured cardiomyocytes or VSMCs as well as in the hearts of transgenic mice(65–68).

PKCs regulate smooth muscle and myocardial contraction through their ability to: 1) activate MAPKs, such as ERK-1/2, which subsequently phosphorylate a thin filament-associated protein caldesmon, reversing its inhibition of myosin ATPase activity and increasing actin-myosin interaction (cross-bridge cycling)(30), 2) phosphorylate calponin, another thin filament-associated protein, and disrupt its ability to block cross-bridge cycling(69), 3) phosphorylate Thr38 on PKC-potentiated inhibitory protein of 17 kDa (CPI-17), which in turn inhibits MLC phosphatase, increasing MLC phosphorylation and smooth muscle contraction(70), and 4) directly phosphorylate MLC kinase and MLC to increase actin-myosin interaction (71, 72).

Apart from PKC, DAG also activates protein kinase D (PKD, also known as PKC μ), a serine/threonine kinase family that can also be activated by PKC phosphorylation. Among the targets of PKD are HDACs, whose phosphorylation leads to their nuclear export, permitting pro-hypertrophic gene expression(73, 74). PKD upregulates Ras activity and MEK/ERK signaling by phosphorylating the Ras-binding protein, Ras and Rab interactor-1 (RIN-1)(75). The inhibition of PKD blocks HDAC nuclear transport and development of cardiomyocyte hypertrophy *in vivo* and *in vitro*(74, 76).

2.4.2. The role of PKA and downstream pathways in GPCR regulated

contraction and hypertrophy—Stimulation of Gs by GPCR agonists leads to the activation of adenylyl cyclase, which generates cAMP from ATP and activates PKA. PKA is composed of two regulatory subunits and two catalytic subunits. cAMP can bind to the regulatory subunits and lead to the release of the active catalytic subunits. One of the primary targets of active PKA are the L-type Ca^{2+} channels on the plasma membrane, resulting in an influx of extracellular Ca^{2+} to induce an increase in $[\text{Ca}^{2+}]_i$, promoting the contraction of VSMCs and cardiomyocytes as well as their hypertrophic growth (through the activation of CaM). PKA also phosphorylates the small G protein Rap, which in turn activates isoform B of Raf to trigger the MEK-ERK pathway(77).

Experimentally, cardiac overexpression of β_1 -adrenoceptor, which is usually coupled to Gs proteins, initially increases heart contractility in transgenic mice, but eventually results in progressive cardiomyocyte hypertrophy and fibrosis(78). Cardiac overexpression of the catalytic subunit of PKA also induces the development of dilated cardiomyopathy associated with cardiomyocyte hypertrophy and fibrosis, supporting the pro-hypertrophic role of Gs protein-coupled receptors and their downstream signaling(79).

Other targets of PKA include transcription factors such as cAMP-response element binding protein (CREB) and cAMP-dependent transcription factor-1 (ATF-1). PKA, in addition to other kinases including ERK and CaM-dependent kinase, phosphorylates CREB on Ser133 and triggers its transcriptional activity. The target genes of CREB include those coding for the β -adrenoceptor, TGF- β and atrial natriuretic peptide, which are involved in the regulation of hypertrophic growth in cardiomyocytes. Stimulation by GPCR agonists induces the activation of CREB, the expression of its target genes and cardiomyocyte hypertrophy(80, 81). Moreover, the inhibition of CREB prevents angiotensin II-induced hypertrophy in VSMCs(82).

In addition to PKA, another target protein of cAMP is the exchange protein directly activated by cAMP (Epac), a small G protein GEF. The classic target of Epac is Rap, which can activate B-Raf to trigger MEK/ERK signaling(83). Epac has been reported to mediate β -adrenergic receptor-induced cardiomyocyte hypertrophy involving the activation of Ras, Rac and calcineurin(84, 85).

2.4.3. Four signaling pathways from GPCRs to transcription and translation—

Downstream of PLC, PKC and PKA, the induction of hypertrophy in VSMCs and cardiomyocytes by GPCR agonists is mainly mediated by their ability to stimulate signal pathways that regulate transcription and translation, including Raf-MEK-ERK, PI3k-Akt, calcineurin-NFAT and HDAC signaling pathways.

2.4.3.1. The Raf-MEK-ERK pathway: ERK-1/2, also known as p42/44-MAPKs, are members of the MAPK family of serine/threonine kinases and are major regulators of hypertrophic growth of VSMCs and cardiomyocytes. Their activation requires phosphorylation on threonine and tyrosine residues by the upstream MAPK kinases, MEK-1/2, which are phosphorylated and activated by Raf MAPK kinase kinase.

The significance of the Raf-MEK-ERK pathway in the development of cardiac hypertrophy has been demonstrated by the development of significant cardiac hypertrophy in mice with cardiac-specific overexpression of MEK-1(86). In addition, mice expressing cardiac-specific dominant negative Raf-1 are resistant to the development of cardiac hypertrophy induced by transverse aortic constriction(87).

Upon stimulation by GPCR agonists, the activation of PKC through Gq α -PLC signaling leads to the activation of small GTPase Ras, although the mechanism is not fully understood(60–62). PKC can phosphorylate and inhibit Ras GAP to maintain the activity of Ras(88). Activated Ras (ie., in GTP-bound state) recruits Raf protein (through Raf's Ras interaction domain) to the membrane and facilitates its phosphorylation on Ser338 and

Tyr341, resulting in the full activation of Raf. PKC can also activate Raf independently of Ras(89). Additionally, cAMP generated downstream of $G_{s\alpha}$ activation, can activate B-Raf, one of the Raf isoforms expressed in the heart, through both PKA-dependent and -independent pathways. The serine/threonine kinase, Raf phosphorylates and activates MEK-1/2, which subsequently activate ERK-1/2(90). Activated ERK-1/2 can phosphorylate and trigger transcription factors including c-myc, c-fos, Elk-1, GATA-4 and CREB to mediate gene transcription and cardiomyocyte hypertrophy(91). The MEK-ERK pathway also regulates translation downstream of GPCR activation. In adult cardiomyocytes, phenylephrine (an α -adrenoreceptor agonist) and endothelin-1 can induce an increase in protein synthesis by activating ribosomal protein S6 kinase 1 (S6K-1), and by inducing the release of eIF4E from its inhibitory binding partner eIF4E-binding protein-1 (4EBP-1). These pro-hypertrophic processes can be blocked by inhibitors of MEK or Ras, suggesting that the MEK-ERK pathway also engages translation to mediate GPCR-induced hypertrophy(92).

Another target of ERK is MAPK-activated protein kinase (MAPKAP-K1 or p90RSK/ ribosome protein S6 kinase), which can also phosphorylate S6 to promote ribosomal activity and translation. In addition, p90RSK promotes transcription by phosphorylating and activating transcription factors like CREB and c-fos. Activation of p90RSK has been reported to mediate cardiomyocyte hypertrophy *in vivo* and *in vitro*(93, 94).

The MEK-ERK pathway may also regulate the contraction of VSMCs. For example, ERK-1/2 can phosphorylate caldesmon, reverse its inhibition of myosin MgATPase activity and increase actin-myosin interaction and subsequent VSMC contraction(30).

2.4.3.2. The PI3K-Akt pathway: Since its discovery in the 1980s, PI3K, has been found to be involved in diverse cell processes including cell proliferation, differentiation and survival, mainly through the activation of its downstream serine/threonine kinase Akt (also known as protein kinase B (PKB))(95).

Activation of Ras downstream of GPCR-PLC-PKC can lead to the activation of class I PI3K (composed of a catalytic 110 kDa subunit and a regulatory 85 kDa or 55 kDa subunit), whose catalytic subunit contains a Ras-binding domain(63). Moreover, free $G_{\beta\gamma}$, upon GPCR activation, is able to activate class IB PI3K (probably through their direct interaction) (24). Activated PI3K converts plasma membrane lipid PI(4,5) P_2 into phosphatidylinositol 3,4,5-trisphosphate (PI(3,4,5) P_3) by phosphorylation. This results in the recruitment of Akt and 3-phosphoinositide-dependent protein kinase 1 (PDK-1) to the cell membrane through the interaction between their pleckstrin-homology (PH) domains and PI(3,4,5) P_3 . This forced colocalization of Akt and PDK-1 leads to the phosphorylation (on Thr308 and Ser473) and activation of Akt by PDK-1(96, 97).

In transgenic mice, PI3K or Akt overexpression induces the development of cardiac hypertrophy(98, 99). Expression of a dominant negative form of PI3K results in smaller hearts compared to the wild-type mice(98, 100). Meanwhile, Akt overexpression induces VSMC hypertrophy, and Akt inhibition blocks angiotensin II-induced VSMC hypertrophy *in*

vitro(101). These results confirm the significance of the PI3K-Akt pathway in the development of GPCR agonist-induced hypertrophy.

One of the ways that Akt contributes to the development of hypertrophy is via the activation of another protein kinase, mammalian target of rapamycin (mTOR)(40). Inhibition of mTOR by rapamycin attenuates pathological cardiac hypertrophy and reverses myocardial dysfunction induced by cardiac Akt-1 overexpression.(102, 103). Akt leads to mTOR activation through the phosphorylation of tuberous sclerosis protein 2 (TSC-2 or tuberin). Phosphorylation attenuates TSC-2's GAP activity towards small G protein Rheb, thus maintaining Rheb activity. Rheb can then bind to the N-terminal lobe of the catalytic domain of mTOR and activate mTOR(104). Downstream of mTOR are p70/85 S6K-1 and p54/56 S6K-2. When activated by mTOR via phosphorylation, S6K-1/2 can phosphorylate and activate ribosomal S6 protein, which subsequently stimulates translation of the 5'-terminal oligopyrimidine tract (5'-TOP) mRNAs (including the mRNAs of translation factors and most ribosomal proteins), promoting the synthesis of ribosomes and subsequent hypertrophic growth of cardiomyocytes and VSMCs(40, 105, 106). mTOR also stimulates translation by activating translation initiation factor eIF4E and translation elongation factor eEF-2. mTOR: 1) phosphorylates eIF4E-binding protein 4EBP-1, leading to the release of eIF4E and enabling it to bind 5'-capped mRNA; 2) phosphorylates eEF-2 kinase, inhibiting its ability to phosphorylate and de-activate eEF-2(105). In addition to Akt, ERK-1/2 can also phosphorylate and inhibit TSC-2 to activate mTOR, indicating there is cross-talk between the PI3K-Akt pathway and the MEK-ERK pathway and supporting the pro-hypertrophic effect of the MEK-ERK signaling(107).

Activation of Akt via PI3K signaling also promotes cardiac hypertrophy through the phosphorylation and deactivation of glycogen synthase kinase 3 β (GSK-3 β). Apart from its role in metabolism, active GSK-3 β phosphorylates and inhibits translation initiation factor eIF2B to block translation. The inhibition of GSK-3 β by Akt facilitates translation by activating eIF2B, and promotes transcription by activating transcription factors involved in hypertrophic growth, such as c-Myc, GATA-4 and NFAT(40, 108). In addition, ERK is reported to interact with GSK-3 β to prime GSK-3 β for inactivation(109).

Another target of Akt is the Forkhead box subfamily O (Foxo) transcription factors which mediate the atrophy of cardiomyocytes. Akt phosphorylates Foxos and inhibits their ability to induce transcription of so-called "atrogenes", genes involved in cellular atrophy (*i.e.*, decrease in cell size). One of the atrogenes, atrogin-1, is an E3 ubiquitin ligase which mediates the degradation of calcineurin to inhibit hypertrophic growth (detailed below). Overexpression of Foxo-3a blocks angiotensin II-, insulin-like growth factor(IGF)- or stretch-induced cardiomyocyte hypertrophy *in vitro*(110, 111).

PI3K has also been reported to activate membrane L-type Ca²⁺ channels to promote Ca²⁺ entry into the myocardium and smooth muscle to mediate their contraction(56, 112, 113). In addition, studies show that PI3K inhibition reduces VSMC contraction, and that cardiac specific expression of PI3K α or Akt initially increases myocardial contractility, suggesting the possible involvement of the PI3K-Akt pathway in the regulation of vasoconstriction and myocardial contraction(98, 99, 114).

2.4.3.3. The calcineurin-NFAT pathway: The Ca^{2+} -dependent serine/threonine protein phosphatase, calcineurin, and downstream transcriptional effector, NFAT, are considered critical mediators of cardiac hypertrophy since they connect changes in $[\text{Ca}^{2+}]_i$ with the development of hypertrophy in cardiomyocytes. Calcineurin, discovered in 1979, consists of a ~60kDa catalytic subunit (CnA) and a 19 kDa regulatory subunit (CnB)(115). The increase in $[\text{Ca}^{2+}]_i$ triggered by GPCRs activates calcineurin by 1) binding of Ca^{2+} to CnB and 2) activating CaM to bind with CnA(116). Active calcineurin dephosphorylates multiple phospho-serine residues in the N-terminus of transcription factor NFAT, which then translocates to the nucleus and initiates pro-hypertrophic transcription together with other transcription factors like AP-1, MEF-2 and GATA-4(58, 117). The target genes of NFAT include hypertrophy markers such as atrial natriuretic factor, β -MHC and α -skeletal actin, and proteins promoting the development of hypertrophy, such as tumor necrosis factor- α (TNF- α) and endothelin-1. Mice expressing constitutively active calcineurin or NFAT develop significant cardiac hypertrophy(118). Inhibition of calcineurin by specific inhibitors like cyclosporine can block the development of cardiomyocyte hypertrophy induced by angiotensin II *in vivo* and *in vitro*(119).

2.4.3.4. Histone acetylases and deacetylases: HDACs compete with histone acetylases (HATs) for the binding site of transcription factor MEF-2 and then suppress gene transcription by removing acetyl groups from histone tails and promoting chromatin condensation. GPCR agonists induce VSMC and cardiomyocyte hypertrophic growth through the regulation of class II HDACs (including -4, -5, -6, -7, -9, -10), which can shuttle between the nucleus and the cytoplasm(120). Stimulation by GPCR agonists activates PKA, PKCs, PKD, and CaM kinase (activated by CaM), all of which can phosphorylate HDACs. Phosphorylation triggers HDACs' nuclear export, which prevents their interaction with MEF-2 and chromosomal DNA, permitting the expression of pro-hypertrophic genes(35, 36, 121, 122). Expression of constitutive nuclear mutants of HDAC-5 or HDAC-9 inhibits GPCR agonist-induced hypertrophy in cardiomyocytes *in vitro*, whereas HDAC-5 or HDAC-9 deficient mice display enhanced cardiac hypertrophy(123, 124).

2.4.4. GPCR signaling through reactive oxygen species—Elevated levels of reactive oxygen species (ROS) such as hydrogen peroxide and superoxide damage proteins, DNA and membrane lipids via oxidation and other modifications (including oxidation of sulfhydryl groups, reduction of disulfide bonds, adduction of residues to metal ions, cross-linking and fragmentation). These damages lead to an increase in oxidative stress, energetic deficit and cell death, all of which contribute to the progression towards heart failure(125). At lower levels, ROS can exert more subtle effects by regulating the activity of diverse intracellular molecules and pathways, such as the MAPK and PI3K pathways, mainly through their ability to activate kinases and inhibit tyrosine phosphatases(126, 127). These subtle effects may be more significant in the regulation of contraction and hypertrophic growth in cardiomyocytes and VSMCs. Studies have shown that stimulation by GPCR agonists like angiotensin II can induce an increase in the level of intracellular ROS to mediate contraction and hypertrophic growth in VSMCs and cardiomyocytes. Furthermore, treatment with antioxidants prevents the development of GPCR agonist-induced vasoconstriction and cardiovascular hypertrophy(15, 128–130).

