

Roles of small GTPases in cardiac hypertrophy (Review)

XIN WANG^{1*}, XINWEN NIE^{2,3*}, HAO WANG^{2,3} and ZHANHONG REN²

¹School of Mathematics and Statistics, Hubei University of Science and Technology, Xianning, Hubei 437100, P.R. China;

²Hubei Key Laboratory of Diabetes and Angiopathy, Medicine Research Institute, Xianning Medical College,

Hubei University of Science and Technology, Xianning, Hubei 437100, P.R. China; ³School of Basic Medical Sciences,

Hubei University of Science and Technology, Xianning, Hubei 437100, P.R. China

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Abstract. Cardiac hypertrophy results from the heart reacting and adapting to various pathological stimuli and its persistent development is a major contributing factor to heart failure. However, the molecular mechanisms of cardiac hypertrophy remain unclear. Small GTPases in the Ras, Rho, Rab, Arf and Ran subfamilies exhibit GTPase activity and play crucial roles in regulating various cellular responses. Previous studies have shown that Ras, Rho and Rab are closely linked to cardiac hypertrophy and that their overexpression can induce cardiac hypertrophy. Here, we review the functions of small GTPases in cardiac hypertrophy and provide additional insights and references for the prevention and treatment of cardiac hypertrophy.

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1. Introduction

Heart failure leads to increased morbidity and mortality and cardiac hypertrophy is an independent risk factor for the development of heart failure (1,2). Cardiac hypertrophy results from long-term pressure overload and is characterized by the enlargement of myocardial cells and enhanced contractility, which enable the heart to maintain normal blood pumping

ability (1,3,4). Chronic concentric hypertrophy can lead to changes in the expression of hypertrophy-related genes such as *Nppa*, *Nppb* and myosin heavy chain 7, systolic dysfunction and extracellular remodeling (4,5). Reactivation of *Nppa* and *Nppb* can promote sodium excretion, lower blood pressure and ameliorate the burden on the heart, resulting in antihypertrophic effects. Long-term cardiac hypertrophy eventually leads to heart failure (2,6). Studies on the molecular mechanisms of cardiac hypertrophy are beneficial for the prevention and treatment of heart failure.

Previous studies have shown that small GTPases, a known type of molecular switch in cells, play a critical role in cardiac hypertrophy (7,8). They have a molecular weight of only 20-30 kDa and GTPase activity (9). These proteins can be divided into five families based on their amino acid sequence: Ras, Rho, Rab, Arf and Ran (9,10). Since the discovery of the Ras protein in the early 1980s, more than 100 types of small GTPases have been identified (10,11). Small GTPases are in GDP-bound (inactive) and GTP-bound (active) states, which links upstream signaling molecules with downstream effectors to mediate cell proliferation, differentiation, transportation and cytoskeleton regulation (12). Ras mainly regulates cell signaling pathways, including the Ras/mitogen-activated protein kinase kinase (MEKK)/c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) pathways and plays important roles in cell proliferation, differentiation, morphology and apoptosis (13-17). Rho is not only involved in regulating actin, cell polarity, cell migration, vesicle transport and cytokinesis, but is also involved in hematopoiesis, especially in the classical and noncanonical Wnt signaling pathways (18-21). Rab is involved in vesicle and endocytic membrane trafficking and can regulate plasma membrane delivery, organelle biogenesis and degradation pathways, lysosomes and autophagy (22). In addition to its basic role in membrane transport, Arf also regulates mitosis, plasma membrane signaling, ciliary transport and lipid droplet functions (23). Ran mainly controls the entry and exit of cargoes between the cytoplasm and nucleus via the nuclear pore complex (24).

Since the discovery of the role of Ras in cardiac hypertrophy in 1993, small GTPases, mainly Ras, Rho and Rab, have been confirmed to be involved in cardiac hypertrophy. Ras can mediate cardiac hypertrophy through multiple pathways, including the MAPK and Ca²⁺/calcineurin/nuclear factor of activated T cells (NFAT) pathways (14). The Rho

Correspondence to: Professor Zhanhong Ren, Hubei Key Laboratory of Diabetes and Angiopathy, Medicine Research Institute, Xianning Medical College, Hubei University of Science and Technology, 88 Xianning Avenue, Xianning, Hubei 437100, P.R. China

