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Review Article

# Emerging Molecular Pathways Involved in Pathophysiology of Cardiac Remodeling

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Abstract

Mammalian heart is a dynamic organ that can adapt morphological changes in response to alteration in workload. Various clinical and experimental findings revealed that in response to physiological stimuli or pathological insults, the heart undergoes cardiac remodeling which can be characterized by molecular, cellular or interstitial changes and can be manifested clinically as changes in size, shape and pumping function of the heart. A Sound understanding of changes in hearts cellular and molecular components and to mediators that derive homeostatic control is necessary before a specific intervention is pursued. Summarized data of this review comprises role of various novel emerging molecular pathways involvement in the pathophysiology of cardiac remodeling.

**Keywords:** Cardiac remodeling, hypertrophy, protein kinase, heart failure, molecular targets, pathological insult.

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## INTRODUCTION

Cardiac remodeling is defined as genomic manifestation resulting in molecular, cellular and interstitial changes, i.e. changes in size, shape and function of the heart in response to cardiac load or injury. Which further leads to changes in cellular function, activation of specific neurohormones and peptides leading to ventricular remodeling and finally progression to heart failure. Cardiac remodeling is mainly influenced by hemodynamic load, neurohormones activation and other molecular pathways still under investigation [1]. Cardiomyocytes, a basic unit of heart are the major cells involved in the remodeling process alongside interstitial fibroblasts, collagen and coronary vasculature are also involved in the remodeling. These all mediator leads to cardiac remodeling in response to various stimuli's including ischemia, cell necrosis and apoptosis. Although compensatory remodeling does not necessarily always define as pathological, as it may also be physiological that follows intensive exertion and workload. Compensatory pathological cardiac remodeling in various diseases viz. Diabetes, obesity, myocardial infarction and hypertension is one of the major causes of heart failure. Various cellular and molecular pathways linked to cardiac remodeling may provide potential targets for pharmacological intervention [2].

## MOLECULAR PATHWAYS IN PATHOPHYSIOLOGY OF CARDIAC REMODELING

### 1) TWEAK-Fn14 cytokine

Recent observations demonstrate an essential role of inflammatory cytokines in the progression of heart failure, cardiac hypertrophy and apoptosis. Recently, other members of the TNF/TNF receptor super family (TNFSF/TNFRSF) have been implicated in the pathophysiology of heart failure. TWEAK, the member of the TNFS Family was discovered in 1997 and is primarily synthesized as a type II transmembrane receptor that is further processed by a furin endoprotease into the soluble cytokine sTWEAK and can exist in both forms in cell [2]. Fn14 is a type I transmembrane protein expressed on a broad variety of different cell types and Fn14 trimerizes upon ligand binding recruiting subsequently adapter proteins of the tumor necrosis factor receptor-associated factor (TRAF) family to its cytoplasmic domain. Several members of the TRAF family (TRAF1, TRAF2, TRAF3, and TRAF5) have been shown to bind Fn14 [3]. Fn14 expression is correlated with Cardiomyocytes proliferation during heart development. In neonatal rat Cardiomyocytes, expressing Fn14 endogenously, TWEAK stimulation was found to induce cardiomyocytes proliferation [4]. TWEAK activates extracellular signal-regulated kinase (ERK), phosphatidylinositol 3-kinase (PI3K) and inhibits glycogen synthase kinase-3beta (GSK-3beta) but don't have such effect on p38 mitogen-activated kinase (p38) signaling. TWEAK had a negligible effect on adult cardiomyocytes proliferation, probably due to down regulation of Fn14 but may be a potent inducer of cardiomyocytes cell cycle as adult zebra fish and newborn mice can regenerate their heart through cardiomyocytes proliferation. Further FasL (TNFSF6) and TWEAK/Fn14 have been reported to cause cardiac hypertrophy with pro-

inflammatory consequences. It has been shown that various hypertrophic agonists [Angiotensin II (Ang II), Phenylephrine (PE), and Endothelin-1 (ET-1)] induces Fn14 expression [5]. Moreover, TRAF2 and TRAF5 are possible downstream targets of TWEAK/Fn14 signaling, have been implicated in cardiac hypertrophy. Cardiomyocyte-specific TRAF2 transgenic mice developed a time-dependent increase in cardiac hypertrophy, left ventricular (LV) dilation, and adverse LV remodeling, and a significant decrease in heart function. TRAF5 deficiency in transverse aortic constriction (TAC) animal model aggravated cardiac hypertrophy and cardiac dysfunction [6]. TWEAK/Fn14 axis also plays an important role in extracellular matrix modulation and reported to develops DCM (dilated cardiomyopathy). Collagen expression induced by TWEAK/Fn14 signaling was mediated via RhoA-dependent nuclear translocation of the myocardin-related transcription factor-A (MRTF-A) and up regulation of Fn14 expression in cardiomyocytes by stretch or stimulation with AngII or norepinephrine mediated by RhoA/ROCK signaling. TWEAK is also reported to activate NF-kB signaling and further causing proliferation and collagen synthesis in cardiac fibroblasts. It has been demonstrated that TNF-alpha induced cardiac remodeling and dysfunction depends on MMP activation. Both TNF-alpha and FasL over expression is associated with increased levels of TGF-beta1, which is also an inducer of myocardial fibrosis [7]. In concluded data TWEAK/Fn14 appears to be a naturalistic approach for future studies and would be beneficial in pharmacological interventions of heart disease [8]. Overall effect of TWEAK-Fn14 cytokine in cardiac remodeling is summarized in *Figure 1*.