The cellular sources of ROS include mitochondria, NADPH oxidase, xanthine oxidase and nitric oxide synthase. The main sites of mitochondrial ROS generation are complex I (NADH dehydrogenase) and complex III (coenzyme Q: cytochrome c oxidoreductase) of the electron transport chain, where single electrons are transferred to oxygen molecules (O_2) to generate superoxide radicals ($O_2^{\bullet-}$) as byproducts of respiration. Most studies suggest that mitochondrial ROS are important in models of heart failure and myocardial ischaemia-reperfusion, but less so in the process of contraction and hypertrophy(131–133). Rather, NADPH oxidase, a membrane-associated protein complex which transfers electrons from NADPH to O_2 to generate superoxides, is considered to be the main source of ROS in acute response to GPCR activation(134–137).

Generated $O_2^{\bullet-}$ can be converted into H_2O_2 molecules (which are more stable and diffusible than radicals like $O_2^{\bullet-}$ and thus serve as better signal molecules) and hydroxyl radicals $HO^{\bullet}$ (which are highly reactive damaging molecules)(138). $O_2^{\bullet-}$ can also react with nitric oxide to generate peroxynitrite ($ONOO^-$). This scavenging of nitric oxide, a vasodilator, contributes to the suppression of vasodilation and thus, the development of hypertension. $ONOO^-$, meanwhile, induces protein oxidation and nitration and, at a high concentration, leads to mitochondrial membrane permeabilization and loss of mitochondrial function(139, 140). In addition, ROS generated by NADPH oxidase can further promote ROS production from nitric oxide synthase, xanthine oxidase and mitochondria as well as NADPH oxidase itself(141–144).

Stimulation by GPCR agonists like angiotensin II induces expression of NADPH oxidase subunits and promotes the assembly of NADPH oxidase complex on the plasma membrane, leading to its activation and subsequent ROS production in cardiomyocytes and VSMCs(145–148). The GPCR agonist-induced NADPH oxidase activation and ROS generation can also be mediated by PKC phosphorylation of p47phox, one of the subunits of NADPH oxidase required for the activation of the enzyme(149, 150). Treatment with phorbol-12-myristate-13-acetate (PMA, also known as 12-O-tetradecanoylphorbol-13-acetate or TPA), a DAG homolog that constitutively activates PKCs, induces an increase in the level of intracellular ROS in VSMCs. This increase can be blocked by the inhibition of PKC or NADPH oxidase, but not by inhibition of other ROS-generating enzymes including xanthine oxidase, nitric oxide synthase and the mitochondrial electron transport chain(137).

Another pathway that mediates the activation of NADPH oxidase is the PI3K-Akt pathway. Akt phosphorylates p47phox on S304 and S328 to support NADPH oxidase activation(151). PI3K also mediates the activation of small G protein Rac, one of the regulator of NADPH oxidase, facilitating the recruitment of cytoplasmic components of the NADPH oxidase complex to the membrane(152). Expression of constitutively active Rac increases basal and angiotensin II-induced levels of ROS, whereas inhibition of Rac activity can decrease angiotensin II-induced ROS production and VSMC contraction as well as angiotensin II-induced cardiomyocyte hypertrophy(147, 153, 154). $PI(3,4,5)P_3$ produced by PI3K activates Rac-specific GEFs, such as Vav and SWAP-70(155, 156), which trigger the activation of Rac. Deletion of PI3K- γ blocks Rac activation, ROS production and hypertension induced by angiotensin II stimulation *in vivo*(157).

The effects of ROS in signaling depend on their ability to inhibit protein tyrosine phosphatases (PTP) and to activate kinases (through the oxidation of cysteine residues). In neonatal cardiomyocytes, treatment with H₂O₂ can induce the activation of MAPKs including ERK-1/2(158, 159). ROS can activate Ras by modifying its cys-118 and increasing its GTP/GDP exchange activity (160). Activation of Ras in turn leads to the activation of the MEK-ERK and the PI3K-Akt pathways, which promote contraction and hypertrophic growth in VSMCs and cardiomyocytes. Additionally, ROS can trigger the activation of Akt in several cell types such as VSMCs and fibroblasts(159, 161). Angiotensin II-induced Akt activation can be inhibited by exposure to antioxidants or by overexpressing catalase, the enzyme catalyzing the decomposition of H₂O₂ to water and oxygen(161).

ROS also stimulate contraction and hypertrophic growth through the regulation of [Ca²⁺]_i. ROS can oxidize a cysteine residue on the ryanodine receptor to increase Ca²⁺-dependent Ca²⁺ release from the SR(162). Meanwhile, NADPH oxidase-derived ROS are reported to mediate endothelin-1 and Ang II-induced activation of L-type Ca²⁺ channels(163, 164).

In the hypertrophic heart, accumulation of ROS-damaged proteins, membrane lipids and DNA (including mitochondrial DNA) results in assembly defects and dysfunction of enzymes, including the mitochondrial respiration chain, which is essential for generation of ATP. These modifications also result in membrane potential abnormality, mitochondrial replication inhibition, and can induce apoptosis signals to mediate death of cardiomyocytes. Furthermore, the increase in the level of ROS also mediates the proliferation of cardiac fibroblasts as well as TGF- β expression and secretion, promoting the deposition of ECM proteins and thus the development of fibrosis(165). These pro-apoptotic and pro-fibrotic effects all contribute to the loss of heart contractility and the development of heart failure in the long term(166).

3. Cross-talk between GPCRs and other cell surface receptors

In the last twenty years, studies have uncovered that stimulation by GPCR agonists can also lead to the activation of receptors of growth factors and cytokines to enhance vascular smooth muscle contraction and promote hypertrophic growth in both vascular smooth muscle and the heart(14, 15, 167, 168). This “transactivation” process, first described in 1996, demonstrated that activation of GPCRs leads to the phosphorylation of the EGFR and subsequent MAPK activation and c-Fos expression(169). Further studies suggested that this process is mediated at least in some cases, by MMPs and ADAMs(14, 15, 167, 168, 170, 171). These metalloproteinases, when activated, cleave and release (shed) growth factors (*e.g.*, HB-EGF) and cytokines (*e.g.*, TNF- α), leading to the activation of their cognate receptors and downstream signaling involving MAPKs, PI3K-Akt and ROS. Studies using animal models have shown that non-specific inhibition of metalloproteinases can attenuate the development of systemic and pulmonary hypertension, cardiac hypertrophy and vascular remodeling, confirming the significance of metalloproteinases in these cardiovascular diseases(172–175).

One of the first metalloproteinases identified to mediate hypertensive cardiac disease is ADAM-12, which was found to mediate GPCR agonist-induced cardiac hypertrophy by

shedding heparin-binding EGF-like growth factor (HB-EGF) and transactivating the epidermal growth factor receptor (EGFR)(167). In later studies ADAM-17 (also known as TNF- α converting enzyme or TACE) was also shown to mediate VSMC hypertrophy(168). Our studies suggest that MMP-7 may transactivate the EGFR to maintain agonist-induced vasoconstriction of small arteries and promote hypertension and cardiac hypertrophy(15, 176, 177). We have also observed that MMP-2 is induced in the course of hypertension and might play a specific role in promoting high blood pressure in mice with angiotensin II-induced hypertension(178, 179).

3.1. Agonist-induced activation of metalloproteinases

Metalloproteinases, including MMPs and ADAMs, are zinc-dependent endopeptidases. They are expressed as latent forms and their activation requires the dissociation of the cysteine in the prodomain with the catalytic zinc ion (normally through the cleavage of prodomain or the oxidation of cysteine)(180). Their primary substrates are ECM proteins, and thus metalloproteinases are important for tissue remodeling(181). Among other targets of metalloproteinases are latent receptor ligands such as growth factors, cytokines, apoptotic ligands (*e.g.*, Fas ligand) and anti-angiogenic factors (*e.g.*, chondromodulin-1). Free active ligands subsequently activate cognate receptors to initiate downstream signaling.

Metalloproteinases are also reported to cleave membrane receptors such as insulin receptor and vascular epidermal growth factor (VEGF) receptor leading to their activation or inactivation(182–185).

Several studies have demonstrated that stimulation by GPCR agonists induces the activation of metalloproteinases (including MMPs and ADAMs), mediating vasoconstriction as well as the development of hypertension and cardiovascular hypertrophy(14, 15, 167, 168, 177, 178, 186).

PKC may be a key mediator of the GPCR agonist-induced activation of metalloproteinases. Studies have shown that treatment with the phorbol ester PMA to induce PKC activation also activates the shedding ability of metalloproteinases such as ADAM-17/TACE(187, 188). In ADAM-17^{-/-} (*i.e.*, TACE^{-/-}) fibroblasts, TGF- α and neuregulin shedding induced by PMA is absent(189). PMA-induced neuregulin release is also lost in ADAM-19 deficient fibroblasts(190) and PMA-triggered HB-EGF shedding is impaired in ADAM-12 deficient cardiomyocytes(167). Additionally, activation of the Ras-Raf-MEK-ERK pathways by GPCR agonists has also been reported to activate metalloproteinases and subsequent HB-EGF shedding independent of PKC(171). The mechanisms of GPCR agonist-induced metalloproteinase activation may be at both the transcriptional and post-translational level.

At the transcriptional level, GPCR activation induced by agonists such as angiotensin II and catecholamines increases the expression of MMPs including MMP-1, -2, -7, -9 and -14, and ADAMs like ADAM-12(15, 167, 179, 191, 192). Activation of PKC by PMA stimulation induces the expression of MMPs, suggesting PKC may mediate GPCR agonist-induced MMP expression(187, 193). GPCR agonist-induced activation of ERK-1/2 signaling and subsequent activation of transcription factors like AP-1 (consisting of c-Fos and c-Jun, whose expression and activity are triggered by ERK) may also contribute to the activation of

metalloproteinases, since the *cis* element of AP-1 has been found in the promoters of almost all MMPs and some ADAMs(187, 188, 194, 195). Additionally, activation of NFAT has been reported to mediate upregulation of MMP-2 and MMP-9 in cardiac myocytes(196).

At the post-translation level, the activation of metalloproteinases by PKCs or MAPKs downstream of GPCRs may be mediated via phosphorylation. PKC δ is able to interact with ADAM-9 and ADAM-12 and mediate PMA-induced HB-EGF shedding(167, 197). ADAM-17/TACE has been shown to be activated by ERK through phosphorylation at Thr735, which is required for its maturation and trafficking(198). Meanwhile, the activation of ADAM-17/TACE induced by PKC is inhibited by overexpressing PTP-H1, a protein tyrosine phosphatase that is able to interact with ADAM-17/TACE through its PDZ domain, suggesting a role for tyrosine phosphorylation in ADAM activation(199).

Increased levels of ROS downstream of GPCRs can also lead to the activation of metalloproteinases. PMA-induced activation of ADAM-17/TACE is reported to be mediated by ROS generation in monocytic cells(200). In COS7 cells, ADAM-17/TACE activation downstream of AT1R is reported to signal through Ca²⁺ and then through ROS(201). ROS can directly induce the oxidation of the thiol-group on the cysteine residue of the regulatory cysteine switch box “PKVCGY” in the prodomain of metalloproteinases. This modification interferes with the interaction between the cysteine and the Zn²⁺ in the catalytic domain, triggering protease activity(180).

Metalloproteinase activation might also be mediated through protein interactions. PACSIN-3 and eve-1, two docking proteins that contain Src homology 3 (SH3) domains to interact with the proline-rich domain of ADAMs, are able to enhance ADAM-12 activation induced by PMA, suggesting their role downstream of GPCR-induced PKC mediated ADAM activation(202, 203). Consistently, knockdown of PACSIN-3 or eve-1 inhibits ADAM-12 activation and subsequent HB-EGF shedding induced by either PMA or angiotensin II.

Active metalloproteinases can activate other metalloproteinases through cleavage of the N-terminal pro-domain. MMP-3 cleaves and activates pro-MMP-1, pro-MMP-7, pro-MMP-8 pro-MMP-9 and pro-MMP-13(204, 205). Similarly, MMP-7 activates pro-MMP-1, pro-MMP-2, pro-MMP-8 and pro-MMP-9(206) whereas MMP-10 activates pro-MMP-7, pro-MMP-8 and pro-MMP-9. In our studies using angiotensin II-induced hypertension and cardiac remodeling, we have observed that the inhibition of MMP-7 or ADAM-17/TACE by siRNA blocks angiotensin II-induced upregulation of both MMP-2 and ADAM-12 *in vivo*, suggesting early activation of MMP-7 and ADAM-17/TACE is necessary for subsequent upregulation of MMP-2 and ADAM-12(177–179). Conceivably, GPCR agonist-induced activation of MMP-7 and ADAM-17/TACE triggers the transcription of MMP-2 and ADAM-12 by transactivating EGFR and MAPK signaling. These data suggest the existence of a feed-forward loop for *de novo* expression of metalloproteinases under the control of other metalloproteinases. This differential expression and mutual regulation of metalloproteinases may be key to the development of hypertension and cardiovascular remodeling.

3.2. Transactivation of membrane receptors by metalloproteinases and resulting downstream signaling

The significance of membrane receptor transactivation via the activation of metalloproteinases has been suggested in the contraction and hypertrophic growth of VSMCs and cardiomyocytes. Activated MMPs and ADAMs, downstream of GPCR stimulation, can shed several growth factors and cytokines from their latent proteins located on the plasma membrane (*e.g.*, HB-EGF, TNF- α)(167, 207). They can also release some growth factors and cytokines from the ECM as well as from their inhibitory binding proteins (*e.g.*, TGF- β , IGF)(30, 208–210). These cytokines and growth factors then bind to their cognate receptors to trigger downstream pathways such as MEK-ERK and PI3K-Akt to mediate contraction and hypertrophy.

3.2.1. The EGF receptor family—ErbB family receptors, including the EGFR (ErbB-1), ErbB-3 and ErbB-4, are the most studied membrane receptors that are transactivated by GPCR agonists. These receptors are single transmembrane receptors and have intracellular protein tyrosine kinase (PTK) activity(211, 212). The significance of the ErbB family receptors in hypertrophy has been demonstrated by the induction of hypertrophic growth of cardiomyocytes in response to stimulation by their ligands *in vitro*(213, 214). EGFR ligands are also known to induce vasoconstriction *in vivo* and *ex vivo*(215, 216). In addition, inhibition of the EGFR blocks the development of angiotensin II-induced hypertension and cardiac hypertrophy(217). The ligands of ErbB family receptors, most of which have been suggested to be shed by metalloproteinases, include EGF, TGF- α , HB-EGF, amphiregulin and epiregulin (all prefer binding to the EGFR) as well as neuregulins (prefer binding to ErbB3 and ErbB4)(167, 190, 197, 207, 218).

The binding of ligands to the extracellular ligand-binding domains of ErbB family receptors leads to their homo- or hetero-dimerization, triggering their intracellular PTK activity(219). This results in the trans-phosphorylation of multiple tyrosines on the C-terminus, which is catalyzed by the PTK sub-domains in the receptor dimer. These phospho-tyrosine residues serve as the docking sites for the binding of effector proteins, such as proteins containing SH2 domains or phospho-tyrosine binding (PTB) domains.

Studies also show that, in VSMCs, the Ang II-induced activation of AT1R triggers its trafficking from plasma membrane into caveolin-1-enriched lipid rafts. This translocation is essential for the activation of Rac1 and NADPH oxidases, ROS production, subsequent EGFR transactivation and VSMC hypertrophy, although the exact mechanism of EGFR activation is unclear(220, 221).