E-mail: renzhanhong@hbust.edu.cn

*Contributed equally

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A/Rho-associated protein kinase (ROCK) pathway is the main pathway involved in Rho A-mediated cardiac hypertrophy (25). Rac1, a member of the Rho family, can cause cardiac hypertrophy by regulating the MAPK signaling pathway and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity (26-30). By contrast, Cdc42 can antagonize cardiac hypertrophy by activating JNK and inhibiting calcineurin-NFAT activity (31). At present, Rab1a, Rab4 and Rab4a from the Rab family have been found to lead to myocardial hypertrophy (32). However, the relationships between the other two small GTPases (Arf and Ran) and cardiac hypertrophy remain unreported. The present study reviewed recent research progress on the roles of small GTPases in cardiac hypertrophy, which is helpful for the prevention and treatment of cardiac hypertrophy and heart failure.

2. Small GTPases in cardiac hypertrophy

Ras family. The Ras family contains 36 members that can be classified into six types: Ras, Ral, Rit, Rap, Rheb and Rad (Ras associated with diabetes) (14). Ras is involved in regulating cell division, cell migration, adhesion, differentiation and apoptosis (Table I) (33). Ral can regulate neuronal plasticity, the immune response and glucose and lipid homeostasis (34). Rit is involved in cell transformation, cell survival, neuromorphogenesis and the regulation of dopamine transporters and plays important roles in autism and schizophrenia (35). Rap plays a role in the normal tissue system, cancer, immune system and hematopoietic system (36). Rheb is highly expressed in the brain, can activate mammalian target of rapamycin complex 1 in response to a variety of growth factor stimuli and is associated with cell growth, protein synthesis and regeneration (37,38). Rad acts as a calcium channel regulator in the normal heart by interacting with cardiac L-type calcium (Table I) (39). Ras and Rad play important roles in cardiac hypertrophy (14,40). However, the relationships between the other four members of the Ras subfamily and cardiac hypertrophy are still unknown.

Ras can induce cardiac hypertrophy (41,42). Microinjection of activated Ras into primary neonatal rat ventricular cardiomyocytes (NRCMs) can upregulate the expression of hypertrophy-related genes, including c-Fos and atrial natriuretic factor (ANF), *in vitro* (41). In addition, dominant negative mutant Ras (DN-Ras) has an antihypertrophic effect *in vitro* in NRCMs and plays a protective role in the rat heart *in vivo* by inhibiting nuclear factor of activated T-cells (NFATs) (42).

Ras can induce cardiac hypertrophy mainly through activating the JNK, p38 MAPK and Ca^{2+} /calcineurin/NFAT signaling pathways both *in vitro* and *in vivo* (Fig. 1) (15,17,43,44). The activation of the Ras/MEKK/JNK pathway can mediate ANF gene expression, which causes cardiac hypertrophy both *in vitro* and *in vivo* (15). MEKK is a serine triphosphate kinase that can be activated by active Ras *in vitro* and can regulate stress-activated protein kinases and mitogen-activated protein kinases (45,46). The activation of JNK by MEKK can promote c-Jun transcriptional activity and upregulate ANF gene expression in phenylephrine-treated cardiomyocytes *in vitro* (15). In activated Ras transgenic mice, JNK activity is upregulated in the left ventricle, which can result in increased ventricular ANF and cardiac hypertrophy *in vivo* (15). Mechanical stress-activated integrins can trigger autophosphorylation of focal adhesion

kinase (FAK) at Tyr397, which can promote the interaction between FAK and c-Src *in vitro* (17). Tyr925 of FAK is then phosphorylated by c-Src (17). It has been confirmed that the phosphorylation of Tyr397 and Tyr925 in FAK is required for p38 MAPK activation (17). In addition, p38 MAPK activation induced by integrins also requires activated Ras (17). Therefore, it has been suggested that mechanical stress can activate p38 MAPK through the integrin/FAK/Src/Ras pathway to induce cardiomyocyte hypertrophy *in vitro* (17). In addition, the down-regulation of Ras can inhibit the calcineurin activity stimulated by phenylephrine and its upregulation can lead to an increase in the activity of the cytosolic transcription factor NFAT *in vitro*, which indicates that Ras can activate the Ca^{2+} /calcineurin/NFAT signaling pathway in cardiomyocytes (44). Rad was originally identified in the skeletal muscle of patients with type 2 diabetes (47). Rad is significantly downregulated in pressure overload and phenylephrine-induced cardiac hypertrophy and decreasing Rad can lead to significant enhancement of the phosphorylation and activity of CaMKII, which induces cardiac hypertrophy both *in vitro* and *in vivo* (40). Therefore, Rad can antagonize cardiac hypertrophy by inhibiting the activity of CaMKII (40).