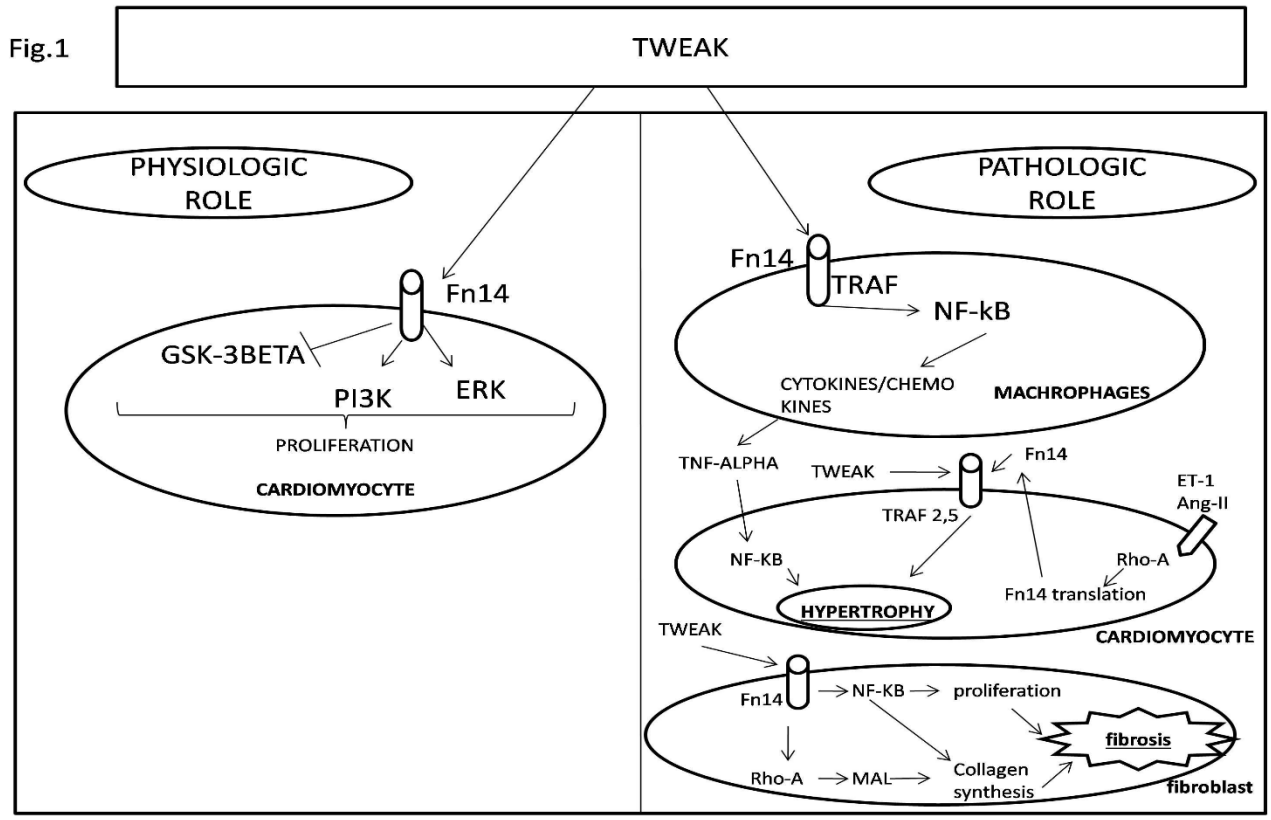


**Figure 1 Role of TWEAK/Fn14 in pathophysiology of cardiac remodeling**

TWEAK/Fn14 (tumor necrosis factor-like weak inducer of apoptosis) acts on cardiomyocyte and inhibits GSK-3 beta (glycogen synthase kinase) and activates Pi3k (Phosphoinositide 3-kinase) and ERK (Extracellular signal-regulated kinases) has physiology and pathology and leads to physiological development of heart. Where as in pathological condition TWEAK/Fn14 acts upon TRAF (TNF receptor associated factors) and activates NFkB (Nuclear factor kB) and leads to release of cytokines/chemokines from macrophages and other inflammatory cells. These cytokines [TNF-alpha (tumor necrosis factor)] further acts on cardio myocyte and cause hypertrophy by NFkB. TWEAK/Fn14 acts upon TRAF2/5 on cardiomyocyte and further leads to hypertrophy. In cardiomyocyte translation of Fn14 is also conducted by receptor ET1 (endothelin receptor 1) and ANGII (angiotensin II receptor 1) through RhoA pathway. In fibroblast TWEAK/Fn14 promote NFkB and RhoA/MAL (Ras homolog A) pathways, which contributes to collagen synthesis and fibroblast proliferation and ultimately fibrosis and cardiac remodeling.

**2) Nuclear factor-erythroid-2- (NF-E2-) related factor 2 (Nrf2)-Mediated Pathway**

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) both contributes to biological oxidative stress in Cardiomyocytes which ultimately leads to heart failure. Numerous cardiovascular diseases including HF, coronary heart disease, and cardiac arrhythmias was found to be triggered by biological oxidative stress [9]. Beside it superoxide anion like ($\cdot O^{2-}$), hydrogen peroxide ($H_2O_2$), hydroxyl radical ($\cdot OH$), and hypochlorite ($OCl\cdot-$), was reported in cardiac dysfunction through their direct toxic effect, resulting in increased necrosis and apoptosis of cardiomyocytes. Transcription factor, nuclear factor-erythroid-2- (NF-E2-) related factor 2 (Nrf2) is highly conserved major regulator of the antioxidant response elements AREs (Adenylate-uridylate-rich elements). Down regulation of Nrf2-regulated enzymes have been recognized to be essential in the pathogenesis of heart Failure. Nrf2/ARE signaling pathway plays an significant role in

preventing oxidative cardiac cell injury in various maladaptive remodeling and cardiac dysfunction [10]. Nrf2, a member of the cap-n-collar (CNC) family of transcription factors, which include Nrf1-3 and Bach1-2 is the master regulator of the oxidative stress signaling. Normally Nrf2 is preserved in the cytoplasm by Kelch-like-ECH-associated protein1 (Keap1) and Cullin 3. Nrf2 is ubiquitinated (Attachment of ubiquitin protein to a substrate protein) by Cullin 3 and this ubiquitination of Nrf2 is facilitated by Keap1 (a substrate adaptor). Conversely, Nrf2 has a short half-life that lasts only for 20 min under normal conditions [11, 12]. Oxidative stress destroys critical cysteine residues in Keap1 which in turn destroys Keap1-Cul3 ubiquitination system and translocated Nrf2 into the nucleus. Nrf2 than combines with a small protein called Maf to form a heterodimer, and this heterodimer complex binds to ARE in the upstream promoter region leading to formation of number of antioxidant enzymes including heme oxygenase-1 (HO-1), NAD(P)H dehydrogenase (quinone 1) (NQO1), superoxide dismutases (SODs), catalase (CAT), glutathione-S-transferase (GST), $\gamma$-glutamyl cysteine synthase ($\gamma$-GCS), and glutathione peroxidase (GPx) by the process of gene translation including as summarized in *figure 2* [11]. These all enzymes are shown to prevent oxidative stress by neutralizing free radicals [9]. Nrf2 and its target genes provide a novel mechanism to protect oxidative stress induced apoptosis and necrosis during pathological cardiac remodeling. Nrf2 expression was also shown to increase transiently and then decline to basal level after transverse aortic constriction (TAC). Over expression of Nrf2 dramatically inhibited hypertrophic factor induced ROS production and growth in cardiomyocytes and cardiac fibroblasts [10].