Activation of the ErbB family receptors triggers signaling through the MEK-ERK pathway. In brief, adaptor proteins such as growth factor receptor-bound protein 2 (GRB-2) or Shc bind to the phospho-tyrosine residues of ErbB family receptors, via their SH2 and/or PTB domains(222, 223). The PTK activity of these receptors also phosphorylates the Shc docking protein, enabling it to bind to GRB-2 or other adaptor proteins. GRB-2 then recruits the Sos protein to the plasma membrane via the interaction between two SH3 domains on the N-terminus of GRB-2 and the proline-rich domain of Sos. Sos serves as a GEF, to activates Ras and downstream Raf-MEK-ERK signaling.

The activation of Ras downstream of ErbB receptors also leads to the activation of PI3K through the interaction between Ras and the regulatory subunit of PI3K. Moreover, the phospho-tyrosine residues of active ErbB family receptors can interact with the SH2 domain of the regulatory subunit of PI3K, recruiting PI3K to the plasma membrane(223). This binding allows PI3K to interact with its substrate PI(4,5)P₂ in the plasma membrane and convert it into PI(3,4,5)P₃, which activates Akt and initiates downstream signaling through mTOR and GSK-3 β , as described above.

The activation of ErbB family receptors can also trigger the activation of NADPH oxidase to induce the production of ROS(224, 225). ErbB-triggered NADPH oxidase activation may involve PI3K, which mediates the activation of the NADPH oxidase through Rac. The induction in ROS and downstream signaling, as well as the direct activation of the PI3K-mTOR pathway and the MEK-ERK pathway downstream of ErbB receptor transactivation promote the contraction and hypertrophy of VSMCs and cardiomyocytes.

3.2.2. Other growth factor receptors—Apart from the EGFR, transactivation of the platelet-derived growth factor (PDGF) receptor and the IGF receptor has also been reported(226–228). Their activation triggers downstream signaling through pathways including the MEK-ERK pathway and the PI3K pathway via their own PTK activity, or via their interaction with and activation of the EGFR family receptors(229, 230). IGF alone is able to induce hypertrophic growth in cultured cardiomyocytes(231–233). Mice overexpressing PDGF develop significant cardiac hypertrophy(234), supporting the pro-hypertrophic role of these receptors and their ligands. Although direct evidence of the shedding of IGF and PDGF by metalloproteinases in the cardiovascular system is limited, metalloproteinases including MMP-3, MMP-9, ADAM-9 and ADAM-12 have been reported to cleave insulin-like growth factor binding proteins (IGFBPs) and release active IGF(208–210). PDGF-C and PDGF-D, new members of PDGF family, are reported to be activated by protease cleavage extracellularly(235–237). However, GPCR-triggered PDGFR transactivation has mostly been suggested to be mediated intracellularly via ROS and tyrosine kinase Src, independent of metalloproteinases.

3.2.3. Cytokines and their receptors—GPCR agonist-induced activation of metalloproteinases could also lead to the activation of cytokines such as TGF- β and TNF- α to mediate the development of hypertension and cardiovascular hypertrophy and fibrosis.

TGF- β , whose expression is promoted in response to GPCR agonists, has been primarily recognized for its role in the activation of Smad signaling and the development of fibrosis (as described above). Recent studies have also shown that TGF- β mediates the development of cardiac hypertrophy. It has been observed that treatment with TGF- β 1 can induce hypertrophy in cultured cardiomyocytes, and that transgenic mice overexpressing TGF- β 1 develop significant cardiac hypertrophy(238, 239). In addition, TGF- β 1 deficient mice are protected from cardiac hypertrophy induced by angiotensin II infusion(17).

Several metalloproteinases have been suggested to activate TGF- β . MMP-2, MMP-3 and MMP-7 can cleave the ECM proteoglycan decorin, which sequesters TGF- β in the

extracellular milieu away from its cell surface receptors(46). In cultured cells, MMP-2 and MMP-9 have been reported to cleave and release active TGF- β from its latent form(45, 240).

The role of TGF- β in hypertrophic growth has been proposed to be mediated through TGF- β activated kinase-1 (TAK-1), a member of the MAPKKK family. The activation of TAK-1 is triggered by the direct interaction between T β RII and TAK-1, or via the activation of PKCs(238, 241). TAK-1 in turn phosphorylates MKK-3 and MKK-6, which then phosphorylate and activate p38 MAPK (242). p38 activates the transcription factors activating transcription factor-2 (ATF-2) and ATF-6(243, 244). ATF-2 promotes transcription of β -MHC, which has negative inotropic effects, mediating the development of cardiac hypertrophy and subsequent heart failure(245, 246).

Another cytokine which is activated by metalloproteinase cleavage and mediates the development of hypertension and cardiovascular hypertrophy is TNF- α . Treatment with TNF- α induces hypertrophy in cultured adult cardiomyocytes(247). and cardiac overexpression of TNF- α in mice triggers the development of cardiac hypertrophy while TNF- α knockout mice show attenuated angiotensin II-induced hypertension and cardiac hypertrophy(248, 249). The release of TNF- α by metalloproteinases is mainly mediated by ADAM-17/TACE, as its name suggests(250). Moreover, several other metalloproteinases, including MMP-7 and ADAM-10, can cleave and release active TNF- α *in vivo* and *in vitro*(251–253). The development of cardiac hypertrophy in response to TNF- α may be mediated through the activation of Akt and ROS since expression of dominant negative Akt or use of antioxidants prevents the TNF- α -induced hypertrophic response(130, 254, 255).

3.2.4. Cleavage and modulation of receptors—Receptor cleavage by metalloproteinases is a novel concept that vastly expands the mechanisms of MMP and ADAM action. In addition to shedding growth factors and cytokines, MMPs and ADAMs can directly cleave cell surface receptors including those of growth factors, cytokines and GPCR agonists implicated in cardiovascular function and disease. ADAM-10 can cleave ErbB-2 to promote its ligand-independent activation which has been shown to lead to proliferation of breast cancer cells(256). In spontaneously hypertensive rats, an established animal model of human essential hypertension, increased metalloproteinase activity results in the cleavage and inactivation of vascular endothelial growth factor receptor-2 (VEGFR-2) (which in turn causes endothelial apoptosis and capillary rarefaction), β_2 -adrenoceptor (which mediates vascular smooth muscle relaxation to reduce peripheral resistance), insulin receptor (which leads to the increase of blood glucose level and insulin resistance) and neutrophil formyl peptide receptor (which inhibits leukocyte pseudopod retraction and thus elevates microvascular resistance) contributing to the development of hypertension and hypertensive cardiac disease(184, 185, 257, 258).

3.3 Therapeutic inhibition of metalloproteinases

Increased metalloproteinase activity has been associated with various cancers, arthritis and cardiovascular disease, making metalloproteinases candidates for therapeutic intervention. Although the etiology of these pathological conditions is often complex or unknown, therapeutic strategies aimed at targeting active signaling agonists, including growth factors

and pro-hypertensive hormones either by receptor antagonism, inhibition of agonist synthesis or acceleration of agonist degradation(10, 11, 259). The effectiveness of this agonist-specific approach is limited as the targeted agonist is seldomly the only agonist active and a high degree of cross talk exists between these agonist systems(260, 261). Since metalloproteinases have been indentified as common downstream mediators in multiple pro-growth and pro-hypertensive pathways, they have been identified as viable therapeutic targets in pathological conditions engaging these signaling pathways.

Despite numerous trials targeting metalloproteinases clinically, there is currently only one metalloproteinase inhibitor (doxycycline, a tetracycline) that is approved for clinical use. Tetracyclines, initially serving as antibiotics, were indentified as metalloproteinase inhibitors at sub-antimicrobial doses in 1983(262). Since then, tetracyclines, including chemically modified tetracyclines (CMTs, whose antimicrobial activity is removed but metalloproteinase inhibitory activity is retained) have been studied for their metalloproteinase inhibitory activity in different disease models including diseases of the cardiovascular system(263–265). The metalloproteinase inhibitor CMT-3 has demonstrated dramatic improvement in the tumor burden of patients with Kaposi Sarcoma in clinical trials(266, 267). Other metalloproteinase inhibitors, including GM6001, batimastat and marimastat have also proven effective in experimental disease models, however success has also been limited in the clinical trials(177, 268–272). The synthetic MMP inhibitor PG-116800 failed to improve any clinical outcomes and showed no reduction in myocardial infarction-induced ventricular remodeling in post-myocardial infarction patients(273). Coronary artery disease patients receiving doxycycline for 6 month showed decreased plasma levels of high sensitivity C-reactive protein, interleukin-6 and pro-MMP-9 levels, but are not protected from cardiovascular death or myocardial infarction(274). The lack of success in targeting metalloproteinases clinically is at least partially attributable to the prescence of adverse side effects including musculoskeletal syndrome, likely due to the lack of specificity of these metalloproteinase inhibitors. Therefore, many studies are now focusing on the design of more selective metalloproteinase inhibitors, including natural products and their dirivatives as well as low molecular weight synthetic conpounds incorporating different Zn^{2+} -binding groups(275, 276).

Importantly, recent research shows that expression of metalloproteinases changes during the progress of disease. Therefore, timely targeting should be as important as specific inhibition of metalloproteinases in cardiovascular and other diseases.

4. Conclusions

Hypertensive cardiac disease is a major cause of morbidity and mortality worldwide. Conditions such as stress, environmental as well as certain genetic factors, obesity and diabetes all trigger hypertensive cardiac disease, at least in part, due to the characteristic excessive production in these conditions of hypercontractile, hypertrophic and pro-fibrotic GPCR agonists. The emergence of metalloproteinases as common mediators of multiple agonists makes them candidate therapeutic targets for the treatment of hypertensive cardiac disease in these conditions. In support of this notion, the inhibition of specific metalloproteinases has been shown to ameliorate the development of cardiovascular disease

in various animal models(172–175). Important challenges remain before a robust therapeutic approach based on metalloproteinase-inhibitors is developed. Precise activity and expression profiles of metalloproteinases remain to be determined and functionally assessed in health vs. in different stages of cardiovascular disease development and progression. Specific pharmacological or RNAi-based metalloproteinase inhibitors should be developed and next evaluated with regards to targeting one or multiple metalloproteinases in a disease stage-specific fashion.

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Abbreviations

GPCR	G protein-coupled receptor
VSMC	vascular smooth muscle cell
MMP	matrix metalloproteinase
ECM	extracellular matrix
ADAM	a disintegrin and metalloproteinase
ACE	angiotensin converting enzyme
SARA	Smad anchor for receptor activation
AC	adenylyl cyclase
PKA	cAMP-dependent protein kinase
Epac	exchange protein directly activated by cAMP
PLC	phospholipase C
PKC	protein kinase C
PS	phosphatidylserine
PKD	protein kinase D
RIN-1	Ras and Rab interactor-1
MAPK	mitogen-activated protein kinase
MEK/MKK/MAPKK	MAPK kinase
ERK	extracellular signal-regulated kinases
PI3K	phosphatidylinositol 3-kinase
PKB	protein kinase B

NFAT	nuclear factor of activated T-cell
HDAC	histone deacetylase
HAT	histone acetylase
ROS	reactive oxygen species
EGF	epidermal growth factor
EGFR	EGF receptor
HB-EGF	heparin-binding EGF-like growth factor
PDGF	platelet-derived growth factor
IGF	insulin-like growth factor
TGF	transforming growth factor
TNF-α	tissue necrosis factor- α
TACE	TNF- α converting enzyme
AT1R	type-1 angiotensin II receptor
ET_{A/B}	endothelin receptor type A/B
GEF	guanine nucleotide exchange factor
GAP	GTPase-activating protein
MEF-2	myocyte enhancer factor-2
AP-1	activating protein-1
MLC	myosin light chain
MHC	myosin heavy chain
eIF4E	eukaryotic translation initiation factor 4E
eEF-2	eukaryotic translation elongation factor 2
eIF2B	eukaryotic translation initiation factor 2B
CaM	calmodulin
PI(4,5)P₂	phosphatidylinositol (4,5)-bisphosphate
IP₃	inositol 1,4,5-triphosphate
DAG	1,2-diacylglycerol
SR	sarcoplasmic reticulum
CPI-17	PKC potential inhibitor of 17kDa

CREB	cAMP response element binding protein
S6K	ribosomal protein S6 kinase
4EBP-1	eIF4E binding protein-1
mTOR	mammalian target of rapamycin
PI(3,4,5)P₃	Phosphatidylinositol (3,4,5)-trisphosphate
PDK-1	phosphoinositide-dependent protein kinase 1
PH	pleckstrin-homology
5'-TOP mRNA	5'-terminal oligopyrimidine tract mRNA
TSC-2	tuberous sclerosis protein 2
GSK-3β	glycogen synthase kinase-3 β
PMA/TPA	phorbol 12-myristate 13-acetate/12-O-tetradecanoylphorbol-13-acetate
PTP	protein tyrosine phosphatase
SERCA	sarco/endoplasmic reticulum Ca ²⁺ -ATPase
VEGF	vascular endothelial growth factor
PTK	protein tyrosine kinase
SH2	Src homology 2
SH3	Src homology 3
PTB	phosphor-tyrosine binding
GRB2	growth factor receptor-bound protein 2
Sos	son of sevenless
MAPKAP-K1	MAPK-activated protein kinase
p90RSK	p90 ribosome S6 kinase
TβRI/II	type-I/II TGF- β receptor
TAK-1	TGF- β activated kinase-1

References

1. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, et al. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension*. 2003; 42(6):1206–52. [PubMed: 14656957]
2. The World Health report 2002. Midwifery. 2003; 19(1):72–3. [PubMed: 12691085]