Statins or farnesyltransferase inhibitors can suppress Ras by intervening with its farnesylation or geranylgeranylation at its carboxy terminus both *in vitro* and *in vivo* (48-50). Previous studies have shown that simvastatin can inhibit the progression of left ventricular hypertrophy and that the farnesyltransferase inhibitor FTI-276 can ameliorate cardiac remodeling in experimental animal models (48-50). However, it has been proven that statins may cause muscle problems, liver dysfunction, renal insufficiency, poor uptake and short persistence in clinical application (51). A study on farnesyltransferase inhibitors is still at the experimental stage and they have not undergone clinical tests (49). At present, there are fewer effective Ras-targeting drugs with few side effects. Therefore, it is worth developing more Ras-targeting drugs to prevent cardiac hypertrophy.

Rho family. The human Rho family consists of 22 genes encoding >20 proteins (52). According to sequence homology, the Rho family includes the Rho, Rac, Cdc42, RhoD, Rnd and RhoH subfamilies (20). Rho is closely linked to the development of cardiovascular disease through promoting endothelial cell barrier dysfunction (53). Rac is involved in various cellular processes, such as cell division and migration (54). Cdc42 has been shown to play an important role in regulating actin dynamics, filopodia extension, cell polarity and migration (55,56). RhoD and Rnd can both mediate actin cytoskeleton dynamics (57,58). RhoH is beneficial for cell survival, migration and invasion (59). It has been reported that the RhoA, Rac1 and Cdc42 proteins of the Rho family play important roles in cardiac hypertrophy (7,60). However, the relationships between other members of the Rho family and cardiac hypertrophy remain to be elucidated.

RhoA can mediate cardiac hypertrophy through the ROCK signaling pathway (Fig. 2) (25,61,62). The inhibition of RhoA can alleviate cardiac hypertrophy both *in vitro* and *in vivo* (63-65). ROCK is a downstream molecule of RhoA and RhoA can participate in regulating cardiac hypertrophy by regulating the ROCK signaling pathway (25,61,62). ROCK is divided into two types, ROCK1 and ROCK2, which play different roles in

Table I. Small GTPases.

First author/s, year	Small GTPases		Downstream effectors	Basic Function	(Refs.)
Tomazini and Shifman, 2023	Ras family	Ras	(JNK, P38, calcineurin, NFAT)	Cell division, migration, adhesion, differentiation and apoptosis	(33)
Li <i>et al</i> , 2020		Rad	CaMK II	Cardiomyocyte calcium signaling	(39)
Kilian <i>et al</i> , 2021	Rho family	RhoA	ROCK	Actin dynamics, signal transduction, gene transcription and cell cycle	(60)
Higuchi <i>et al</i> , 2003		Rac1	(MEK, ERK, JNK, P38, ASK, NADPH oxidase)	Actin dynamics, cell morphology, adhesion and migration	(28)
Liu <i>et al</i> , 2022		Cdc42	JNK, NAFT	Actin dynamics, filopodia extension, cell polarity and migration	(32)
Filipeanu <i>et al</i> , 2008	Rab family	Rab1a	/	Endoplasmic reticulum to Golgi apparatus transport	(99)
Filipeanu <i>et al</i> , 2006		Rab4	/	Endosome to plasma membrane transport	(89)
Etzion <i>et al</i> , 2010		Rab4a	/	Translocation of glucose transporter 4 (GLUT4) and endosomal recycling	(100)

/, no reports; JNK, c-Jun N-terminal kinase; NFAT, nuclear factor of activated T cells; ROCK, RhoA/Rho-associated protein kinase; MEK, mitogen-activated protein kinase kinase; ERK, extracellular signal-regulated kinase; ASK, apoptosis signal-regulated kinase; GLUT4, glucose transporter 4.