Furthermore, some antioxidants have also been reported to protect the heart from ischemia induced cardiac injury through the Nrf2 pathway. ά- lipoic acid and prostaglandin $D_2$. Significant increase in Nrf2 nuclear translocation and the expression of its downstream genes reduced lactate dehydrogenase (LDH) and creatine kinase (CK) release, attenuated myocardial infarct size, decreased cardiomyocytes apoptosis and partially preserve heart function. Nrf2 and its target genes were also reported to be useful for protection against cardiotoxicity of anticancer drugs [13]. Hence from the existing data Nrf2 with its target genes was found to play essential role in maintaining cellular redox homeostasis by attenuating oxidative stress-associated pathological processes [14] (Figure 2).

Fig. 2

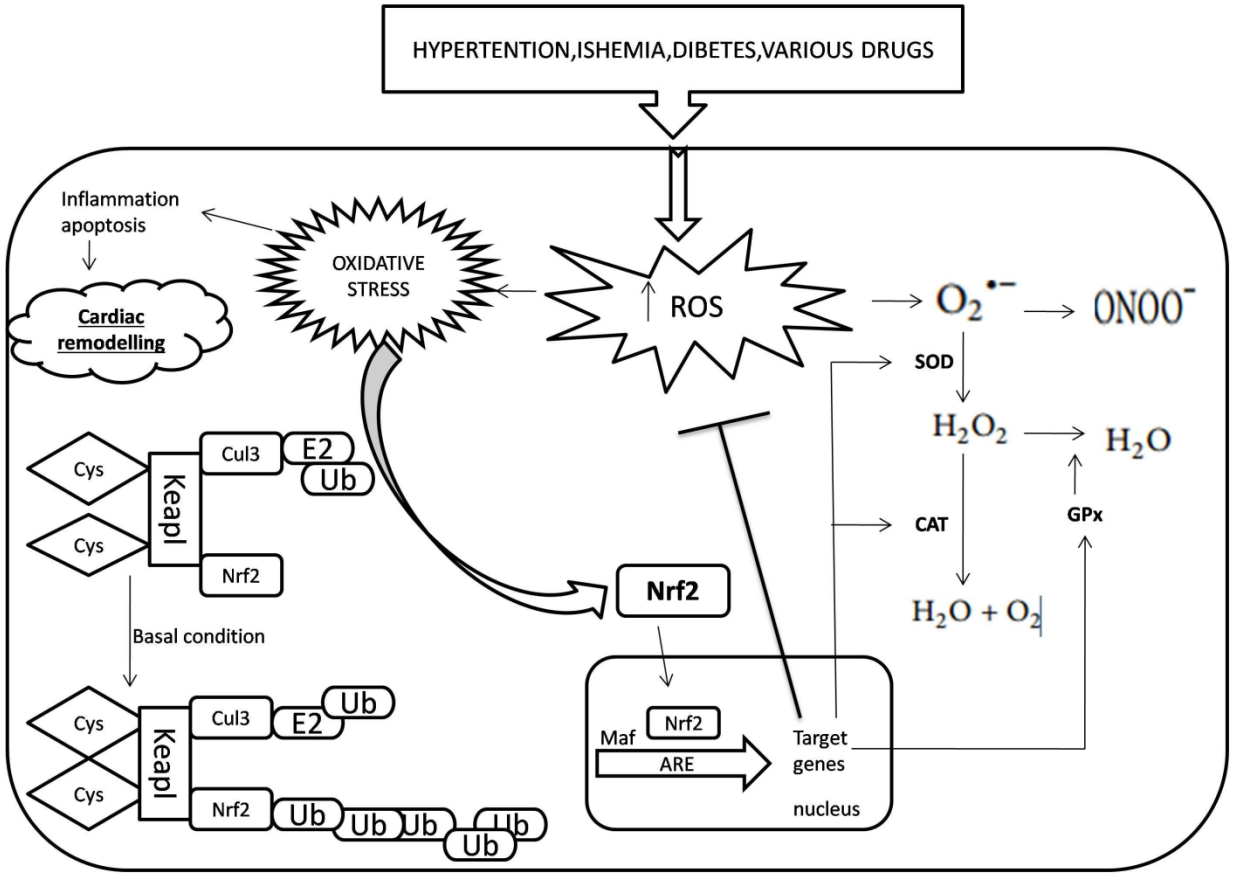


**Figure 2 Role of NRF2 in cardiac remodeling**

Pathological condition like hypertension and ischemia leads to increase in generation of ROS, which contribute to oxidative stress causing inflammation and apoptosis in heart and cause cardiac remodeling. NRf2 (nuclear factor erythroid-2) also has been found to be activated by ROS which interact with cysteine residues in Keap1, disrupting the Keap1-Cu13 system and activates Nrf2. which further acts along with MAF protein and ARE promoting antioxidant generation. Including SOD (superoxide dismutase), CAT (catalase) and GPx and abolishes the effects of ROS.

### 3) Stress-Activated MAP Kinases (SAPKs) mediated pathway

SAPKs are the subset of the MAP kinase family that comprises members of c-Jun N Terminal Kinases (JNKs) and p38 Kinases. SAPKs consist of mainly two parallel kinase cascades; each includes upstream MAP kinase kinase kinases (e.g., MEKK1, TAK-1, and ASK-1), downstream MAP kinase kinases (including MKK4, MKK7, MKK3, and MKK6) and corresponding MAP kinases (JNKs and p38s). The MEKK1-MKK4/ MKK7 cascade can specifically activate JNK, whereas TAK-1-MKK3/MKK6 leads to stimulation of p38 [15] . ASK-1/MAPKKK5 is able to activate both JNK and p38 pathways via direct phosphorylation of MKK4/7 and MKK3/6. In addition, SAPK activation has been observed to be a part of essential cellular signaling events mediated through adrenergic receptors and tyrosine kinase receptors. Alongside the targeted transgene (MKK3bE or MKK6bE) expression induced p38 activation in double transgenic hearts and lethal cardiac abnormality. p38 activity was found to inhibits calcineurin- induced nuclear translocation of nuclear factor of activated T-cells which is the possible molecular mechanism for the inhibitory function of p38 in cardiac hypertrophy. However, p38 can still play an integral role in apoptotic signaling in heart by modulating pro-apoptotic proteins such as p53 [16]. p38 activation also leads to induction of fetal gene expression profile, loss of contractility, and induced interstitial fibrosis, which contribute to a restrictive type of cardiomyopathy [17] . Further studies demonstrated that p38 mediates a negative inotropic effect without affecting intracellular calcium homeostasis. p38 has been associated in the cardiac metabolic stress response by regulating the transcriptional activity of peroxisome proliferators-activated receptor alpha [18]. Transient activation of SAPKs at early stages may function as protective/modulatory pathways, whereas prolonged activation may contribute to aggravate pathologic changes in the heart which may finally contributes to cardiac remodeling. Stress-Activated MAP Kinases (SAPKs) mediated pathway is merged in *Figure 3*.