3. Frey N, Olson EN. Cardiac hypertrophy: the good, the bad, and the ugly. *Annu Rev Physiol.* 2003; 65:45–79. [PubMed: 12524460]
4. Khan R, Sheppard R. Fibrosis in heart disease: understanding the role of transforming growth factor-beta in cardiomyopathy, valvular disease and arrhythmia. *Immunology.* 2006; 118(1):10–24. [PubMed: 16630019]
5. Rizzoni D, Agabiti-Rosei E. Relationships of cardiac function and structure to blood pressure rhythms. *Ann N Y Acad Sci.* 1996; 783:159–71. [PubMed: 8853640]
6. Zhang Y, Griendling KK, Dikalova A, Owens GK, Taylor WR. Vascular hypertrophy in angiotensin II-induced hypertension is mediated by vascular smooth muscle cell-derived H₂O₂. *Hypertension.* 2005; 46(4):732–7. [PubMed: 16172434]
7. Schiffrin EL. Vascular stiffening and arterial compliance. Implications for systolic blood pressure. *Am J Hypertens.* 2004; 17(12 Pt 2):39S–48S. [PubMed: 15607434]
8. Sorescu D. Smad3 mediates angiotensin II- and TGF-beta1-induced vascular fibrosis: Smad3 thickens the plot. *Circ Res.* 2006; 98(8):988–9. [PubMed: 16645147]
9. Intengan HD, Schiffrin EL. Vascular remodeling in hypertension: roles of apoptosis, inflammation, and fibrosis. *Hypertension.* 2001; 38(3 Pt 2):581–7. [PubMed: 11566935]
10. Rockman HA, Wachhorst SP, Mao L, Ross J Jr. ANG II receptor blockade prevents ventricular hypertrophy and ANF gene expression with pressure overload in mice. *Am J Physiol.* 1994; 266(6 Pt 2):H2468–75. [PubMed: 8024008]
11. Muller P, Kazakov A, Jagoda P, Semenov A, Bohm M, Laufs U. ACE inhibition promotes upregulation of endothelial progenitor cells and neoangiogenesis in cardiac pressure overload. *Cardiovasc Res.* 2009; 83(1):106–14. [PubMed: 19380417]
12. Berk BC, Vekshtein V, Gordon HM, Tsuda T. Angiotensin II-stimulated protein synthesis in cultured vascular smooth muscle cells. *Hypertension.* 1989; 13(4):305–14. [PubMed: 2466788]
13. Geisterfer AA, Peach MJ, Owens GK. Angiotensin II induces hypertrophy, not hyperplasia, of cultured rat aortic smooth muscle cells. *Circ Res.* 1988; 62(4):749–56. [PubMed: 3280155]
14. Prenzel N, Zwick E, Daub H, Leserer M, Abraham R, Wallasch C, et al. EGF receptor transactivation by G-protein-coupled receptors requires metalloproteinase cleavage of proHB-EGF. *Nature.* 1999; 402(6764):884–8. [PubMed: 10622253]
15. Hao L, Du M, Lopez-Campistrous A, Fernandez-Patron C. Agonist-induced activation of matrix metalloproteinase-7 promotes vasoconstriction through the epidermal growth factor-receptor pathway. *Circ Res.* 2004; 94(1):68–76. [PubMed: 14656925]
16. Akhter SA, Luttrell LM, Rockman HA, Iaccarino G, Lefkowitz RJ, Koch WJ. Targeting the receptor-Gq interface to inhibit in vivo pressure overload myocardial hypertrophy. *Science.* 1998; 280(5363):574–7. [PubMed: 9554846]
17. Schultz Jel J, Witt SA, Glascock BJ, Nieman ML, Reiser PJ, Nix SL, et al. TGF-beta1 mediates the hypertrophic cardiomyocyte growth induced by angiotensin II. *J Clin Invest.* 2002; 109(6):787–96. [PubMed: 11901187]
18. Lu Y, Yang S. Angiotensin II induces cardiomyocyte hypertrophy probably through histone deacetylases. *Tohoku J Exp Med.* 2009; 219(1):17–23. [PubMed: 19713680]
19. Griffin SA, Brown WC, MacPherson F, McGrath JC, Wilson VG, Korsgaard N, et al. Angiotensin II causes vascular hypertrophy in part by a non-pressor mechanism. *Hypertension.* 1991; 17(5): 626–35. [PubMed: 2022407]
20. Liao Y, Asakura M, Takashima S, Ogai A, Asano Y, Shintani Y, et al. Celiprolol, a vasodilatory beta-blocker, inhibits pressure overload-induced cardiac hypertrophy and prevents the transition to heart failure via nitric oxide-dependent mechanisms in mice. *Circulation.* 2004; 110(6):692–9. [PubMed: 15262839]
21. Biaggioni I. Should we target the sympathetic nervous system in the treatment of obesity-associated hypertension? *Hypertension.* 2008; 51(2):168–71. [PubMed: 18086947]
22. Rockman HA, Koch WJ, Lefkowitz RJ. Seven-transmembrane-spanning receptors and heart function. *Nature.* 2002; 415(6868):206–12. [PubMed: 11805844]
23. Cook B, Bar-Yaacov M, Cohen Ben-Ami H, Goldstein RE, Paroush Z, Selinger Z, et al. Phospholipase C and termination of G-protein-mediated signalling in vivo. *Nat Cell Biol.* 2000; 2(5):296–301. [PubMed: 10806481]

24. Brock C, Schaefer M, Reusch HP, Czupalla C, Michalke M, Spicher K, et al. Roles of G beta gamma in membrane recruitment and activation of p110 gamma/p101 phosphoinositide 3-kinase gamma. *J Cell Biol.* 2003; 160(1):89–99. [PubMed: 12507995]
25. Crespo P, Xu N, Simonds WF, Gutkind JS. Ras-dependent activation of MAP kinase pathway mediated by G-protein beta gamma subunits. *Nature.* 1994; 369(6479):418–20. [PubMed: 8196770]
26. Inglese J, Koch WJ, Touhara K, Lefkowitz RJ. G beta gamma interactions with PH domains and Ras-MAPK signaling pathways. *Trends Biochem Sci.* 1995; 20(4):151–6. [PubMed: 7770915]
27. Lorenz K, Schmitt JP, Schmitteckert EM, Lohse MJ. A new type of ERK1/2 autophosphorylation causes cardiac hypertrophy. *Nat Med.* 2009; 15(1):75–83. [PubMed: 19060905]
28. Sun Y, Yamauchi J, Kaziro Y, Itoh H. Activation of c-fos promoter by Gbetagamma-mediated signaling: involvement of Rho and c-Jun N-terminal kinase. *J Biochem.* 1999; 125(3):515–21. [PubMed: 10050039]
29. Kamm KE, Stull JT. Dedicated myosin light chain kinases with diverse cellular functions. *J Biol Chem.* 2001; 276(7):4527–30. [PubMed: 11096123]
30. Kordowska J, Huang R, Wang CL. Phosphorylation of caldesmon during smooth muscle contraction and cell migration or proliferation. *J Biomed Sci.* 2006; 13(2):159–72. [PubMed: 16453176]
31. Gordon AM, Regnier M, Homsher E. Skeletal and cardiac muscle contractile activation: tropomyosin “rocks and rolls”. *News Physiol Sci.* 2001; 16:49–55. [PubMed: 11390948]
32. Kanaya N, Gable B, Murray PA, Damron DS. Propofol increases phosphorylation of troponin I and myosin light chain 2 via protein kinase C activation in cardiomyocytes. *Anesthesiology.* 2003; 98(6):1363–71. [PubMed: 12766644]
33. Monasky MM, Biesiadecki BJ, Janssen PM. Increased phosphorylation of tropomyosin, troponin I, and myosin light chain-2 after stretch in rabbit ventricular myocardium under physiological conditions. *J Mol Cell Cardiol.* 2010; 48(5):1023–8. [PubMed: 20298699]
34. Magid NM, Borer JS, Young MS, Wallerson DC, DeMonteiro C. Suppression of protein degradation in progressive cardiac hypertrophy of chronic aortic regurgitation. *Circulation.* 1993; 87(4):1249–57. [PubMed: 8462151]
35. Lu J, McKinsey TA, Nicol RL, Olson EN. Signal-dependent activation of the MEF2 transcription factor by dissociation from histone deacetylases. *Proc Natl Acad Sci U S A.* 2000; 97(8):4070–5. [PubMed: 10737771]
36. Vega RB, Harrison BC, Meadows E, Roberts CR, Papst PJ, Olson EN, et al. Protein kinases C and D mediate agonist-dependent cardiac hypertrophy through nuclear export of histone deacetylase 5. *Mol Cell Biol.* 2004; 24(19):8374–85. [PubMed: 15367659]
37. Tardiff JC, Hewett TE, Factor SM, Vikstrom KL, Robbins J, Leinwand LA. Expression of the beta (slow)-isoform of MHC in the adult mouse heart causes dominant-negative functional effects. *Am J Physiol Heart Circ Physiol.* 2000; 278(2):H412–9. [PubMed: 10666070]
38. Hewett TE, Grupp IL, Grupp G, Robbins J. Alpha-skeletal actin is associated with increased contractility in the mouse heart. *Circ Res.* 1994; 74(4):740–6. [PubMed: 8137509]
39. Krenz M, Sanbe A, Bouyer-Daloz F, Gulick J, Klevitsky R, Hewett TE, et al. Analysis of myosin heavy chain functionality in the heart. *J Biol Chem.* 2003; 278(19):17466–74. [PubMed: 12626511]
40. Proud CG. Ras, PI3-kinase and mTOR signaling in cardiac hypertrophy. *Cardiovasc Res.* 2004; 63(3):403–13. [PubMed: 15276465]
41. Dorn GW 2nd, Force T. Protein kinase cascades in the regulation of cardiac hypertrophy. *J Clin Invest.* 2005; 115(3):527–37. [PubMed: 15765134]
42. Leask A. Potential therapeutic targets for cardiac fibrosis: TGFbeta, angiotensin, endothelin, CCN2, and PDGF, partners in fibroblast activation. *Circ Res.* 2010; 106(11):1675–80. [PubMed: 20538689]
43. Rosenkranz S. TGF-beta1 and angiotensin networking in cardiac remodeling. *Cardiovasc Res.* 2004; 63(3):423–32. [PubMed: 15276467]

44. Fukuda N, Hu WY, Kubo A, Kishioka H, Satoh C, Soma M, et al. Angiotensin II upregulates transforming growth factor-beta type I receptor on rat vascular smooth muscle cells. *Am J Hypertens*. 2000; 13(2):191–8. [PubMed: 10701820]
45. Yu Q, Stamenkovic I. Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. *Genes Dev*. 2000; 14(2):163–76. [PubMed: 10652271]
46. Imai K, Hiramatsu A, Fukushima D, Pierschbacher MD, Okada Y. Degradation of decorin by matrix metalloproteinases: identification of the cleavage sites, kinetic analyses and transforming growth factor-beta1 release. *Biochem J*. 1997; 322 (Pt 3):809–14. [PubMed: 9148753]
47. Lonn P, Moren A, Raja E, Dahl M, Moustakas A. Regulating the stability of TGFbeta receptors and Smads. *Cell Res*. 2009; 19(1):21–35. [PubMed: 19030025]
48. Rodriguez-Vita J, Sanchez-Lopez E, Esteban V, Ruperez M, Egido J, Ruiz-Ortega M. Angiotensin II activates the Smad pathway in vascular smooth muscle cells by a transforming growth factor-beta-independent mechanism. *Circulation*. 2005; 111(19):2509–17. [PubMed: 15883213]
49. Wang W, Huang XR, Canlas E, Oka K, Truong LD, Deng C, et al. Essential role of Smad3 in angiotensin II-induced vascular fibrosis. *Circ Res*. 2006; 98(8):1032–9. [PubMed: 16556868]
50. Lee CH, Park D, Wu D, Rhee SG, Simon MI. Members of the Gq alpha subunit gene family activate phospholipase C beta isozymes. *J Biol Chem*. 1992; 267(23):16044–7. [PubMed: 1322889]
51. Boyer JL, Waldo GL, Harden TK. Beta gamma-subunit activation of G-protein-regulated phospholipase C. *J Biol Chem*. 1992; 267(35):25451–6. [PubMed: 1460039]
52. Camps M, Carozzi A, Schnabel P, Scheer A, Parker PJ, Gierschik P. Isozyme-selective stimulation of phospholipase C-beta 2 by G protein beta gamma-subunits. *Nature*. 1992; 360(6405):684–6. [PubMed: 1465133]
53. Kelso EJ, McDermott BJ, Silke B, Spiers JP. Endothelin(A) receptor subtype mediates endothelin-induced contractility in left ventricular cardiomyocytes isolated from rabbit myocardium. *J Pharmacol Exp Ther*. 2000; 294(3):1047–52. [PubMed: 10945858]
54. Schelling JR, Nkemere N, Konieczkowski M, Martin KA, Dubyak GR. Angiotensin II activates the beta 1 isoform of phospholipase C in vascular smooth muscle cells. *Am J Physiol*. 1997; 272(5 Pt 1):C1558–66. [PubMed: 9176147]
55. Filtz TM, Grubb DR, McLeod-Dryden TJ, Luo J, Woodcock EA. Gq-initiated cardiomyocyte hypertrophy is mediated by phospholipase Cbeta1b. *Faseb J*. 2009; 23(10):3564–70. [PubMed: 19564249]
56. Viard P, Exner T, Maier U, Mironneau J, Nurnberg B, Macrez N. Gbetagamma dimers stimulate vascular L-type Ca2+ channels via phosphoinositide 3-kinase. *Faseb J*. 1999; 13(6):685–94. [PubMed: 10094929]
57. Zucchi R, Ronca-Testoni S. The sarcoplasmic reticulum Ca2+ channel/ryanodine receptor: modulation by endogenous effectors, drugs and disease states. *Pharmacol Rev*. 1997; 49(1):1–51. [PubMed: 9085308]
58. Wilkins BJ, Molkentin JD. Calcium-calmodulin signaling in the regulation of cardiac hypertrophy. *Biochem Biophys Res Commun*. 2004; 322(4):1178–91. [PubMed: 15336966]
59. Naruse K, King GL. Protein kinase C and myocardial biology and function. *Circ Res*. 2000; 86(11):1104–6. [PubMed: 10850959]
60. Rusanescu G, Gotoh T, Tian X, Feig LA. Regulation of Ras signaling specificity by protein kinase C. *Mol Cell Biol*. 2001; 21(8):2650–8. [PubMed: 11283245]
61. Clerk A, Sugden PH. Signaling through the extracellular signal-regulated kinase 1/2 cascade in cardiac myocytes. *Biochem Cell Biol*. 2004; 82(6):603–9.
62. Clerk A, Bogoyevitch MA, Anderson MB, Sugden PH. Differential activation of protein kinase C isoforms by endothelin-1 and phenylephrine and subsequent stimulation of p42 and p44 mitogen-activated protein kinases in ventricular myocytes cultured from neonatal rat hearts. *J Biol Chem*. 1994; 269(52):32848–57. [PubMed: 7806510]
63. Pacold ME, Suire S, Perisic O, Lara-Gonzalez S, Davis CT, Walker EH, et al. Crystal structure and functional analysis of Ras binding to its effector phosphoinositide 3-kinase gamma. *Cell*. 2000; 103(6):931–43. [PubMed: 11136978]