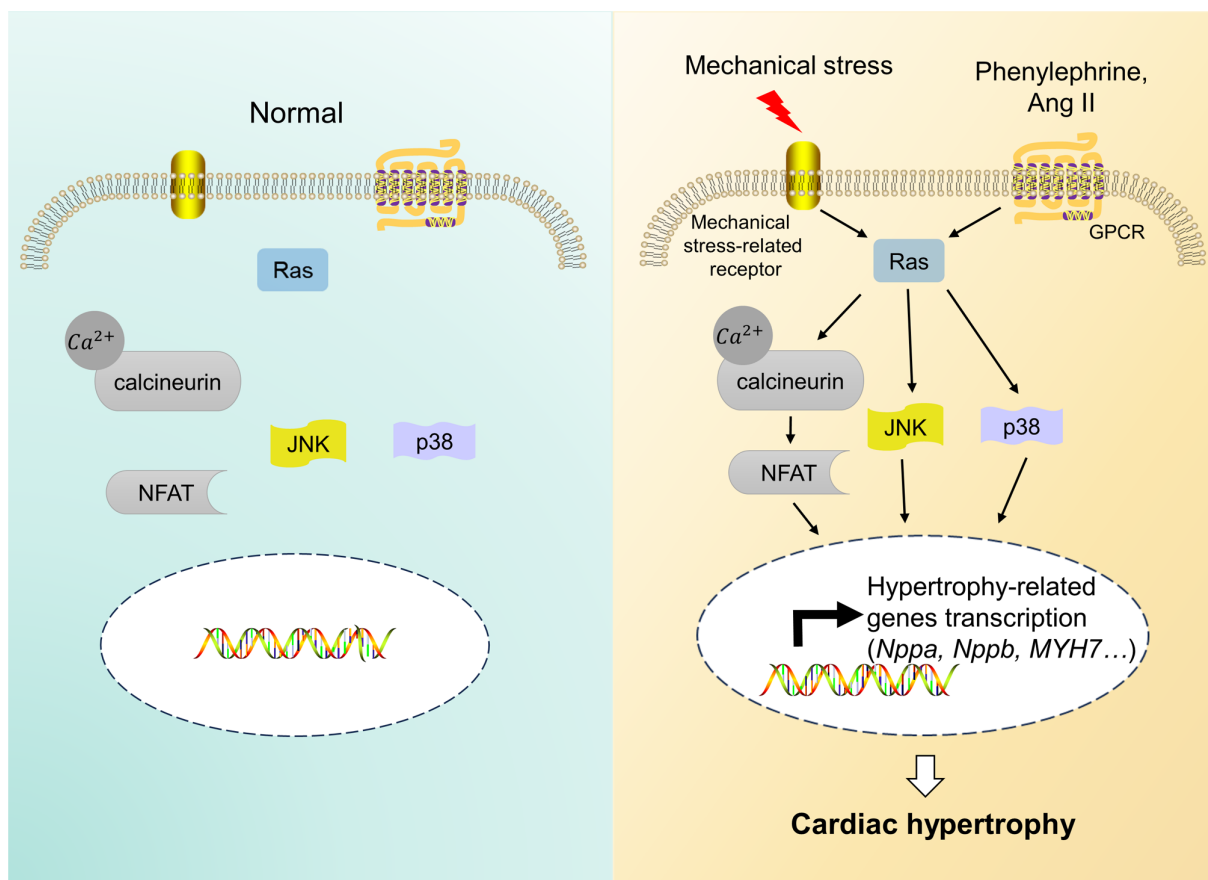


Figure 1. The role of Ras in cardiac hypertrophy. Ras can induce cardiac hypertrophy by activating the Ras/calcineurin/NFAT, Ras/JNK and Ras/p38 MAPK pathways. Phenylephrine, angiotensin II and mechanical stress can induce the Ca^{2+} /calcineurin/NFAT, Ras/JNK and Ras/p38 MAPK signaling pathways by binding to their specific receptors. NFAT, nuclear factor of activated T cells; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; GPCR, G protein-coupled receptor.