Fig. 3

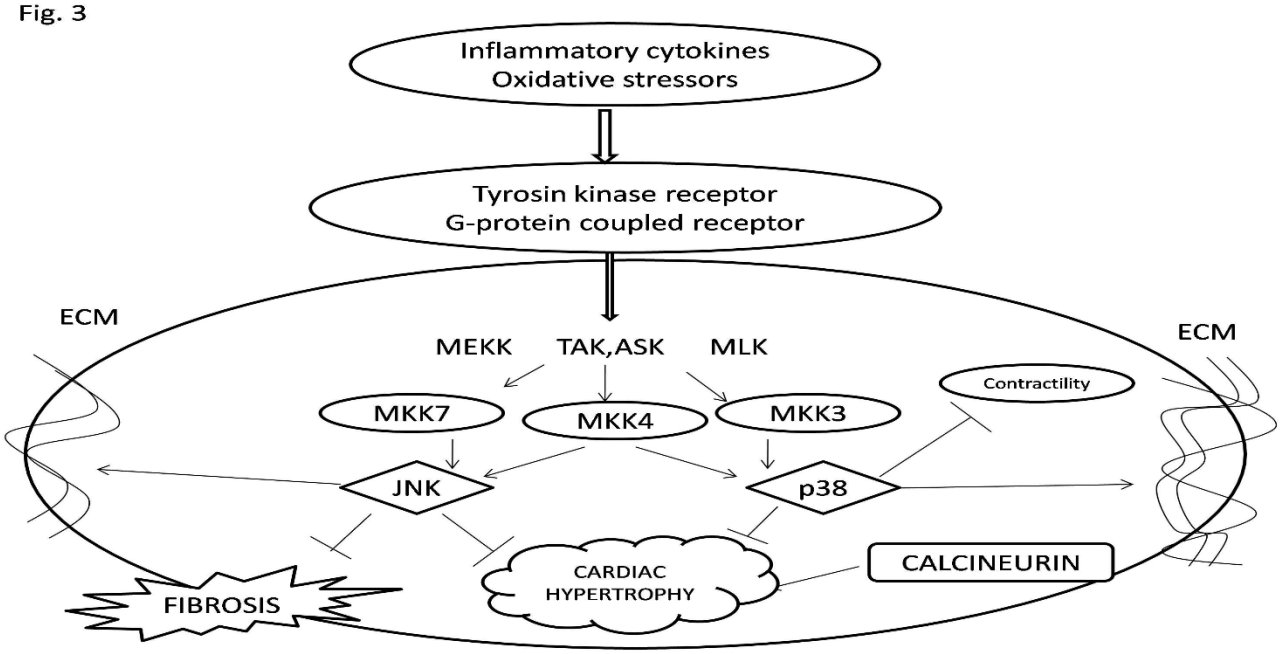


**Figure 3 Role of Stress-Activated MAP Kinases (SAPKs) in cardiac remodeling**

In pathological conditions inflammatory cytokines and oxidative stress leads to the activation of various stress receptors on myocyte contributing to the activation of various stress kinases –MEKK (MAP kinase kinase kinase),TAK ( Tat-associated kinase) ASK (**Apoptosis signal-regulating kinase** )and MLK (**Mixed-lineage kinase**).These all involved in the activation of JNK (**c-Jun N-terminal kinases** )and p38.JNK inhibits fibrosis and cardiac hypertrophy and promotes ECM deposition. On the other hand, p38 inhibits contractility and cardiac hypertrophy and involved in ECM deposition causing restrictive cardiomyopathy without hypertrophy and apoptosis.

### 4) Ghrelin / GHSR-1a mediated pathway.

Ghrelin, a 28 amino acid containing peptide firstly found in rat and human stomach and was identified to be a strong stimulator of growth hormone (GH). Ghrelin and its various receptors were found to be ubiquitous in numerous organ and tissue and was demonstrated to have role in regulation of cardiovascular system [19]. Ghrelin was also reported to inhibit cardiac remodeling through an anti-inflammatory, anti- apoptotic and inhibitory effect on myocardial fibrosis. Beyond growth hormone receptors, type 1a (GHSR-1a) has been found to be distributed in vascular endothelium, myocardium and monocytes. Ghrelin transduce cellular signal by GHSR-1a receptor. Cultured cardiomyocytes are reported to produce Ghrelin indicating autocrine function of ghrelin in cardiac muscle [20]. Ghrelin induces GH release by enhancing activity of phospholipase C, protein kinase C and intracellular calcium mobilization by acting on GHSR-1a receptor in somatotroph cells. Ghrelin has been publicized to act by several signaling mechanisms involving both classical G-protein effectors and G-protein-independent pathways. Beside ERK1/2 and Akt/protein kinase B signaling, β-arrestins and PPAR is also found to be involved in ghrelin-activated signaling networks [21]. Ghrelin was also reported to promotes nitric oxide (NO) synthesis by GHSR-1a, PI3K/Akt and endothelial NO synthase (eNOS) pathways in aortic endothelial cells [22]. This action of ghrelin is dependent on a ghrelin receptor/Gq protein/calcium dependent pathway,

leading to stimulation of AMPK and Akt activation. GHRH (growth hormone releasing hormone) agonist GHRH-A was also shown to reverse cardiac remodeling after myocardial infraction without involving the GH/IGF-1 axis, as the circulating levels of these hormones were not increased by GHRH-A treatment. Ghrelin expression was decreased in the atrium and ventricles of the hearts of patients with CHF, whereas GHSR-1a expression was increased [23], these finding suggest significant role of maladaptive processes (Reduction in Ghrelin level) and compensatory mechanism (Increase in GHSR-1a receptor level) in process of cardiac remodeling [24].