64. Marais R, Light Y, Mason C, Paterson H, Olson MF, Marshall CJ. Requirement of Ras-GTP-Raf complexes for activation of Raf-1 by protein kinase C. *Science*. 1998; 280(5360):109–12. [PubMed: 9525855]
65. Nakashima H, Frank GD, Shirai H, Hinoki A, Higuchi S, Ohtsu H, et al. Novel role of protein kinase C-delta Tyr 311 phosphorylation in vascular smooth muscle cell hypertrophy by angiotensin II. *Hypertension*. 2008; 51(2):232–8. [PubMed: 18180404]
66. Vijayan K, Szotek EL, Martin JL, Samarel AM. Protein kinase C-alpha-induced hypertrophy of neonatal rat ventricular myocytes. *Am J Physiol Heart Circ Physiol*. 2004; 287(6):H2777–89. [PubMed: 15271671]
67. Takeishi Y, Ping P, Bolli R, Kirkpatrick DL, Hoit BD, Walsh RA. Transgenic overexpression of constitutively active protein kinase C epsilon causes concentric cardiac hypertrophy. *Circ Res*. 2000; 86(12):1218–23. [PubMed: 10864911]
68. Wakasaki H, Koya D, Schoen FJ, Jirousek MR, Ways DK, Hoit BD, et al. Targeted overexpression of protein kinase C beta2 isoform in myocardium causes cardiomyopathy. *Proc Natl Acad Sci U S A*. 1997; 94(17):9320–5. [PubMed: 9256480]
69. Walsh MP, Horowitz A, Clement-Chomienne O, Andrea JE, Allen BG, Morgan KG. Protein kinase C mediation of Ca(2+)-independent contractions of vascular smooth muscle. *Biochem Cell Biol*. 1996; 74(4):485–502. [PubMed: 8960355]
70. Walsh MP, Susnjar M, Deng J, Sutherland C, Kiss E, Wilson DP. Phosphorylation of the protein phosphatase type 1 inhibitor protein CPI-17 by protein kinase C. *Methods Mol Biol*. 2007; 365:209–23. [PubMed: 17200564]
71. Venema RC, Raynor RL, Noland TA Jr, Kuo JF. Role of protein kinase C in the phosphorylation of cardiac myosin light chain 2. *Biochem J*. 1993; 294 (Pt 2):401–6. [PubMed: 8396912]
72. Clement O, Puceat M, Walsh MP, Vassort G. Protein kinase C enhances myosin light-chain kinase effects on force development and ATPase activity in rat single skinned cardiac cells. *Biochem J*. 1992; 285 (Pt 1):311–7. [PubMed: 1386218]
73. Xu X, Ha CH, Wong C, Wang W, Hausser A, Pfizenmaier K, et al. Angiotensin II stimulates protein kinase D-dependent histone deacetylase 5 phosphorylation and nuclear export leading to vascular smooth muscle cell hypertrophy. *Arterioscler Thromb Vasc Biol*. 2007; 27(11):2355–62. [PubMed: 17823368]
74. Monovich L, Vega RB, Meredith E, Miranda K, Rao C, Capparelli M, et al. A novel kinase inhibitor establishes a predominant role for protein kinase D as a cardiac class IIa histone deacetylase kinase. *FEBS Lett*. 2010; 584(3):631–7. [PubMed: 20018189]
75. Wang Y, Waldron RT, Dhaka A, Patel A, Riley MM, Rozengurt E, et al. The RAS effector RIN1 directly competes with RAF and is regulated by 14-3-3 proteins. *Mol Cell Biol*. 2002; 22(3):916–26. [PubMed: 11784866]
76. Fielitz J, Kim MS, Shelton JM, Qi X, Hill JA, Richardson JA, et al. Requirement of protein kinase D1 for pathological cardiac remodeling. *Proc Natl Acad Sci U S A*. 2008; 105(8):3059–63. [PubMed: 18287012]
77. Nagao S, Yamaguchi T, Kusaka M, Maser RL, Takahashi H, Cowley BD, et al. Renal activation of extracellular signal-regulated kinase in rats with autosomal-dominant polycystic kidney disease. *Kidney Int*. 2003; 63(2):427–37. [PubMed: 12631108]
78. Bisognano JD, Weinberger HD, Bohlmeier TJ, Pende A, Reynolds MV, Sastravaha A, et al. Myocardial-directed overexpression of the human beta(1)-adrenergic receptor in transgenic mice. *J Mol Cell Cardiol*. 2000; 32(5):817–30. [PubMed: 10775486]
79. Antos CL, Frey N, Marx SO, Reiken S, Gaburjakova M, Richardson JA, et al. Dilated cardiomyopathy and sudden death resulting from constitutive activation of protein kinase a. *Circ Res*. 2001; 89(11):997–1004. [PubMed: 11717156]
80. Harrison JG, Sugden PH, Clerk A. Endothelin-1 promotes phosphorylation of CREB transcription factor in primary cultures of neonatal rat cardiac myocytes: implications for the regulation of c-jun expression. *Biochim Biophys Acta*. 2004; 1644(1):17–25. [PubMed: 14741741]
81. Markou T, Hadzopoulou-Cladaras M, Lazou A. Phenylephrine induces activation of CREB in adult rat cardiac myocytes through MSK1 and PKA signaling pathways. *J Mol Cell Cardiol*. 2004; 37(5):1001–11. [PubMed: 15522277]

82. Ichiki T. Role of cAMP response element binding protein in cardiovascular remodeling: good, bad, or both? *Arterioscler Thromb Vasc Biol.* 2006; 26(3):449–55. [PubMed: 16293792]
83. Wang Z, Dillon TJ, Pokala V, Mishra S, Labudda K, Hunter B, et al. Rap1-mediated activation of extracellular signal-regulated kinases by cyclic AMP is dependent on the mode of Rap1 activation. *Mol Cell Biol.* 2006; 26(6):2130–45. [PubMed: 16507992]
84. Metrich M, Lucas A, Gastineau M, Samuel JL, Heymes C, Morel E, et al. Epac mediates beta-adrenergic receptor-induced cardiomyocyte hypertrophy. *Circ Res.* 2008; 102(8):959–65. [PubMed: 18323524]
85. Morel E, Marcantoni A, Gastineau M, Birkedal R, Rochais F, Garnier A, et al. cAMP-binding protein Epac induces cardiomyocyte hypertrophy. *Circ Res.* 2005; 97(12):1296–304. [PubMed: 16269655]
86. Bueno OF, De Windt LJ, Tymitz KM, Witt SA, Kimball TR, Klevitsky R, et al. The MEK1-ERK1/2 signaling pathway promotes compensated cardiac hypertrophy in transgenic mice. *Embo J.* 2000; 19(23):6341–50. [PubMed: 11101507]
87. Harris IS, Zhang S, Treskov I, Kovacs A, Weinheimer C, Muslin AJ. Raf-1 kinase is required for cardiac hypertrophy and cardiomyocyte survival in response to pressure overload. *Circulation.* 2004; 110(6):718–23. [PubMed: 15289381]
88. Downward J, Graves JD, Warne PH, Rayter S, Cantrell DA. Stimulation of p21ras upon T-cell activation. *Nature.* 1990; 346(6286):719–23. [PubMed: 2201921]
89. Ueda Y, Hirai S, Osada S, Suzuki A, Mizuno K, Ohno S. Protein kinase C activates the MEK-ERK pathway in a manner independent of Ras and dependent on Raf. *J Biol Chem.* 1996; 271(38):23512–9. [PubMed: 8798560]
90. Garrington TP, Johnson GL. Organization and regulation of mitogen-activated protein kinase signaling pathways. *Curr Opin Cell Biol.* 1999; 11(2):211–8. [PubMed: 10209154]
91. Babu GJ, Lalli MJ, Sussman MA, Sadoshima J, Periasamy M. Phosphorylation of elk-1 by MEK/ERK pathway is necessary for c-fos gene activation during cardiac myocyte hypertrophy. *J Mol Cell Cardiol.* 2000; 32(8):1447–57. [PubMed: 10900171]
92. Wang L, Proud CG. Ras/Erk signaling is essential for activation of protein synthesis by Gq protein-coupled receptor agonists in adult cardiomyocytes. *Circ Res.* 2002; 91(9):821–9. [PubMed: 12411397]
93. Siddiqui RA, Shaikh SR, Kovacs R, Stillwell W, Zaloga G. Inhibition of phenylephrine-induced cardiac hypertrophy by docosahexaenoic acid. *J Cell Biochem.* 2004; 92(6):1141–59. [PubMed: 15258898]
94. Takeishi Y, Huang Q, Abe J, Glassman M, Che W, Lee JD, et al. Src and multiple MAP kinase activation in cardiac hypertrophy and congestive heart failure under chronic pressure-overload: comparison with acute mechanical stretch. *J Mol Cell Cardiol.* 2001; 33(9):1637–48. [PubMed: 11549343]
95. Whitman M, Downes CP, Keeler M, Keller T, Cantley L. Type I phosphatidylinositol kinase makes a novel inositol phospholipid, phosphatidylinositol-3-phosphate. *Nature.* 1988; 332(6165):644–6. [PubMed: 2833705]
96. Cantley LC. The phosphoinositide 3-kinase pathway. *Science.* 2002; 296(5573):1655–7. [PubMed: 12040186]
97. Toker A, Newton AC. Akt/protein kinase B is regulated by autophosphorylation at the hypothetical PDK-2 site. *J Biol Chem.* 2000; 275(12):8271–4. [PubMed: 10722653]
98. Condorelli G, Drusco A, Stassi G, Bellacosa A, Roncarati R, Iaccarino G, et al. Akt induces enhanced myocardial contractility and cell size in vivo in transgenic mice. *Proc Natl Acad Sci U S A.* 2002; 99(19):12333–8. [PubMed: 12237475]
99. Yano N, Tseng A, Zhao TC, Robbins J, Padbury JF, Tseng YT. Temporally controlled overexpression of cardiac-specific PI3K α induces enhanced myocardial contractility--a new transgenic model. *Am J Physiol Heart Circ Physiol.* 2008; 295(4):H1690–4. [PubMed: 18723766]
100. Shioi T, Kang PM, Douglas PS, Hampe J, Yballe CM, Lawitts J, et al. The conserved phosphoinositide 3-kinase pathway determines heart size in mice. *Embo J.* 2000; 19(11):2537–48. [PubMed: 10835352]

101. Hixon ML, Muro-Cacho C, Wagner MW, Obejero-Paz C, Millie E, Fujio Y, et al. Akt1/PKB upregulation leads to vascular smooth muscle cell hypertrophy and polyploidization. *J Clin Invest.* 2000; 106(8):1011–20. [PubMed: 11032861]
102. Shiojima I, Sato K, Izumiya Y, Schiekofer S, Ito M, Liao R, et al. Disruption of coordinated cardiac hypertrophy and angiogenesis contributes to the transition to heart failure. *J Clin Invest.* 2005; 115(8):2108–18. [PubMed: 16075055]
103. McMullen JR, Sherwood MC, Tarnavski O, Zhang L, Dorfman AL, Shioi T, et al. Inhibition of mTOR signaling with rapamycin regresses established cardiac hypertrophy induced by pressure overload. *Circulation.* 2004; 109(24):3050–5. [PubMed: 15184287]
104. Inoki K, Li Y, Xu T, Guan KL. Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. *Genes Dev.* 2003; 17(15):1829–34. [PubMed: 12869586]
105. Inoki K, Ouyang H, Li Y, Guan KL. Signaling by target of rapamycin proteins in cell growth control. *Microbiol Mol Biol Rev.* 2005; 69(1):79–100. [PubMed: 15755954]
106. Hannan RD, Jenkins A, Jenkins AK, Brandenburger Y. Cardiac hypertrophy: a matter of translation. *Clin Exp Pharmacol Physiol.* 2003; 30(8):517–27. [PubMed: 12890171]
107. Ma L, Chen Z, Erdjument-Bromage H, Tempst P, Pandolfi PP. Phosphorylation and functional inactivation of TSC2 by Erk implications for tuberous sclerosis and cancer pathogenesis. *Cell.* 2005; 121(2):179–93. [PubMed: 15851026]
108. Kwon OY, Kwon IC, Song HK, Jeon H. Real-time imaging of NF-AT nucleocytoplasmic shuttling with a photoswitchable fluorescence protein in live cells. *Biochim Biophys Acta.* 2008; 1780(12):1403–7. [PubMed: 18771710]
109. Ding Q, Xia W, Liu JC, Yang JY, Lee DF, Xia J, et al. Erk associates with and primes GSK-3 β for its inactivation resulting in upregulation of β -catenin. *Mol Cell.* 2005; 19(2):159–70. [PubMed: 16039586]
110. Ni YG, Berenji K, Wang N, Oh M, Sachan N, Dey A, et al. Foxo transcription factors blunt cardiac hypertrophy by inhibiting calcineurin signaling. *Circulation.* 2006; 114(11):1159–68. [PubMed: 16952979]
111. Skurk C, Izumiya Y, Maatz H, Razeghi P, Shiojima I, Sandri M, et al. The FOXO3a transcription factor regulates cardiac myocyte size downstream of AKT signaling. *J Biol Chem.* 2005; 280(21):20814–23. [PubMed: 15781459]
112. Le Blanc C, Mironneau C, Barbot C, Henaff M, Bondeva T, Wetzker R, et al. Regulation of vascular L-type Ca²⁺ channels by phosphatidylinositol 3,4,5-trisphosphate. *Circ Res.* 2004; 95(3):300–7. [PubMed: 15242973]
113. Quignard JF, Mironneau J, Carricaburu V, Fournier B, Babich A, Nurnberg B, et al. Phosphoinositide 3-kinase gamma mediates angiotensin II-induced stimulation of L-type calcium channels in vascular myocytes. *J Biol Chem.* 2001; 276(35):32545–51. [PubMed: 11443116]
114. Komalavilas P, Mehta S, Wingard CJ, Dransfield DT, Bhalla J, Woodrum JE, et al. PI3-kinase/Akt modulates vascular smooth muscle tone via cAMP signaling pathways. *J Appl Physiol.* 2001; 91(4):1819–27. [PubMed: 11568168]
115. Klee CB, Crouch TH, Krinks MH. Calcineurin: a calcium- and calmodulin-binding protein of the nervous system. *Proc Natl Acad Sci U S A.* 1979; 76(12):6270–3. [PubMed: 293720]
116. Perrino BA, Ng LY, Soderling TR. Calcium regulation of calcineurin phosphatase activity by its B subunit and calmodulin. Role of the autoinhibitory domain. *J Biol Chem.* 1995; 270(12):7012. [PubMed: 7896854]
117. Suzuki E, Nishimatsu H, Satonaka H, Walsh K, Goto A, Omata M, et al. Angiotensin II induces myocyte enhancer factor 2- and calcineurin/nuclear factor of activated T cell-dependent transcriptional activation in vascular myocytes. *Circ Res.* 2002; 90(9):1004–11. [PubMed: 12016267]
118. Molken JD, Lu JR, Antos CL, Markham B, Richardson J, Robbins J, et al. A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. *Cell.* 1998; 93(2):215–28. [PubMed: 9568714]
119. Goldspink PH, McKinney RD, Kimball VA, Geenen DL, Buttrick PM. Angiotensin II induced cardiac hypertrophy in vivo is inhibited by cyclosporin A in adult rats. *Mol Cell Biochem.* 2001; 226(1–2):83–8. [PubMed: 11768242]