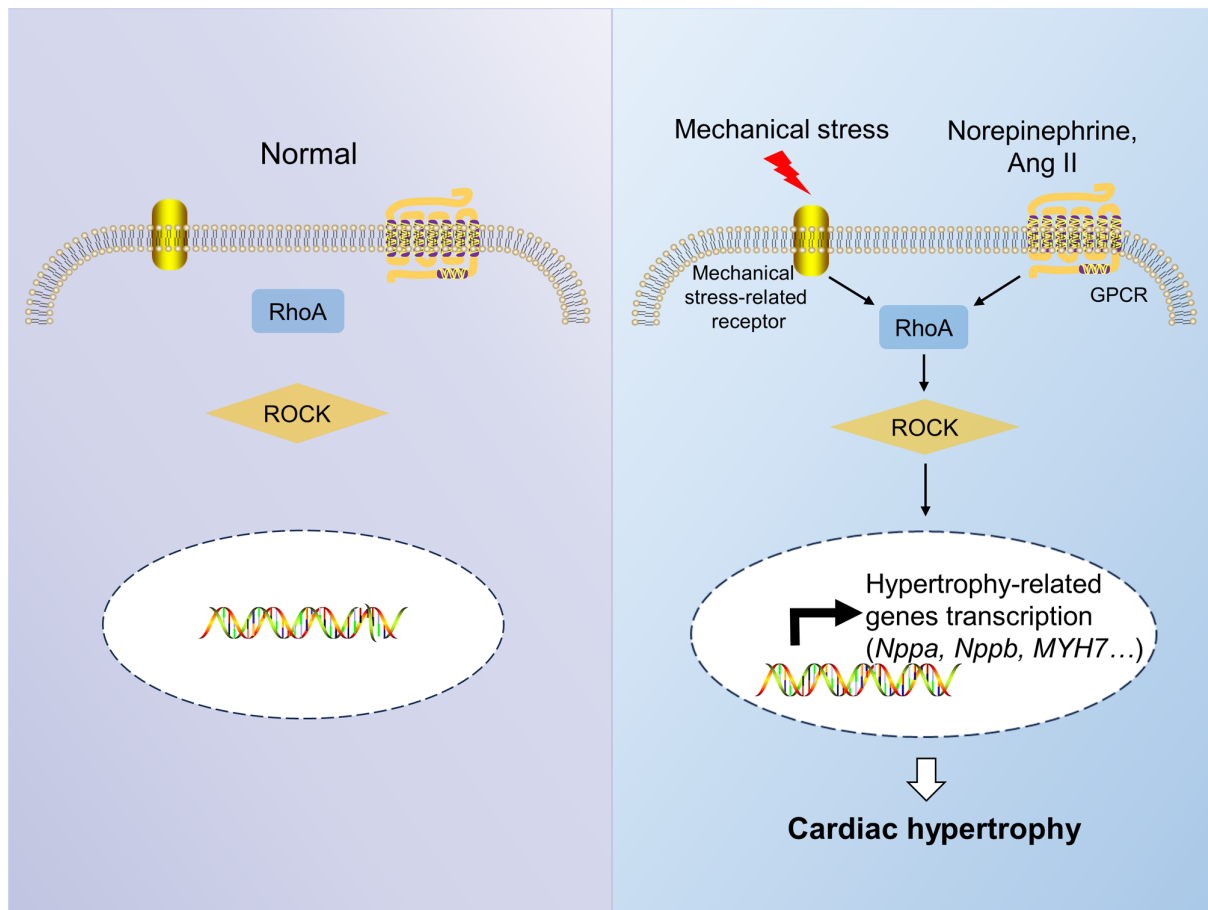


Figure 2. The role of RhoA in cardiac hypertrophy. RhoA can lead to cardiac hypertrophy by activating ROCK. The RhoA/ROCK pathway can be induced by various factors, such as mechanical stress, norepinephrine and angiotensin II. Norepinephrine and angiotensin II activate RhoA by binding to specific GPCRs. Mechanical stress can mediate RhoA by activating mechanical stress-related receptors. ROCK, RhoA/Rho-associated protein kinase; GPCRs, G protein-coupled receptors; Ang, angiotensin.

maintaining normal cardiac structure and function (66). A lack of ROCK1 can inhibit cardiac fibrosis and cell apoptosis (67). In addition to cardiac fibrosis and apoptosis, ROCK2 depletion can also ameliorate cardiac hypertrophy (68,69). In addition, the activity of ROCK2 in pressure overload-induced hypertrophied hearts is upregulated, which contributes to cardiac hypertrophy (70). Therefore, it is hypothesized that RhoA may be involved in cardiac hypertrophy through regulating ROCK2. However, further studies are needed to reveal whether RhoA regulates cardiac hypertrophy through ROCK2 and other downstream signaling molecules.