**5) Protein kinase G mediated pathway**

cGMP-dependent protein kinase or Protein Kinase G (PKG) is a serine/threonine-specific protein kinase that is stimulated by cGMP and phosphorylate a number of biologically important compounds implicated in the regulation of metabolism, cell division and nucleic acid synthesis. PKG type I (PKG-I) and type II (PKG-II), have been identified in mammals. Mainly PKG-I isoform has been detected in cardiac myocytes, Most of which are found cardio protective during cardiac hypertrophy [25]. Recent studies reveal that genetic deletion of particulate guanylcyclase (pGC) action in cardiac myocyte (CM) worsens pathological remodeling and cause functional deterioration whereas knock-in of pGC normalized the condition after pressure overload [26]. PKG prompts protective effect in cardiac remodeling by activating regulator of G-protein signaling (RGS)-2/4 and protein phosphatase I (PPI). Alongside protein Kinase-G inhibits TRP6 (transient receptor potential channel) and L-type Ca2 + channel (LTCC) activity. Thereby reducing Calcium-induced interacellular Calcium release and phosphorylation of troponin I [27]. Soluble guanylcyclase (sGK) was also reported to inhibit hypertrophy, apoptosis and reduces the peregrine actions of connective tissue growth factor (CTGF) on fibroblasts which ultimately decreases fibrosis of cardiomyocytes. In addition, cGKI in endothelial cells acts downstream of natriuretic peptide receptor type A (NPRA) to promote angiogenesis, and in fibroblasts cGKI has been shown to inhibit profibrotic pathways, which would be additive to the reduction of paracrine CTGF production [28]. Further cGKI expressed in cardiac progenitors may promote differentiation of cardiac myocytes thereby enhancing regeneration as previously cGKI was also shown to mediate cardiac myocyte survival [29], [30]. (Figure 4).

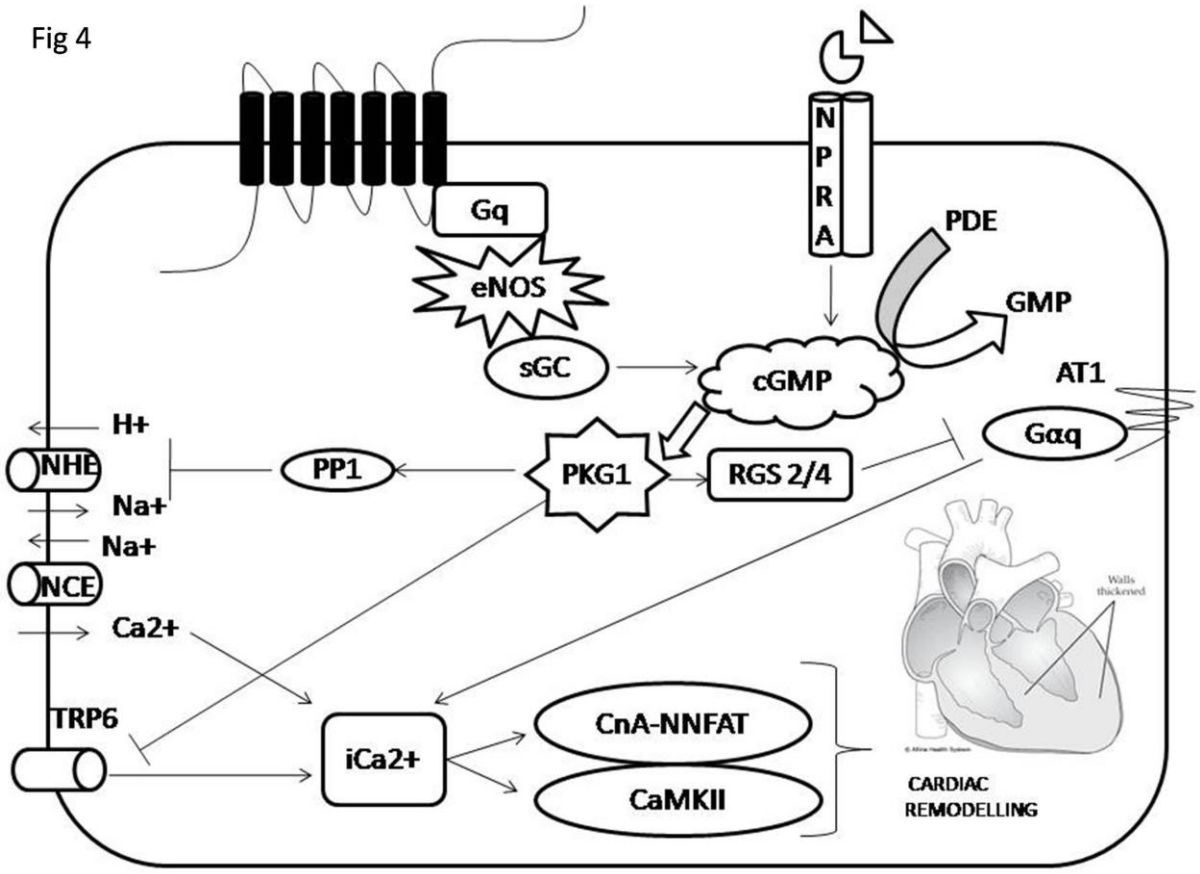


**Figure 4 Role of Protein Kinase G in cardiac remodeling**

Protein kinase G1 (PKG1) activated by cGMP (Cyclic guanosine monophosphate) which is activated by eNOS (endothelial nitric oxide synthase) activated sGC (soluble guanylyl cyclase) and NPRA (natriuretic peptide receptor A). Upon activation PKG1 stimulate PP1(protein phosphatase 1) which further inhibits NHE (sod. Hydrogen exchange Pump) eventually leads to decrease in NCE (Sod. Calcium exchange) and also inhibits TRP6 (Transient receptor protein) preventing release of $iCa^{2+}$ which is activating can-NNFAT (calcineurin-NFAT) and CaMKII ($Ca^{2+}$/calmodulin (CaM)-dependent protein kinase II) and leads to cardiac remodeling. PKG1 also stimulate RGS2/4 (Regulator of G protein signaling) which inhibits action of AT1 (angiotensin II receptor 1) which also attenuates levels of iCa2+ and prevents cardiac remodeling.