120. de Ruijter AJ, van Gennip AH, Caron HN, Kemp S, van Kuilenburg AB. Histone deacetylases (HDACs): characterization of the classical HDAC family. *Biochem J.* 2003; 370(Pt 3):737–49. [PubMed: 12429021]
121. Ha CH, Kim JY, Zhao J, Wang W, Jhun BS, Wong C, et al. PKA phosphorylates histone deacetylase 5 and prevents its nuclear export, leading to the inhibition of gene transcription and cardiomyocyte hypertrophy. *Proc Natl Acad Sci U S A.* 2010; 107(35):15467–72. [PubMed: 20716686]
122. Li H, Li W, Gupta AK, Mohler PJ, Anderson ME, Grumbach IM. Calmodulin kinase II is required for angiotensin II-mediated vascular smooth muscle hypertrophy. *Am J Physiol Heart Circ Physiol.* 2010; 298(2):H688–98. [PubMed: 20023119]
123. Chang S, McKinsey TA, Zhang CL, Richardson JA, Hill JA, Olson EN. Histone deacetylases 5 and 9 govern responsiveness of the heart to a subset of stress signals and play redundant roles in heart development. *Mol Cell Biol.* 2004; 24(19):8467–76. [PubMed: 15367668]
124. Zhang CL, McKinsey TA, Chang S, Antos CL, Hill JA, Olson EN. Class II histone deacetylases act as signal-responsive repressors of cardiac hypertrophy. *Cell.* 2002; 110(4):479–88. [PubMed: 12202037]
125. Giordano FJ. Oxygen, oxidative stress, hypoxia, and heart failure. *J Clin Invest.* 2005; 115(3):500–8. [PubMed: 15765131]
126. Finkel T. Oxidant signals and oxidative stress. *Curr Opin Cell Biol.* 2003; 15(2):247–54. [PubMed: 12648682]
127. Frank GD, Mifune M, Inagami T, Ohba M, Sasaki T, Higashiyama S, et al. Distinct mechanisms of receptor and nonreceptor tyrosine kinase activation by reactive oxygen species in vascular smooth muscle cells: role of metalloprotease and protein kinase C- δ . *Mol Cell Biol.* 2003; 23(5):1581–9. [PubMed: 12588978]
128. Yoshizumi M, Tsuchiya K, Suzaki Y, Kirima K, Kyaw M, Moon JH, et al. Quercetin glucuronide prevents VSMC hypertrophy by angiotensin II via the inhibition of JNK and AP-1 signaling pathway. *Biochem Biophys Res Commun.* 2002; 293(5):1458–65. [PubMed: 12054679]
129. Garrido AM, Griendling KK. NADPH oxidases and angiotensin II receptor signaling. *Mol Cell Endocrinol.* 2009; 302(2):148–58. [PubMed: 19059306]
130. Nakamura K, Fushimi K, Kouchi H, Mihara K, Miyazaki M, Ohe T, et al. Inhibitory effects of antioxidants on neonatal rat cardiac myocyte hypertrophy induced by tumor necrosis factor- α and angiotensin II. *Circulation.* 1998; 98(8):794–9. [PubMed: 9727550]
131. Ide T, Tsutsui H, Hayashidani S, Kang D, Suematsu N, Nakamura K, et al. Mitochondrial DNA damage and dysfunction associated with oxidative stress in failing hearts after myocardial infarction. *Circ Res.* 2001; 88(5):529–35. [PubMed: 11249877]
132. Ide T, Tsutsui H, Kinugawa S, Suematsu N, Hayashidani S, Ichikawa K, et al. Direct evidence for increased hydroxyl radicals originating from superoxide in the failing myocardium. *Circ Res.* 2000; 86(2):152–7. [PubMed: 10666410]
133. Kimura S, Zhang GX, Nishiyama A, Shokoji T, Yao L, Fan YY, et al. Mitochondria-derived reactive oxygen species and vascular MAP kinases: comparison of angiotensin II and diazoxide. *Hypertension.* 2005; 45(3):438–44. [PubMed: 15699441]
134. Li JM, Wheatcroft S, Fan LM, Kearney MT, Shah AM. Opposing roles of p47phox in basal versus angiotensin II-stimulated alterations in vascular O₂⁻ production, vascular tone, and mitogen-activated protein kinase activation. *Circulation.* 2004; 109(10):1307–13. [PubMed: 14993144]
135. Hirono Y, Yoshimoto T, Suzuki N, Sugiyama T, Sakurada M, Takai S, et al. Angiotensin II receptor type 1-mediated vascular oxidative stress and proinflammatory gene expression in aldosterone-induced hypertension: the possible role of local renin-angiotensin system. *Endocrinology.* 2007; 148(4):1688–96. [PubMed: 17218415]
136. Murdoch CE, Zhang M, Cave AC, Shah AM. NADPH oxidase-dependent redox signalling in cardiac hypertrophy, remodelling and failure. *Cardiovasc Res.* 2006; 71(2):208–15. [PubMed: 16631149]
137. Inoguchi T, Li P, Umeda F, Yu HY, Kakimoto M, Imamura M, et al. High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C--dependent

- activation of NAD(P)H oxidase in cultured vascular cells. *Diabetes*. 2000; 49(11):1939–45. [PubMed: 11078463]
138. Castilho RF, Kowaltowski AJ, Meinicke AR, Bechara EJ, Vercesi AE. Permeabilization of the inner mitochondrial membrane by Ca²⁺ ions is stimulated by t-butyl hydroperoxide and mediated by reactive oxygen species generated by mitochondria. *Free Radic Biol Med*. 1995; 18(3):479–86. [PubMed: 9101238]
 139. Gadelha FR, Thomson L, Fagian MM, Costa AD, Radi R, Vercesi AE. Ca²⁺-independent permeabilization of the inner mitochondrial membrane by peroxynitrite is mediated by membrane protein thiol cross-linking and lipid peroxidation. *Arch Biochem Biophys*. 1997; 345(2):243–50. [PubMed: 9308896]
 140. Radi R, Beckman JS, Bush KM, Freeman BA. Peroxynitrite oxidation of sulfhydryls. The cytotoxic potential of superoxide and nitric oxide. *J Biol Chem*. 1991; 266(7):4244–50. [PubMed: 1847917]
 141. Djordjevic T, Pogrebniak A, BelAiba RS, Bonello S, Wotzlaw C, Acker H, et al. The expression of the NADPH oxidase subunit p22phox is regulated by a redox-sensitive pathway in endothelial cells. *Free Radic Biol Med*. 2005; 38(5):616–30. [PubMed: 15683718]
 142. Landmesser U, Dikalov S, Price SR, McCann L, Fukai T, Holland SM, et al. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. *J Clin Invest*. 2003; 111(8):1201–9. [PubMed: 12697739]
 143. McNally JS, Saxena A, Cai H, Dikalov S, Harrison DG. Regulation of xanthine oxidoreductase protein expression by hydrogen peroxide and calcium. *Arterioscler Thromb Vasc Biol*. 2005; 25(8):1623–8. [PubMed: 15905466]
 144. Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial ROS-induced ROS release: an update and review. *Biochim Biophys Acta*. 2006; 1757(5–6):509–17. [PubMed: 16829228]
 145. Zafari AM, Ushio-Fukai M, Akers M, Yin Q, Shah A, Harrison DG, et al. Role of NADH/NADPH oxidase-derived H₂O₂ in angiotensin II-induced vascular hypertrophy. *Hypertension*. 1998; 32(3):488–95. [PubMed: 9740615]
 146. Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. *Circ Res*. 1994; 74(6):1141–8. [PubMed: 8187280]
 147. Hingtgen SD, Tian X, Yang J, Dunlay SM, Peek AS, Wu Y, et al. Nox2-containing NADPH oxidase and Akt activation play a key role in angiotensin II-induced cardiomyocyte hypertrophy. *Physiol Genomics*. 2006; 26(3):180–91. [PubMed: 16670255]
 148. Nakagami H, Takemoto M, Liao JK. NADPH oxidase-derived superoxide anion mediates angiotensin II-induced cardiac hypertrophy. *J Mol Cell Cardiol*. 2003; 35(7):851–9. [PubMed: 12818576]
 149. Reeves EP, Dekker LV, Forbes LV, Wientjes FB, Grogan A, Pappin DJ, et al. Direct interaction between p47phox and protein kinase C: evidence for targeting of protein kinase C by p47phox in neutrophils. *Biochem J*. 1999; 344(Pt 3):859–66. [PubMed: 10585874]
 150. Fontayne A, Dang PM, Gougerot-Pocidalo MA, El-Benna J. Phosphorylation of p47phox sites by PKC alpha, beta II, delta, and zeta: effect on binding to p22phox and on NADPH oxidase activation. *Biochemistry*. 2002; 41(24):7743–50. [PubMed: 12056906]
 151. Hoyal CR, Gutierrez A, Young BM, Catz SD, Lin JH, Tschlis PN, et al. Modulation of p47PHOX activity by site-specific phosphorylation: Akt-dependent activation of the NADPH oxidase. *Proc Natl Acad Sci U S A*. 2003; 100(9):5130–5. [PubMed: 12704229]
 152. Gregg D, Rauscher FM, Goldschmidt-Clermont PJ. Rac regulates cardiovascular superoxide through diverse molecular interactions: more than a binary GTP switch. *Am J Physiol Cell Physiol*. 2003; 285(4):C723–34. [PubMed: 12958025]
 153. Seshiah PN, Weber DS, Rocic P, Valppu L, Taniyama Y, Griendling KK. Angiotensin II stimulation of NAD(P)H oxidase activity: upstream mediators. *Circ Res*. 2002; 91(5):406–13. [PubMed: 12215489]
 154. Satoh M, Ogita H, Takeshita K, Mukai Y, Kwiatkowski DJ, Liao JK. Requirement of Rac1 in the development of cardiac hypertrophy. *Proc Natl Acad Sci U S A*. 2006; 103(19):7432–7. [PubMed: 16651530]

155. Welch HC, Coadwell WJ, Stephens LR, Hawkins PT. Phosphoinositide 3-kinase-dependent activation of Rac. *FEBS Lett.* 2003; 546(1):93–7. [PubMed: 12829242]
156. Price MO, Atkinson SJ, Knaus UG, Dinauer MC. Rac activation induces NADPH oxidase activity in transgenic COSphox cells, and the level of superoxide production is exchange factor-dependent. *J Biol Chem.* 2002; 277(21):19220–8. [PubMed: 11896053]
157. Vecchione C, Patrucco E, Marino G, Barberis L, Poulet R, Aretini A, et al. Protection from angiotensin II-mediated vasculotoxic and hypertensive response in mice lacking PI3Kgamma. *J Exp Med.* 2005; 201(8):1217–28. [PubMed: 15824082]
158. Kwon SH, Pimentel DR, Remondino A, Sawyer DB, Colucci WS. H(2)O(2) regulates cardiac myocyte phenotype via concentration-dependent activation of distinct kinase pathways. *J Mol Cell Cardiol.* 2003; 35(6):615–21. [PubMed: 12788379]
159. Blanc A, Pandey NR, Srivastava AK. Synchronous activation of ERK 1/2, p38mapk and PKB/Akt signaling by H2O2 in vascular smooth muscle cells: potential involvement in vascular disease (review). *Int J Mol Med.* 2003; 11(2):229–34. [PubMed: 12525883]
160. Pimentel DR, Adachi T, Ido Y, Heibeck T, Jiang B, Lee Y, et al. Strain-stimulated hypertrophy in cardiac myocytes is mediated by reactive oxygen species-dependent Ras S-glutathiolation. *J Mol Cell Cardiol.* 2006; 41(4):613–22. [PubMed: 16806262]
161. Ushio-Fukai M, Alexander RW, Akers M, Yin Q, Fujio Y, Walsh K, et al. Reactive oxygen species mediate the activation of Akt/protein kinase B by angiotensin II in vascular smooth muscle cells. *J Biol Chem.* 1999; 274(32):22699–704. [PubMed: 10428852]
162. Kawakami M, Okabe E. Superoxide anion radical-triggered Ca²⁺ release from cardiac sarcoplasmic reticulum through ryanodine receptor Ca²⁺ channel. *Mol Pharmacol.* 1998; 53(3):497–503. [PubMed: 9495817]
163. Zeng Q, Zhou Q, Yao F, O'Rourke ST, Sun C. Endothelin-1 regulates cardiac L-type calcium channels via NAD(P)H oxidase-derived superoxide. *J Pharmacol Exp Ther.* 2008; 326(3):732–8. [PubMed: 18539650]
164. Amberg GC, Earley S, Glapa SA. Local Regulation of Arterial L-Type Calcium Channels by Reactive Oxygen Species. *Circ Res.*
165. Li PF, Dietz R, von Harsdorf R. Superoxide induces apoptosis in cardiomyocytes, but proliferation and expression of transforming growth factor-beta1 in cardiac fibroblasts. *FEBS Lett.* 1999; 448(2–3):206–10. [PubMed: 10218477]
166. Sorescu D, Griendling KK. Reactive oxygen species, mitochondria, and NAD(P)H oxidases in the development and progression of heart failure. *Congest Heart Fail.* 2002; 8(3):132–40. [PubMed: 12045381]
167. Asakura M, Kitakaze M, Takashima S, Liao Y, Ishikura F, Yoshinaka T, et al. Cardiac hypertrophy is inhibited by antagonism of ADAM12 processing of HB-EGF: metalloproteinase inhibitors as a new therapy. *Nat Med.* 2002; 8(1):35–40. [PubMed: 11786904]
168. Ohtsu H, Dempsey PJ, Frank GD, Brailoiu E, Higuchi S, Suzuki H, et al. ADAM17 mediates epidermal growth factor receptor transactivation and vascular smooth muscle cell hypertrophy induced by angiotensin II. *Arterioscler Thromb Vasc Biol.* 2006; 26(9):e133–7. [PubMed: 16840716]
169. Daub H, Weiss FU, Wallasch C, Ullrich A. Role of transactivation of the EGF receptor in signalling by G-protein-coupled receptors. *Nature.* 1996; 379(6565):557–60. [PubMed: 8596637]
170. Lautrette A, Li S, Alili R, Sunnarborg SW, Burtin M, Lee DC, et al. Angiotensin II and EGF receptor cross-talk in chronic kidney diseases: a new therapeutic approach. *Nat Med.* 2005; 11(8):867–74. [PubMed: 16041383]
171. Shah BH, Shah FB, Catt KJ. Role of metalloproteinase-dependent EGF receptor activation in alpha-adrenoceptor-stimulated MAP kinase phosphorylation in GT1-7 neurons. *J Neurochem.* 2006; 96(2):520–32. [PubMed: 16336626]
172. Castro MM, Rizzi E, Figueiredo-Lopes L, Fernandes K, Bendhack LM, Pitol DL, et al. Metalloproteinase inhibition ameliorates hypertension and prevents vascular dysfunction and remodeling in renovascular hypertensive rats. *Atherosclerosis.* 2008; 198(2):320–31. [PubMed: 18054360]

173. Herget J, Novotna J, Bibova J, Povysilova V, Vankova M, Hampl V. Metalloproteinase inhibition by Batimastat attenuates pulmonary hypertension in chronically hypoxic rats. *Am J Physiol Lung Cell Mol Physiol*. 2003; 285(1):L199–208. [PubMed: 12665462]
174. Guimaraes DA, Rizzi E, Ceron CS, Oliveira AM, Oliveira DM, Castro MM, et al. Doxycycline dose-dependently Inhibits MMP-2-mediated vascular changes in 2K1C hypertension. *Basic Clin Pharmacol Toxicol*. 2011; 108(5):318–25. [PubMed: 21176109]
175. Miura S, Ohno I, Suzuki J, Suzuki K, Okada S, Okuyama A, et al. Inhibition of matrix metalloproteinases prevents cardiac hypertrophy induced by beta-adrenergic stimulation in rats. *J Cardiovasc Pharmacol*. 2003; 42(2):174–81. [PubMed: 12883319]
176. Hao L, Nishimura T, Wo H, Fernandez-Patron C. Vascular responses to alpha1-adrenergic receptors in small rat mesenteric arteries depend on mitochondrial reactive oxygen species. *Arterioscler Thromb Vasc Biol*. 2006; 26(4):819–25. [PubMed: 16424353]
177. Wang X, Chow FL, Oka T, Hao L, Lopez-Campistrous A, Kelly S, et al. Matrix Metalloproteinase-7 and ADAM-12 (a Disintegrin and Metalloproteinase-12) Define a Signaling Axis in Agonist-Induced Hypertension and Cardiac Hypertrophy. *Circulation*. 2009
178. Wang X, Oka T, Chow FL, Cooper SB, Odenbach J, Lopaschuk GD, et al. Tumor necrosis factor-alpha-converting enzyme is a key regulator of agonist-induced cardiac hypertrophy and fibrosis. *Hypertension*. 2009; 54(3):575–82. [PubMed: 19581512]
179. Odenbach J, Wang X, Cooper S, Chow FL, Oka T, Lopaschuk G, et al. MMP-2 Mediates Angiotensin II-Induced Hypertension Under the Transcriptional Control of MMP-7 and TACE. *Hypertension*.
180. Van Wart HE, Birkedal-Hansen H. The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. *Proc Natl Acad Sci U S A*. 1990; 87(14):5578–82. [PubMed: 2164689]
181. Spinale FG. Myocardial matrix remodeling and the matrix metalloproteinases: influence on cardiac form and function. *Physiol Rev*. 2007; 87(4):1285–342. [PubMed: 17928585]
182. Powell WC, Fingleton B, Wilson CL, Boothby M, Matrisian LM. The metalloproteinase matrilysin proteolytically generates active soluble Fas ligand and potentiates epithelial cell apoptosis. *Curr Biol*. 1999; 9(24):1441–7. [PubMed: 10607586]
183. Hayami T, Funaki H, Yaoeda K, Mitui K, Yamagiwa H, Tokunaga K, et al. Expression of the cartilage derived anti-angiogenic factor chondromodulin-I decreases in the early stage of experimental osteoarthritis. *J Rheumatol*. 2003; 30(10):2207–17. [PubMed: 14528519]
184. DeLano FA, Schmid-Schonbein GW. Proteinase activity and receptor cleavage: mechanism for insulin resistance in the spontaneously hypertensive rat. *Hypertension*. 2008; 52(2):415–23. [PubMed: 18606910]
185. Tran ED, DeLano FA, Schmid-Schonbein GW. Enhanced matrix metalloproteinase activity in the spontaneously hypertensive rat: VEGFR-2 cleavage, endothelial apoptosis, and capillary rarefaction. *J Vasc Res*. 47(5):423–31.
186. Nagareddy PR, Chow FL, Hao L, Wang X, Nishimura T, MacLeod KM, et al. Maintenance of adrenergic vascular tone by MMP transactivation of the EGFR requires PI3K and mitochondrial ATP synthesis. *Cardiovasc Res*. 2009; 84(3):368–77. [PubMed: 19578070]
187. Benbow U, Brinckerhoff CE. The AP-1 site and MMP gene regulation: what is all the fuss about? *Matrix Biol*. 1997; 15(8–9):519–26. [PubMed: 9138284]
188. Lin CW, Georgescu HI, Evans CH. The role of AP-1 in matrix metalloproteinase gene expression. *Agents Actions*. 1993; 39(Spec No):C215–8. [PubMed: 8273573]
189. Sahin U, Weskamp G, Kelly K, Zhou HM, Higashiyama S, Peschon J, et al. Distinct roles for ADAM10 and ADAM17 in ectodomain shedding of six EGFR ligands. *J Cell Biol*. 2004; 164(5):769–79. [PubMed: 14993236]
190. Kurohara K, Komatsu K, Kurisaki T, Masuda A, Irie N, Asano M, et al. Essential roles of Meltrin beta (ADAM19) in heart development. *Dev Biol*. 2004; 267(1):14–28. [PubMed: 14975714]
191. Ancha HR, Kurella RR, Stewart CA, Damera G, Ceresa BP, Harty RF. Histamine stimulation of MMP-1(collagenase-1) secretion and gene expression in gastric epithelial cells: role of EGFR transactivation and the MAP kinase pathway. *Int J Biochem Cell Biol*. 2007; 39(11):2143–52. [PubMed: 17656145]