Rac1 can induce cardiac hypertrophy mainly through the MAPK pathway, the apoptosis signal-regulated kinase 1 (ASK1)/NF- κ B pathway and NADPH oxidase both *in vitro* and *in vivo* (26-30,71,72). To reveal the role of Rac1 in cardiac hypertrophy, an activating mutant (V12 Rac1) and a negative mutant (N17 Rac1) were used in a previous *in vitro* study (71). The overexpression of V12 Rac1 can cause cardiac hypertrophy, while that of N17 Rac1 can inhibit the hypertrophic responses induced by phenylephrine stimulation (71). Previous studies have shown that Rac1 can mediate cardiac hypertrophy by phosphorylating mitogen-activated protein kinase kinase 1/2 (MEK1/2) and extracellular signal-regulated kinase (ERK)1/2 (Fig. 3) both *in vitro* and *in vivo* (26,73). The inhibition of the Rac1/JNK pathway can reduce the reactivation of ANF

and ameliorate cardiomyocyte hypertrophy *in vitro* (27,74). Mechanical stress can induce cardiac hypertrophy through the Rac1/reactive oxygen species (ROS)/p38 MAPK pathway *in vitro* (30). In addition, the overexpression of Rac1 can induce the activation of ASK1 and NF- κ B, which leads to cardiomyocyte hypertrophy *in vitro* (28). ASK1 and NF- κ B play important roles in mediating the hypertrophic response of cardiomyocytes (28). NADPH oxidase activity is also closely linked to cardiac hypertrophy induced by Rac1 both *in vitro* and *in vivo* (29,75). Rac1 can regulate NADPH oxidase activity through interaction with its components, including gp91^{phox} and p67^{phox} and the upregulation of NADPH oxidase activity can lead to the development of cardiac hypertrophy (29).

In contrast to other small GTPases, Cdc42 can antagonize cardiac hypertrophy *in vivo* (31). Cdc42 can reduce calcineurin/NFAT activity by stimulating JNK, which leads to cardiac hypertrophy and prevents the development of heart failure (31). Whether there are other signaling pathways through which Cdc2 antagonizes cardiac hypertrophy remains unclear.

The use of Rho-targeting medicines for treating cardiac hypertrophy has been reported. Statins are powerful cardiac protectants that have been found to inhibit cardiac hypertrophy by blocking Rho isopentenylation as inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase both *in vitro* and *in vivo* (76,77). 1,25-Dihydroxyvitamin D3 (VitD) exerts