**6) PPAR (Peroxisome Proliferator-Activated Receptors) mediated pathway**

PPARs are type of nuclear receptors that heterodimerize with the retinoid X receptor. These receptors function as transcription factor and modulate functions of many target genes related to lipid and glucose metabolism [31] . PPARs have been shown to be involved in the regulation of cell growth, migration, oxidative stress and inflammation in the cardiovascular system [32]. PPARs are of three type PPARs-α, PPARs-β/δ and PPARs-γ out of which PPAR-α and PPAR-γ are mainly involved in functioning of cardiovascular system which may be activated by different synthetic agonists. Both are present in variable amounts in cardiovascular tissues including endothelium, smooth muscle cells, macrophages, and Cardiomyocytes [33]. The activators of PPAR-α (fibrates) and PPAR-γ (thiazolidinediones or glitazones), by exerting antioxidant and anti-inflammatory effects attenuate the actions of angiotensin II in cardiovascular system. However recently PPAR- β/δ was also reported to be involved in functioning of cardiovascular system. [34]. PPAR-α controls cardiac energy, lipid metabolism and play important role in mitochondrial fatty acid β-oxidation, which is the process for fuel generation for heart. Pathological inhibition of PPAR-α have been documented to reduce the capacity of hypertrophied myocytes to metabolize myocardial lipids which results in intracellular fat accumulation in heart [35]. Beside it PPAR-α activation inhibits cardiac expression of TNF-α, NF-kB, prepro-ET-1 mRNA, collagen type I and type III mRNA and shown to increase expression of anti-inflammatory cytokine (IL-10) associated with decreased interstitial and perivascular cardiac fibrosis [36]. PPARs activation has been demonstrated to decrease the expression of VCAM-1, platelet, endothelial cell adhesion molecule, ICAM-1, ED-1 (macrophage antigen) and AT1 receptors, where as it upreguates the expression of AT2 receptors, which inhibits the hypertrophic and fibrotic action of AT1 receptor [37].

PPAR-γ isoforms has been reported to inhibit hypertrophy and brain natriuretic peptide expression in cultured cardiomyocytes and shown to inhibit cardiac hypertrophy in experimental aortic banding. However, In some cases PPAR-γ activators may trigger an aggravation of congestive heart failure [38], that appears mainly due to fluid retention as a consequence of their insulinomimetic action on the kidney rather than a negative inotropic effect. These agents may have cardiovascular protective properties in less advanced cardiovascular disease. In hypertrophic heart there is an increase in glucose utilization and decrease in fatty acid oxidation. PPAR-γ has unclear role in fatty acid metabolism but has overlapping ligand binding profile with PPAR-α. Hence it could attenuate cardiac remodeling via metabolic mechanisms secondary to reduction in lipid and glucose level. Further PPAR- γ has been reported to have anti-inflammatory and anti-hypertrophic effects in cardiac remodeling by its inhibitory action on Nuclear factor κβ (NFκβ) and AP-1. So from the collected data it can be concluded that PPAR may be the potential target involved in cardiac remodeling [39].

**7) Protein Kinase C (PKC) mediated pathway**

Protein kinase C (PKC) is a group of closely related serine-threonine protein kinases that phosphorylates serine–threonine groups on proteins. Protein kinases C are widely distributed and are distinguished into three classes viz.

A) The classical PKCs (ά, βI, βII and γ), which require diacylglycerol (DAG)-, and calcium for activity

B) The novel PKCs (δ, ε, θ and η), which require DAG, but didn't require calcium for activity

C) The atypical PKCs (ζ, λ) which are not stimulated by DAG or calcium, but are stimulated by other lipid-derived second messengers.

PKC isozymes are expressed in all tissues and mRNA expression of ά, δ, ε, η and atypical PKCs is mainly found in cardiomyocytes. Both βI and βII `PKC are abundantly expressed in cardiomyocytes [40]. PKC isozymes have been documented to be involved in a variety of chronic cardiac diseases that contributes to heart failure. The expression and activity of άPKC are unaltered in early HF but were up-regulated in end-stage of HF. άPKC is mainly involved in calcium homeostasis and myocardial contractility. βPKC levels are reported to increase in hypertension-induced HF and inhibition of βIIPKC reduces cardiomyocytes size, thereby indicating antihypertropic action of βIIPKC [41]. Targeted over-expression of βIIPKC in mice resulted in cardiac hypertrophy with myocardial dysfunction.

Transgenic inhibition of εPKC leads to eccentric cardiomyopathy whereas transgenic activation of εPKC exhibit normal cardiac function, increase in cardiac muscle mass, and hyperplasia of cardiomyocytes. Beside it sustain activation of εPKC increases cardiac fibrosis and its inhibition decreases TNF-α production in macrophages, myocyte hypertrophy, fibrosis, and inflammation and prolongs survival [42]. Furthermore, δ PKC and ζ PKC are reported to be involved in TGFβ-induced fibroblast proliferation, however δ PKC-selective peptide inhibitor shows an opposite effect to that of the ζPKC inhibitor; it increases TGF-β1-induced fibroblast proliferation. PKC also regulates the levels and activity of matrix metalloproteinases (MMP), degrades ECM and facilitate the motility of cardiac fibroblast, specifically ά and βI PKC increases the activity of MMP-9 through the JNK-dependent pathway. Other PKCs, such as θ and ζPKC, increase both MMP-2 and MMP-9 via ERK and NFκβ pathways in cardiac fibroblasts [43]. Classical PKC (ά, β) are also reported to be involved in cardiac fibrogenesis. εPKC and δPKC activate mTOR, S6 kinase, TGFβ, TAK1 and activating transcription factor (ATF-2) and subsequently lead to cardiomyocyte hypertrophy [44]. Further PKC's are also reported to be involved in nuclear export of histone deacetylase 5 (HDAC5, a protein regulating myogenesis) in response to hypertrophic agonists hence causing hypertrophy.

Over-expression of dominant negative ά and δ PKC also inhibits phosphorylation of various kinases including MEK1/ERK1/2, MEK kinase, MKK4, and Jun kinase, however β IIPKC mediate phosphorylation of troponin I and decreases myofilament calcium responsiveness, thus causes cardiomyocyte dysfunction. Further εPKC is also documented to regulate β1-integrin complex formation with ECM and further participates in the fibrotic events that leads to cardiac remodeling [45]. Thus various PKC isoforms ubiquitously regulates the events of cardiac remodeling by acting as mediator for various pathways and may act as potent target for attenuating cardiac remodeling as shown in *Figure 5*. [46]