192. Coker ML, Jolly JR, Joffs C, Etoh T, Holder JR, Bond BR, et al. Matrix metalloproteinase expression and activity in isolated myocytes after neurohormonal stimulation. *Am J Physiol Heart Circ Physiol*. 2001; 281(2):H543–51. [PubMed: 11454555]
193. Tsukioka K, Suzuki J, Fujimori M, Wada Y, Yamaura K, Ito K, et al. Expression of matrix metalloproteinases in cardiac allograft vasculopathy and its attenuation by anti MMP-2 ribozyme gene transfection. *Cardiovasc Res*. 2002; 56(3):472–8. [PubMed: 12445888]
194. Moshal KS, Sen U, Tyagi N, Henderson B, Steed M, Ovechkin AV, et al. Regulation of homocysteine-induced MMP-9 by ERK1/2 pathway. *Am J Physiol Cell Physiol*. 2006; 290(3):C883–91. [PubMed: 16251475]
195. Taniguchi Y, Doronbekov K, Yamada T, Sasaki Y, Takano A, Sugimoto Y. Genomic organization and promoter analysis of the bovine ADAM12 gene. *Anim Biotechnol*. 2008; 19(3):178–89. [PubMed: 18607790]
196. Saygili E, Rana OR, Meyer C, Gemein C, Andrzejewski MG, Ludwig A, et al. The angiotensin-calcineurin-NFAT pathway mediates stretch-induced up-regulation of matrix metalloproteinases-2/-9 in atrial myocytes. *Basic Res Cardiol*. 2009; 104(4):435–48. [PubMed: 19148693]
197. Izumi Y, Hirata M, Hasuwa H, Iwamoto R, Umata T, Miyado K, et al. A metalloprotease-disintegrin, MDC9/meltrin-gamma/ADAM9 and PKCdelta are involved in TPA-induced ectodomain shedding of membrane-anchored heparin-binding EGF-like growth factor. *Embo J*. 1998; 17(24):7260–72. [PubMed: 9857183]
198. Diaz-Rodriguez E, Montero JC, Esparis-Ogando A, Yuste L, Pandiella A. Extracellular signal-regulated kinase phosphorylates tumor necrosis factor alpha-converting enzyme at threonine 735: a potential role in regulated shedding. *Mol Biol Cell*. 2002; 13(6):2031–44. [PubMed: 12058067]
199. Zheng Y, Schlondorff J, Blobel CP. Evidence for regulation of the tumor necrosis factor alpha-converter (TACE) by protein-tyrosine phosphatase PTPH1. *J Biol Chem*. 2002; 277(45):42463–70. [PubMed: 12207026]
200. Zhang Z, Oliver P, Lancaster JJ, Schwarzenberger PO, Joshi MS, Cork J, et al. Reactive oxygen species mediate tumor necrosis factor alpha-converting, enzyme-dependent ectodomain shedding induced by phorbol myristate acetate. *Faseb J*. 2001; 15(2):303–5. [PubMed: 11156944]
201. Mifune M, Ohtsu H, Suzuki H, Nakashima H, Brailoiu E, Dun NJ, et al. G protein coupling and second messenger generation are indispensable for metalloprotease-dependent, heparin-binding epidermal growth factor shedding through angiotensin II type-1 receptor. *J Biol Chem*. 2005; 280(28):26592–9. [PubMed: 15905175]
202. Mori S, Tanaka M, Nanba D, Nishiwaki E, Ishiguro H, Higashiyama S, et al. PACSIN3 binds ADAM12/meltrin alpha and up-regulates ectodomain shedding of heparin-binding epidermal growth factor-like growth factor. *J Biol Chem*. 2003; 278(46):46029–34. [PubMed: 12952982]
203. Tanaka M, Nanba D, Mori S, Shiba F, Ishiguro H, Yoshino K, et al. ADAM binding protein Eve-1 is required for ectodomain shedding of epidermal growth factor receptor ligands. *J Biol Chem*. 2004; 279(40):41950–9. [PubMed: 15280379]
204. Beklen A, Tuter G, Sorsa T, Hanemaaijer R, Virtanen I, Tervahartiala T, et al. Gingival tissue and crevicular fluid co-operation in adult periodontitis. *J Dent Res*. 2006; 85(1):59–63. [PubMed: 16373682]
205. Salo J, Mackiewicz Z, Indahl A, Kontinen YT, Holm AK, Sukura A, et al. Plasmin-matrix metalloproteinase cascades in spinal response to an experimental disc lesion in pig. *Spine (Phila Pa 1976)*. 2008; 33(8):839–44. [PubMed: 18404101]
206. Dozier S, Escobar GP, Lindsey ML. Matrix metalloproteinase (MMP)-7 activates MMP-8 but not MMP-13. *Med Chem*. 2006; 2(5):523–6. [PubMed: 17017992]
207. Zhan M, Jin B, Chen SE, Reecy JM, Li YP. TACE release of TNF-alpha mediates mechanotransduction-induced activation of p38 MAPK and myogenesis. *J Cell Sci*. 2007; 120(Pt 4):692–701. [PubMed: 17264149]
208. Mohan S, Thompson GR, Amaar YG, Hathaway G, Tschesche H, Baylink DJ. ADAM-9 is an insulin-like growth factor binding protein-5 protease produced and secreted by human osteoblasts. *Biochemistry*. 2002; 41(51):15394–403. [PubMed: 12484779]

209. Shi Z, Xu W, Loechel F, Wewer UM, Murphy LJ. ADAM 12, a disintegrin metalloprotease, interacts with insulin-like growth factor-binding protein-3. *J Biol Chem.* 2000; 275(24):18574–80. [PubMed: 10849447]
210. Coppock HA, White A, Aplin JD, Westwood M. Matrix metalloprotease-3 and -9 proteolyze insulin-like growth factor-binding protein-1. *Biol Reprod.* 2004; 71(2):438–43. [PubMed: 15070833]
211. Edwin F, Wiepz GJ, Singh R, Peet CR, Chaturvedi D, Bertics PJ, et al. A historical perspective of the EGF receptor and related systems. *Methods Mol Biol.* 2006; 327:1–24. [PubMed: 16780209]
212. Karunagaran D, Tzahar E, Beerli RR, Chen X, Graus-Porta D, Ratzkin BJ, et al. ErbB-2 is a common auxiliary subunit of NDF and EGF receptors: implications for breast cancer. *Embo J.* 1996; 15(2):254–64. [PubMed: 8617201]
213. Clerk A, Aggeli IK, Stathopoulou K, Sugden PH. Peptide growth factors signal differentially through protein kinase C to extracellular signal-regulated kinases in neonatal cardiomyocytes. *Cell Signal.* 2006; 18(2):225–35. [PubMed: 15936927]
214. Zhao YY, Sawyer DR, Baliga RR, Opel DJ, Han X, Marchionni MA, et al. Neuregulins promote survival and growth of cardiac myocytes. Persistence of ErbB2 and ErbB4 expression in neonatal and adult ventricular myocytes. *J Biol Chem.* 1998; 273(17):10261–9. [PubMed: 9553078]
215. Merkel LA, Rivera LM, Colussi DJ, Perrone MH. Inhibition of EGF-induced vasoconstriction in isolated rabbit aortic rings with the tyrosine kinase inhibitor RG50864. *Biochem Biophys Res Commun.* 1993; 192(3):1319–26. [PubMed: 7685166]
216. Florian JA, Watts SW. Epidermal growth factor: a potent vasoconstrictor in experimental hypertension. *Am J Physiol.* 1999; 276(3 Pt 2):H976–83. [PubMed: 10070082]
217. Kagiya S, Eguchi S, Frank GD, Inagami T, Zhang YC, Phillips MI. Angiotensin II-induced cardiac hypertrophy and hypertension are attenuated by epidermal growth factor receptor antisense. *Circulation.* 2002; 106(8):909–12. [PubMed: 12186792]
218. Borrell-Pages M, Rojo F, Albanell J, Baselga J, Arribas J. TACE is required for the activation of the EGFR by TGF- α in tumors. *Embo J.* 2003; 22(5):1114–24. [PubMed: 12606576]
219. Jorissen RN, Walker F, Pouliot N, Garrett TP, Ward CW, Burgess AW. Epidermal growth factor receptor: mechanisms of activation and signalling. *Exp Cell Res.* 2003; 284(1):31–53. [PubMed: 12648464]
220. Zuo L, Ushio-Fukai M, Ikeda S, Hilenski L, Patrushev N, Alexander RW. Caveolin-1 is essential for activation of Rac1 and NAD(P)H oxidase after angiotensin II type 1 receptor stimulation in vascular smooth muscle cells: role in redox signaling and vascular hypertrophy. *Arterioscler Thromb Vasc Biol.* 2005; 25(9):1824–30. [PubMed: 15976327]
221. Zuo L, Ushio-Fukai M, Hilenski LL, Alexander RW. Microtubules regulate angiotensin II type 1 receptor and Rac1 localization in caveolae/lipid rafts: role in redox signaling. *Arterioscler Thromb Vasc Biol.* 2004; 24(7):1223–8. [PubMed: 15142861]
222. Schlessinger J, Lemmon MA. SH2 and PTB domains in tyrosine kinase signaling. *Sci STKE.* 2003; 2003(191):RE12. [PubMed: 12865499]
223. Olayioye MA, Neve RM, Lane HA, Hynes NE. The ErbB signaling network: receptor heterodimerization in development and cancer. *Embo J.* 2000; 19(13):3159–67. [PubMed: 10880430]
224. Touyz RM, Cruzado M, Tabet F, Yao G, Salomon S, Schiffrin EL. Redox-dependent MAP kinase signaling by Ang II in vascular smooth muscle cells: role of receptor tyrosine kinase transactivation. *Can J Physiol Pharmacol.* 2003; 81(2):159–67. [PubMed: 12710530]
225. Bae YS, Kang SW, Seo MS, Baines IC, Tekle E, Chock PB, et al. Epidermal growth factor (EGF)-induced generation of hydrogen peroxide. Role in EGF receptor-mediated tyrosine phosphorylation. *J Biol Chem.* 1997; 272(1):217–21. [PubMed: 8995250]
226. Rao GN, Delafontaine P, Runge MS. Thrombin stimulates phosphorylation of insulin-like growth factor-I receptor, insulin receptor substrate-1, and phospholipase C- γ 1 in rat aortic smooth muscle cells. *J Biol Chem.* 1995; 270(46):27871–5. [PubMed: 7499260]
227. Linseman DA, Benjamin CW, Jones DA. Convergence of angiotensin II and platelet-derived growth factor receptor signaling cascades in vascular smooth muscle cells. *J Biol Chem.* 1995; 270(21):12563–8. [PubMed: 7759503]