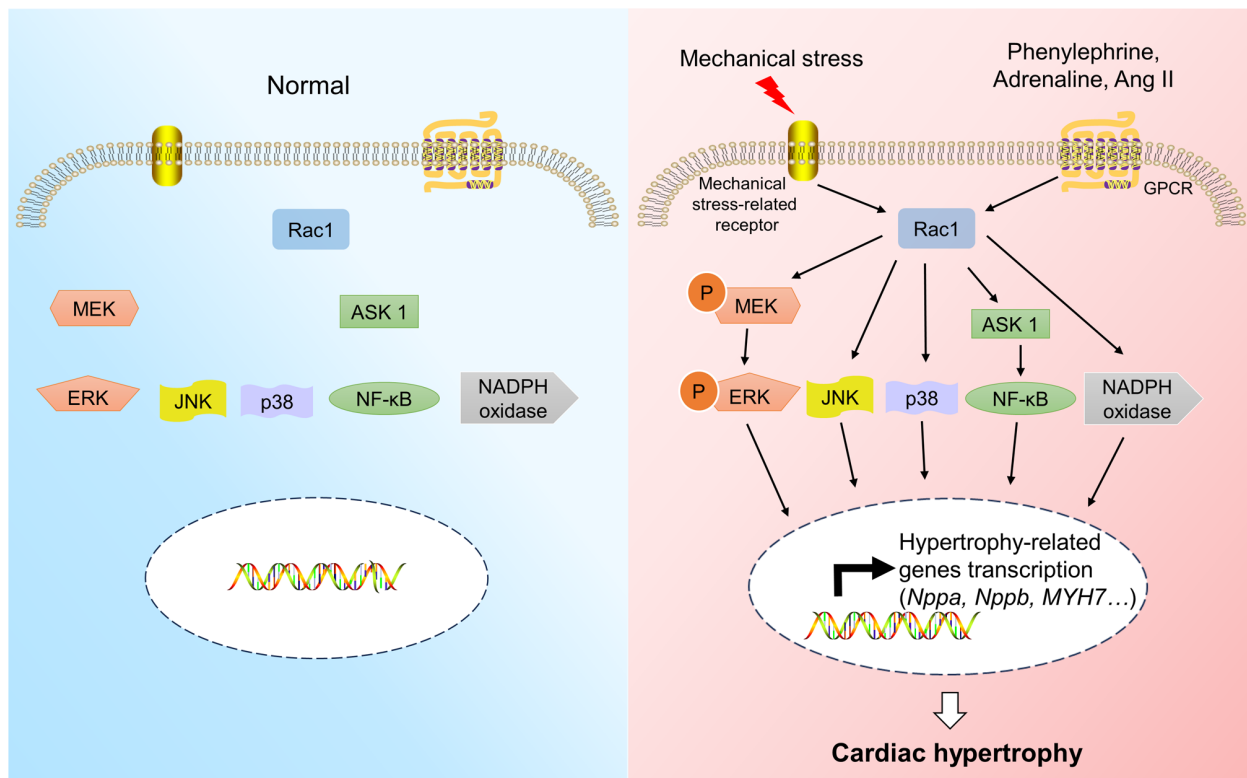


Figure 3. The role of Rac 1 in cardiac hypertrophy. Rac1 is involved in cardiac hypertrophy through the MEK-ERK cascade, the JNK and p38 MAPK pathways, the ASK1/NF- κ B pathway and the regulation of NADPH oxidase activity. Phenylephrine can induce the Rac1/MEK/ERK pathway. The Rac1/p38 MAPK pathway can be induced by mechanical stress. Under adrenaline treatment, the ASK1/NF- κ B pathway can be activated. Angiotensin II can stimulate NADPH oxidase activity and the Rac1/JNK signaling pathway. Phenylephrine, adrenaline and angiotensin II can interact with specific GPCRs, while mechanical stress can activate their corresponding receptors. MEK, mitogen-activated protein kinase kinase; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; GPCR, G protein-coupled receptor; NADPH, nicotinamide adenine dinucleotide phosphate; ASK1, apoptosis signal-regulated kinase 1.

its anti-cardiac hypertrophy effect by downregulating Rac1 *in vivo* (78). Alendronate, a farnesyl pyrophosphate synthase inhibitor, can inhibit angiotensin (Ang) II-induced neonatal cardiomyocyte hypertrophy by inactivating RhoA both *in vitro* and *in vivo* (79). In addition, the RhoA inhibitor *Clostridium botulinum* C3 exozyme and the ROCK inhibitor Y-27632 exhibit anti-cardiac hypertrophy effects *in vitro* (80). Glucagon like peptide 1 may reverse cardiac hypertrophy by inhibiting the RhoA/ROCK2 signaling pathway both *in vitro* and *in vivo* (81). The selective ROCK inhibitor fasudil can treat various cardiovascular diseases, such as cerebral ischemia and pulmonary hypertension, but its effect on cardiac hypertrophy remains unknown (82,83). The Rac1 inhibitor NSC23766 can antagonize cardiac hypertrophy induced by active Rac1 both *in vitro* and *in vivo* (26). There is no Cdc42-targeting medicine for preventing cardiac hypertrophy. The aforementioned Rho-targeting drugs and inhibitors do not undergo clinical tests for treating cardiac hypertrophy and further studies are needed to reveal their detailed roles in ameliorating cardiac hypertrophy.

Rab family. The Rab gene is widely distributed and encodes at least 60 different members of the Rab family in humans (84,85). The Rab family is mainly divided into 10 subfamilies: Rab1, Rab3, Rab4, Rab5, Rab6, Rab8, Rab11, Rab22, Rab27 and Rab40 (85,86). Rab1 is involved in vesicular trafficking from the endoplasmic reticulum to the Golgi apparatus and its dysregulation is associated with the development