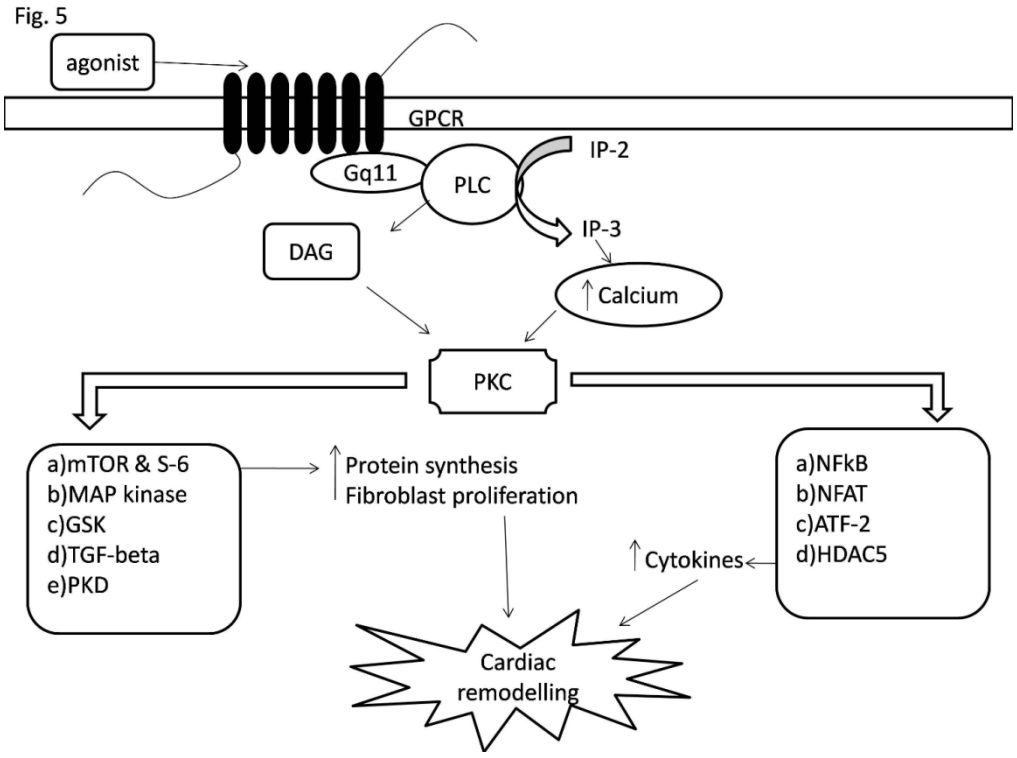


**Figure 5 Role of PKC in cardiac remodelling**

PKC (protein kinase c ) activated by G-protein coupled receptor linked PLC (phospholipase c) which activates DAG (Diacylglycerol) and converts IP2 to IP3(inositol 1,4,5-trisphosphate) leading to increase in calcium levels and upon activation protein kinase C increases protein synthesis and fibroblast proliferation by activating mTOR (mammalian target of rapamycin), protein S6 kinase, mitogen activated protein kinases (MAP), GSK(Glycogen synthase kinase), TGF-beta(Transforming growth factor) , PKD(protein kinase D). And beside it PKC also leads to increase in cytokine production by acting on NFkB (nuclear factor kappa-light-chain-enhancer of activated B cells), NFAT (Nuclear factor of activated T-cells), ATF-2(Activating transcription factor 2) and HDAC5 (histone deacetylase 5) leading to cardiac remodeling.

### 8) PARP (Poly (ADP ribose) polymerase) mediated pathway

Poly ADP ribose polymerase (PARP), is the most abundant nuclear enzyme of eukaryotic cells and has been implicated to maintain the genomic integrity and cell survival [47] . Poly (ADP ribose) Polymerase 1 (PARP-1) is involved in DNA repair that catalyzes the synthesis of poly (ADP-ribose) from nicotine adenine dinucleotide (NAD+). The Hyper activation of PARP-1 from oxidative stress–induced DNA strand breakage can result in NAD+ depletion and prevent efficient synthesis of adenosine triphosphate (ATP) and leads to cell death. The DNA dependent group consists of PARP- 1 and PARP-2, which are activated by DNA strand breaks. PARP-1 is also involved in regulating transcription, stability, integrity of the genome and cell division [48]. PARP inhibition or genetic deletion has been shown to be protective in cardiac hypertrophy and heart failure. Peroxinitrate, a potent activator of the PARP- 1 is formed by the interaction of nitric oxide (NO) with the free radical superoxide is a potent and relatively stable oxidant that directly attacks integrity and performance of cardiac myocytes by damaging proteins, DNA and membrane lipids that determine heart function, including the activation of matrix metalloproteinase (MMPs) and depletion of antioxidant defenses. PARP-1 has also been documented to be a promoter-specific co-activator of the transcription factor nuclear factor κB (NF-κB), which plays a key role in the immune system and inflammatory response. This action of PARP-1 is independent of its enzymatic activity and occurs in response to inflammatory stimuli, such as tumor necrosis factor α [49]. Beside it PARP-1 also acts as a transactivator of inducible NO synthase (iNOS/NOS2) gene. PARP inhibitions are shown to be preventive in experimental cardiac hypertrophy, fibrosis and progression of heart failure. Pharmacological PARP-1 inhibition has also been reported to enhance the activity of GSK-3 by blocking PKc (protein kinase C) .PARP-1 hyperactivation induces cell death by several pathways including mitochondrial depolarization and release of apoptosis-inducing factor (AIF) which leads to chromatinolysis[50]. Beside this PARP also inhibits phosphoinositide-3-kinase (PI3K)-Akt signaling pathway, which couples to multiple survival mechanisms [51]. Moreover, PARP is a nuclear target of angiotensin II-mediated cell signaling and JNK (C-jun N-terminal kinase) activity that leads to myocyte death and cardiac remodeling. Thus modulation of PARP prevents myocyte from necrosis and apoptosis and may be a potential therapeutic target for pathological cardiac remodeling.

### 9) Rho-kinase mediated pathway

Rho-kinase, a serine threonine kinase and target of GTPase protein includes Rho-kinase alpha (Rho-kinase 2) and Rho-kinase beta (Rho kinase 1) and collectively called as Rho-kinase. Rho-kinase is mainly expressed in endothelial cells and contributes to various cellular functions such as vascular smooth muscle cell contraction, actin cytoskeleton organization, cell adhesion and cytokinesis. Further, Rho-kinase upregulates various molecules that accelerate inflammation, oxidative stress, thrombus formation and fibrosis. Rho-kinase has been shown to regulate various cardiovascular functions [52] . Activated Rho kinase can phosphorylate various downstream targets thought to be involved in remodeling. Myosin light chain phosphatase (MLCP) is one such target. Once phosphorylated, the MLCP enzyme is inhibited, thereby stimulating vascular smooth

muscle contraction, stress fiber formation, and cell migration. These effects contribute to diastolic dysfunction, most notably by processes involving inflammatory cell infiltration, adhesion to the endothelium, and activation of fibroblasts into myofibroblasts leading to fibrosis [53]. Rho kinase mediates upregulation of proinflammatory cytokines and mediators such as interleukin-6, monocyte chemo attractant protein-1, and transforming growth factor-1 which are involved in pathological cardiac remodeling. Rho kinase inhibition attenuates ANG II-induced cardiac hypertrophy and fibrosis. Beside it ROCK1 knockout mice exhibited reduced myocardial fibrosis in transverse aortic-banded animals without effects on cardiac hypertrophy and its inhibition upregulates the expression of eNOS mRNA and protein expression [54]. Thereby, ROCK shows hypertrophic and fibrotic actions in pathologic cardiac remodeling by modulation of myosin phosphatase and ERM family member [55]. Thus, modulation of ROCK may be a potential therapy for attenuating cardiac remodeling and preventing heart failure.