228. Kelly DJ, Cox AJ, Gow RM, Zhang Y, Kemp BE, Gilbert RE. Platelet-derived growth factor receptor transactivation mediates the trophic effects of angiotensin II in vivo. *Hypertension*. 2004; 44(2):195–202. [PubMed: 15197170]
229. Roudabush FL, Pierce KL, Maudsley S, Khan KD, Luttrell LM. Transactivation of the EGF receptor mediates IGF-1-stimulated shc phosphorylation and ERK1/2 activation in COS-7 cells. *J Biol Chem*. 2000; 275(29):22583–9. [PubMed: 10807918]
230. Saito Y, Haendeler J, Hojo Y, Yamamoto K, Berk BC. Receptor heterodimerization: essential mechanism for platelet-derived growth factor-induced epidermal growth factor receptor transactivation. *Mol Cell Biol*. 2001; 21(19):6387–94. [PubMed: 11533228]
231. Munoz JP, Collao A, Chiong M, Maldonado C, Adasme T, Carrasco L, et al. The transcription factor MEF2C mediates cardiomyocyte hypertrophy induced by IGF-1 signaling. *Biochem Biophys Res Commun*. 2009; 388(1):155–60. [PubMed: 19654000]
232. Huang CY, Hao LY, Buetow DE. Insulin-like growth factor-II induces hypertrophy of adult cardiomyocytes via two alternative pathways. *Cell Biol Int*. 2002; 26(8):737–9. [PubMed: 12175677]
233. Huang CY, Hao LY, Buetow DE. Insulin-like growth factor-induced hypertrophy of cultured adult rat cardiomyocytes is L-type calcium-channel-dependent. *Mol Cell Biochem*. 2002; 231(1–2): 51–9. [PubMed: 11952165]
234. Ponten A, Li X, Thoren P, Aase K, Sjoblom T, Ostman A, et al. Transgenic overexpression of platelet-derived growth factor-C in the mouse heart induces cardiac fibrosis, hypertrophy, and dilated cardiomyopathy. *Am J Pathol*. 2003; 163(2):673–82. [PubMed: 12875986]
235. Heeneman S, Haendeler J, Saito Y, Ishida M, Berk BC. Angiotensin II induces transactivation of two different populations of the platelet-derived growth factor beta receptor. Key role for the p66 adaptor protein Shc. *J Biol Chem*. 2000; 275(21):15926–32. [PubMed: 10748142]
236. LaRochelle WJ, Jeffers M, McDonald WF, Chillakuru RA, Giese NA, Lokker NA, et al. PDGF-D, a new protease-activated growth factor. *Nat Cell Biol*. 2001; 3(5):517–21. [PubMed: 11331882]
237. Li X, Ponten A, Aase K, Karlsson L, Abramsson A, Uutela M, et al. PDGF-C is a new protease-activated ligand for the PDGF alpha-receptor. *Nat Cell Biol*. 2000; 2(5):302–9. [PubMed: 10806482]
238. Lim JY, Park SJ, Hwang HY, Park EJ, Nam JH, Kim J, et al. TGF-beta1 induces cardiac hypertrophic responses via PKC-dependent ATF-2 activation. *J Mol Cell Cardiol*. 2005; 39(4): 627–36. [PubMed: 16125722]
239. Rosenkranz S, Flesch M, Amann K, Haeuseler C, Kilter H, Seeland U, et al. Alterations of beta-adrenergic signaling and cardiac hypertrophy in transgenic mice overexpressing TGF-beta(1). *Am J Physiol Heart Circ Physiol*. 2002; 283(3):H1253–62. [PubMed: 12181157]
240. Ge G, Greenspan DS. BMP1 controls TGFbeta1 activation via cleavage of latent TGFbeta-binding protein. *J Cell Biol*. 2006; 175(1):111–20. [PubMed: 17015622]
241. Watkins SJ, Jonker L, Arthur HM. A direct interaction between TGFbeta activated kinase 1 and the TGFbeta type II receptor: implications for TGFbeta signalling and cardiac hypertrophy. *Cardiovasc Res*. 2006; 69(2):432–9. [PubMed: 16360132]
242. Moriguchi T, Kuroyanagi N, Yamaguchi K, Gotoh Y, Irie K, Kano T, et al. A novel kinase cascade mediated by mitogen-activated protein kinase kinase 6 and MKK3. *J Biol Chem*. 1996; 271(23):13675–9. [PubMed: 8663074]
243. Zhang D, Gaussin V, Taffet GE, Belaguli NS, Yamada M, Schwartz RJ, et al. TAK1 is activated in the myocardium after pressure overload and is sufficient to provoke heart failure in transgenic mice. *Nat Med*. 2000; 6(5):556–63. [PubMed: 10802712]
244. Sano Y, Harada J, Tashiro S, Gotoh-Mandeville R, Maekawa T, Ishii S. ATF-2 is a common nuclear target of Smad and TAK1 pathways in transforming growth factor-beta signaling. *J Biol Chem*. 1999; 274(13):8949–57. [PubMed: 10085140]
245. Monzen K, Hiroi Y, Kudoh S, Akazawa H, Oka T, Takimoto E, et al. Smads, TAK1, and their common target ATF-2 play a critical role in cardiomyocyte differentiation. *J Cell Biol*. 2001; 153(4):687–98. [PubMed: 11352931]

246. Herron TJ, Vandenboom R, Fomicheva E, Mundada L, Edwards T, Metzger JM. Calcium-independent negative inotropy by beta-myosin heavy chain gene transfer in cardiac myocytes. *Circ Res.* 2007; 100(8):1182–90. [PubMed: 17363698]
247. Yokoyama T, Nakano M, Bednarczyk JL, McIntyre BW, Entman M, Mann DL. Tumor necrosis factor-alpha provokes a hypertrophic growth response in adult cardiac myocytes. *Circulation.* 1997; 95(5):1247–52. [PubMed: 9054856]
248. Dibbs ZI, Diwan A, Nemoto S, DeFreitas G, Abdellatif M, Carabello BA, et al. Targeted overexpression of transmembrane tumor necrosis factor provokes a concentric cardiac hypertrophic phenotype. *Circulation.* 2003; 108(8):1002–8. [PubMed: 12912811]
249. Sriramula S, Haque M, Majid DS, Francis J. Involvement of tumor necrosis factor-alpha in angiotensin II-mediated effects on salt appetite, hypertension, and cardiac hypertrophy. *Hypertension.* 2008; 51(5):1345–51. [PubMed: 18391105]
250. Zheng Y, Saftig P, Hartmann D, Blobel C. Evaluation of the contribution of different ADAMs to tumor necrosis factor alpha (TNFalpha) shedding and of the function of the TNFalpha ectodomain in ensuring selective stimulated shedding by the TNFalpha convertase (TACE/ADAM17). *J Biol Chem.* 2004; 279(41):42898–906. [PubMed: 15292243]
251. Haro H, Crawford HC, Fingleton B, Shinomiya K, Spengler DM, Matrisian LM. Matrix metalloproteinase-7-dependent release of tumor necrosis factor-alpha in a model of herniated disc resorption. *J Clin Invest.* 2000; 105(2):143–50. [PubMed: 10642592]
252. Gearing AJ, Beckett P, Christodoulou M, Churchill M, Clements JM, Crimmin M, et al. Matrix metalloproteinases and processing of pro-TNF-alpha. *J Leukoc Biol.* 1995; 57(5):774–7. [PubMed: 7759957]
253. Gearing AJ, Beckett P, Christodoulou M, Churchill M, Clements J, Davidson AH, et al. Processing of tumour necrosis factor-alpha precursor by metalloproteinases. *Nature.* 1994; 370(6490):555–7. [PubMed: 8052310]
254. Condorelli G, Morisco C, Latronico MV, Claudio PP, Dent P, Tschlis P, et al. TNF-alpha signal transduction in rat neonatal cardiac myocytes: definition of pathways generating from the TNF-alpha receptor. *Faseb J.* 2002; 16(13):1732–7. [PubMed: 12409315]
255. Hiraoka E, Kawashima S, Takahashi T, Rikitake Y, Kitamura T, Ogawa W, et al. TNF-alpha induces protein synthesis through PI3-kinase-Akt/PKB pathway in cardiac myocytes. *Am J Physiol Heart Circ Physiol.* 2001; 280(4):H1861–8. [PubMed: 11247802]
256. Liu PC, Liu X, Li Y, Covington M, Wynn R, Huber R, et al. Identification of ADAM10 as a major source of HER2 ectodomain sheddase activity in HER2 overexpressing breast cancer cells. *Cancer Biol Ther.* 2006; 5(6):657–64. [PubMed: 16627989]
257. Rodrigues SF, Tran ED, Fortes ZB, Schmid-Schonbein GW. Matrix metalloproteinases cleave the beta2-adrenergic receptor in spontaneously hypertensive rats. *Am J Physiol Heart Circ Physiol.* 2009; 297(1):H25–35.
258. Chen AY, DeLano FA, Valdez SR, Ha JN, Shin HY, Schmid-Schonbein GW. Receptor cleavage reduces the fluid shear response in neutrophils of the spontaneously hypertensive rat. *Am J Physiol Cell Physiol.* 2010; 299(6):C1441–9. [PubMed: 20861466]
259. Yamazato Y, Ferreira AJ, Hong KH, Sriramula S, Francis J, Yamazato M, et al. Prevention of pulmonary hypertension by Angiotensin-converting enzyme 2 gene transfer. *Hypertension.* 2009; 54(2):365–71. [PubMed: 19564552]
260. Boadle MC, Hughes J, Roth RH. Angiotensin accelerates catecholamine biosynthesis in sympathetically innervated tissues. *Nature.* 1969; 222(5197):987–8. [PubMed: 4307048]
261. Cox SL, Ben A, Story DF, Ziogas J. Evidence for the involvement of different receptor subtypes in the pre- and postjunctional actions of angiotensin II at rat sympathetic neuroeffector sites. *Br J Pharmacol.* 1995; 114(5):1057–63. [PubMed: 7780640]
262. Golub LM, Lee HM, Lehrer G, Nemiroff A, McNamara TF, Kaplan R, et al. Minocycline reduces gingival collagenolytic activity during diabetes. Preliminary observations and a proposed new mechanism of action. *J Periodontal Res.* 1983; 18(5):516–26. [PubMed: 6315909]
263. Maitra SR, Bhaduri S, Valane PD, Tervahartiala T, Sorsa T, Ramamurthy N. Inhibition of matrix metalloproteinases by chemically modified tetracyclines in sepsis. *Shock.* 2003; 20(3):280–5. [PubMed: 12923502]

264. Steinberg J, Halter J, Schiller HJ, Dasilva M, Landas S, Gatto LA, et al. Metalloproteinase inhibition reduces lung injury and improves survival after cecal ligation and puncture in rats. *J Surg Res*. 2003; 111(2):185–95. [PubMed: 12850461]
265. Salo T, Soini Y, Oiva J, Kariylitalo, Nissinen A, Biancari F, et al. Chemically modified tetracyclines (CMT-3 and CMT-8) enable control of the pathologic remodelling of human aortic valve stenosis via MMP-9 and VEGF inhibition. *Int J Cardiol*. 2006; 111(3):358–64. [PubMed: 16242193]
266. Lokeshwar BL. Chemically modified non-antimicrobial tetracyclines are multifunctional drugs against advanced cancers. *Pharmacol Res*. 63(2):146–50.
267. Richards C, Pantanowitz L, Dezube BJ. Antimicrobial and non-antimicrobial tetracyclines in human cancer trials. *Pharmacol Res*. 63(2):151–6.
268. Peterson JT. Matrix metalloproteinase inhibitor development and the remodeling of drug discovery. *Heart Fail Rev*. 2004; 9(1):63–79. [PubMed: 14739769]
269. Sparano JA, Bernardo P, Stephenson P, Gradishar WJ, Ingle JN, Zucker S, et al. Randomized phase III trial of marimastat versus placebo in patients with metastatic breast cancer who have responding or stable disease after first-line chemotherapy: Eastern Cooperative Oncology Group trial E2196. *J Clin Oncol*. 2004; 22(23):4683–90. [PubMed: 15570070]
270. Guimaraes DA, Rizzi E, Ceron CS, Oliveira AM, Oliveira DM, Castro MM, et al. Doxycycline dose-dependently inhibits MMP-2-mediated vascular changes in 2K1C hypertension. *Basic Clin Pharmacol Toxicol*. 108(5):318–25.
271. Errami M, Galindo CL, Tassa AT, Dimaio JM, Hill JA, Garner HR. Doxycycline attenuates isoproterenol- and transverse aortic banding-induced cardiac hypertrophy in mice. *J Pharmacol Exp Ther*. 2008; 324(3):1196–203. [PubMed: 18089841]
272. Camp TM, Tyagi SC, Aru GM, Hayden MR, Mehta JL. Doxycycline ameliorates ischemic and border-zone remodeling and endothelial dysfunction after myocardial infarction in rats. *J Heart Lung Transplant*. 2004; 23(6):729–36. [PubMed: 15366434]
273. Hudson MP, Armstrong PW, Ruzyllo W, Brum J, Cusmano L, Krzeski P, et al. Effects of selective matrix metalloproteinase inhibitor (PG-116800) to prevent ventricular remodeling after myocardial infarction: results of the PREMIER (Prevention of Myocardial Infarction Early Remodeling) trial. *J Am Coll Cardiol*. 2006; 48(1):15–20. [PubMed: 16814643]
274. Brown DL, Desai KK, Vakili BA, Nouneh C, Lee HM, Golub LM. Clinical and biochemical results of the metalloproteinase inhibition with subantimicrobial doses of doxycycline to prevent acute coronary syndromes (MIDAS) pilot trial. *Arterioscler Thromb Vasc Biol*. 2004; 24(4):733–8. [PubMed: 14962945]
275. Sang QX, Jin Y, Newcomer RG, Monroe SC, Fang X, Hurst DR, et al. Matrix metalloproteinase inhibitors as prospective agents for the prevention and treatment of cardiovascular and neoplastic diseases. *Curr Top Med Chem*. 2006; 6(4):289–316. [PubMed: 16611144]
276. Dorman G, Kocsis-Szommer K, Spadoni C, Ferdinandy P. MMP inhibitors in cardiac diseases: an update. *Recent Pat Cardiovasc Drug Discov*. 2007; 2(3):186–94. [PubMed: 18221118]

Figure 1.

Postulated model whereby GPCR agonists signal hypertension and pathological cardiovascular remodeling through induction of contraction and gene expression in vasculature and the heart.

GPCR: G protein-coupled receptor.

MP: metalloproteinase.

MMP: matrix metalloproteinase.

ADAM: a disintegrin and metalloproteinase.

Figure 2.
Major signal transduction pathways linking GPCR agonists to contractile processes.

GPCR: G protein-coupled receptor.

MP: metalloproteinase.

PI3K: phosphatidylinositol 3-kinase.

DAG: 1,2-diacylglycerol.

GRB-2: growth factor receptor-bound protein 2.

Sos: son of sevenless.

PIP₂: phosphatidylinositol (4,5)-bisphosphate.

PIP₃: phosphatidylinositol (3,4,5)-bisphosphate.

PDK-1: phosphoinositide-dependent protein kinase 1.

GSK-3 β : glycogen synthase kinase-3 β .

ERK: extracellular signal-regulated kinases.

MEK: mitogen-activated protein kinase kinase.

ROS: reactive oxygen species.

AC: adenylyl cyclase.

PKA: cAMP-dependent protein kinase.

PLC-β: phospholipase C isoform β.

IP₃: inositol 1,4,5-triphosphate.

PKC: protein kinase C.

PKD: protein kinase D.

CaM: calmodulin.

Red arrows: adenylyl cyclase-PKA pathway.

Purple arrows: PLC-pathway.

Green arrows: Ca^{2+} pathway.

Light blue arrows: PKC pathway.

Blue arrows: Raf-MEK-ERK pathway.

Pink arrows: PI3K-Akt pathway.

Orange arrows: ROS pathway.

Figure 3.

Major signal transduction pathways in GPCR agonist-induced hypertrophy and fibrosis.

GPCR: G protein-coupled receptor.

MP: metalloproteinase.

PI3K: phosphatidylinositol 3-kinase.

DAG: 1,2-diacylglycerol.

GRB-2: growth factor receptor-bound protein 2.

Sos: son of sevenless.

PIP₂: phosphatidylinositol (4,5)-bisphosphate.

PIP₃: phosphatidylinositol (3,4,5)-bisphosphate.

PDK-1: phosphoinositide-dependent protein kinase 1.

TSC-2: Tuberous sclerosis protein 2.

GSK-3β: glycogen synthase kinase-3β.

mTOR: mammalian target of rapamycin.

eIF4E: eukaryotic translation initiation factor 4E.

eIF2B: eukaryotic translation initiation factor 2B.

eEF-2: eukaryotic translation elongation factor 2.

eEF2K: eEF2 kinase.

4EBP-1: eIF4E binding protein-1.

S6K: ribosomal protein S6 kinase.
ERK: extracellular signal-regulated kinases.
MEK: mitogen-activated protein kinase kinase.
ROS: reactive oxygen species.
AC: adenylyl cyclase.
PKA: cAMP-dependent protein kinase.
PLC- β : phospholipase C isoform β .
IP₃: inositol 1,4,5-triphosphate.
PKC: protein kinase C.
PKD: protein kinase D.
CaN: calcineurin.
CREB: cAMP response element binding protein.
HDAC: histone deacetylase.
AP-1: activator protein 1.
NFAT: nuclear factor of activated T-cell.
Red arrows: adenylyl cyclase-PKA pathway.
Purple arrows: PLC-pathway.
Green arrows: Ca²⁺ pathway.
Light blue arrows: PKC pathway.
Blue arrows: Raf-MEK-ERK pathway.
Pink arrows: PI3K-Akt pathway.
Orange arrows: ROS pathway.
Purple arrows: Smad pathway.

Table 1

Classification of adult blood pressure (Adopted from (1)).

Classification	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)
Normal	<120	and <80
Prehypertension	120–139	or 80–89
Stage 1 hypertension	140–159	or 90–99
Stage 2 hypertension	160	or 100