of cardiomyopathy (87). Rab3 can promote large dense-core vesicle fusion (88). Rab4 can mediate protein transport from the endosome to the plasma membrane (Table I) (89). Rab5 plays a critical role in the early steps of endocytosis (90). Rab6 can regulate not only anterograde transport pathways from the Golgi apparatus but also retrograde trafficking pathways to the Golgi apparatus (91). Rab8 can mediate anterograde membrane trafficking and plays an important role in cell morphogenesis and cell migration (92). Rab11 can regulate the recycling of endosomal cargo proteins to the plasma membrane and is involved in cell migration (93,94). Rab22 plays a critical role in early endosomal recycling (95). Rab27 can promote exocytosis (96). Rab40 can mediate actin dynamics during cell migration (97). In addition, there are some Rab proteins. The roles of Rab1a, Rab4 and Rab4a in cardiac hypertrophy have been reported (89,98,99). However, the relationship between other Rab proteins and cardiac hypertrophy remains unclear.

In transgenic mice overexpressing Rab1a, the endoplasmic reticulum and Golgi apparatus stack significantly enlarged and secretory cardiac natriuretic peptide granules increased, indicating that overexpression of Rab1a can lead to cardiac hypertrophy both *in vitro* and *in vivo* (98). Rab4 participates in the transport of proteins from early endosomes to the plasma membrane and promotes the cycle and activation of β -adrenergic receptor (β -AR) (89,100). Cardiac-specific overexpression of Rab4 can enhance β -AR signaling and lead to cardiac hypertrophy both *in vitro* and *in vivo* (89). Rab4a

participates in glucose transport and induces β -AR recycling to the plasma membrane (99). Increased myocardial Rab4a expression activates β -adrenergic hypersensitivity and leads to cardiac hypertrophy both *in vitro* and *in vivo* (99). Other proteins in the Rab family also play different roles in the heart, but their relationship with cardiac hypertrophy remains to be further studied.

3. Concluding remarks

Cardiac hypertrophy is an irreversible myocardial injury accompanied by myocardial dysfunction and fibrosis due to long-term pathological overload, eventually leading to heart failure (4). As a molecular switch in cell, small GTPases play an important role in the development of cardiac hypertrophy (7,8). The roles of Ras, Rad, RhoA, Rac1, Cdc42, Rab1a, Rab4 and Rab4a in cardiac hypertrophy have been reported (14,31,40,65,71,89,98,99). The roles of Ras, Rad, RhoA, Rac1 and Cdc42 in cardiac hypertrophy are relatively clear, but further studies are needed (14,25,26,31,40). By comparison, the molecular mechanisms of Rab1a, Rab4 and Rab4a in cardiac hypertrophy remain elusive (89,98,99). However, the associations between other small GTPases, including Arf and Ran and cardiac hypertrophy remain unclear. Further studies are needed.

Previous studies have shown that regulators of G-protein signaling can suppress small GTPases by inactivating the heterotrimeric G-proteins of G protein-coupled receptors (GPCRs), which act as GTPase-activating proteins (101-103). In addition, it has been reported that RGS2 and RGS4 can ameliorate cardiac hypertrophy by suppressing hypertrophy-related signaling initiated by GPCRs in response to upstream agonists such as angiotensin II and phenylephrine in myocardial cells (102,104-106). However, direct evidence showing that RGS can exert protective effects on cardiac hypertrophy through mediating small GTPases is lacking. This topic is worthy of further study.

Currently, statins are used in clinical treatment as powerful cardioprotectants because they inhibit Ras and RhoA (48). It has been reported that Rac1-targeting VitD, RhoA-targeting alendronate and inhibitors of Ras, RhoA and Rac1 can exhibit antihypertrophic effects in the experimental stage, but these agents have not been tested clinically for treating cardiac hypertrophy (26,49,78,79,83). There are no reports on the use of Rad, Cdc42 and Rab-targeting medicines for treating cardiac hypertrophy. Further studies on the roles of small GTPases in cardiac hypertrophy and the development of small GTPase-targeting medicines for treating cardiac hypertrophy are needed. The present study is beneficial for further studies on the molecular mechanisms of cardiac hypertrophy and provided more references for the development of prospective therapeutic targets for cardiac hypertrophy and heart failure.

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Authors' contributions

XW, XN and HW edited the manuscript. ZR conceived, edited and finalized the manuscript. Data authentication is not applicable. All authors read and approved the final manuscript.

Ethics approval and consent to participate

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Patient consent for publication

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Competing interests

The authors declare that they have no competing interests.

Authors' information

ORCID: Zhanhong Ren, <https://orcid.org/0000-0002-3582-008X>.

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