### 10) Sphingosine-1-Phosphate (S1P) mediated pathway

Sphingosine 1-Phosphate (S1P) is a lipid signaling molecule formed by catalytic action of enzyme sphingosine kinase (SK) which catalyzes the addition of a phosphate group to sphingosine (a membrane derived lipid). Among two isoforms of SK viz SK1 and SK2, Optimal SK1 activity and S1P synthesis has been documented to be required for cardiac fibroblast proliferation and collegen production. Excesive SK1 activity may lead to S1P2-mediated fibrosis and is associated with degranulation, chemotaxis, and cytokine production from macrophages. Morover, inhibition of SK1 has also shown to exert antihypertrophic and antifibrotic actions in experimental cardiac remodeling. Signals generated by sphingosine 1-phosphate pathway are critical for cell motility, cytoskeletal organization, vasculogenesis and cell growth [56]. Sphingolipids receptors are distinguished as S1P1, S1P2, S1P3, S1P4 and S1P5. All are highly expressed in endothelium and blood plasma indicating its role in cardiovascular functions. S1P1 interacts with Gαi, whereas S1P2 and S1P3 couple with Gαq, Gα13 and Gαi in a ligand-dependent manner [57]. S1P1 receptors are highly expressed in cardiomyocytes and have been linked to proliferative/survival and migratory signaling in various cell types. S1P1 and S1P3 receptors induce rac dependent chemotaxis and stimulates the expression of IL-1β and TNF-α from macrophages [58]. S1P2 mediate rho dependent inhibition of rac induced cell migration and has been reported to inhibit neutrophill chemotaxis and migration. However, S1P1 and S1P3 have also been reported to activate eNOS where as SIP2 inhibits its activation [59]. S1P1 and S1P3 induces fibrosis by ROCK MRTF-A (Myocardin-related transcription factor-A). Beside it, S1P2 and S1P3 receptors also have an important role in regulation of vascular tone [60]. Collectively, these studies indicate multiple functions of S1P on cardiac remodeling and further investigations may provide therapeutic approach by modulation of S1P pathway [59] (Figure 6).

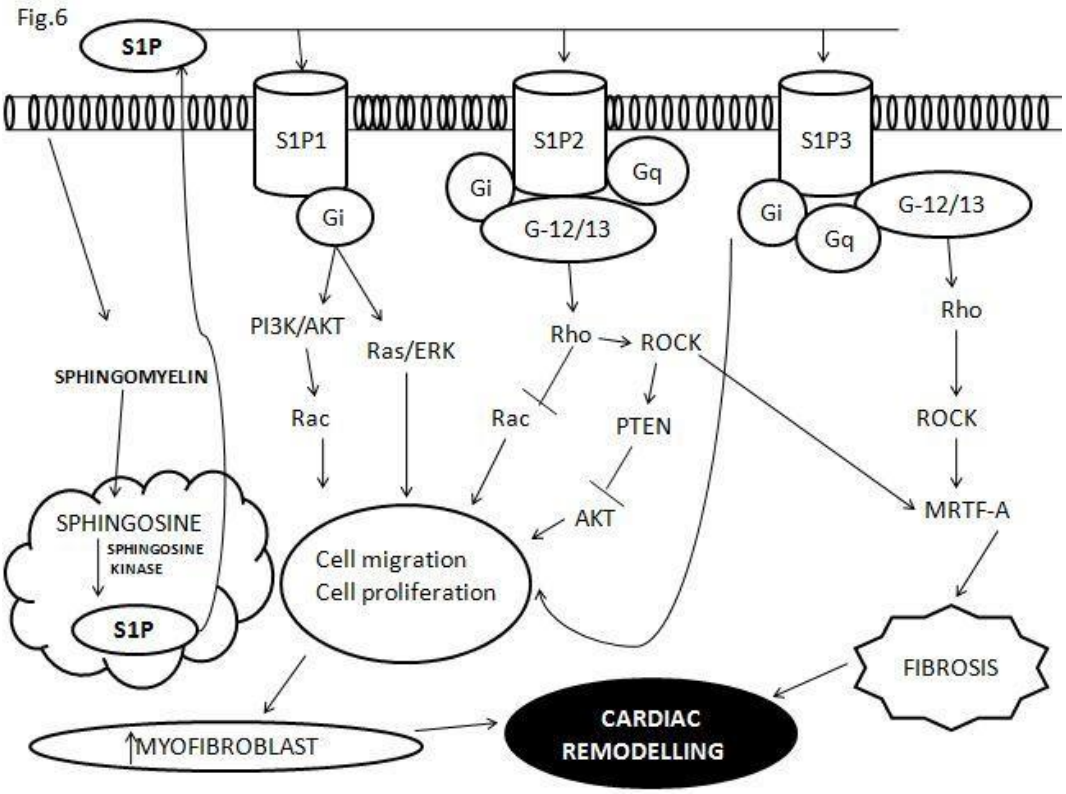


**Figure 6 Role of SIP in cardiac remodelling**

S1P (sphingosine-1-phosphate) derived by sphingomyeline by enzyme spingosine kinase acts on its three receptor and shows different action. by acting on S1P1 receptor it activates protein kinase b (akt) by acting on Pi3K (Phosphatidylinositol-4, 5-bisphosphate 3-kinase) which further activates rac protein beside it Ras protein associated with ERK (extracellular-signal-regulated kinases) is also activated both of which leads to migration and proliferation of cells including myofibroblasts. Further by acting on S1P2 SIP has opposite action on cell migration and proliferation by inhibiting rac protein and akt by rho/ROCK (Rho-associated protein kinase) dependent pathways which activates PTEN gene which further inhibits akt (protein kinase B) beside it S1P2 and S1P3 receptor increases `levels of MRTF-A (Myocardin-related transcription factor-a) which further leads to fibrosis these all action leads to cardiac remodeling.

## CONCLUSION

Recent advances in pathophysiology of cardiac remodeling and Identification of targets offer a new possibility in developing new therapeutic agents for attenuating cardiac remodeling. Role and involvement of various molecular targets Viz. Rho-kinase, PARP, PKC, PPAR, S1P, SMAPK, TWEAK-Fn14 cytokine, Nrf2, ghrelin and PKG have been delineated in pathological cardiac remodeling. These targets comprise both positive and negative regulator of cardiac remodeling. The new strategic pharmacological agents modulating these molecular targets may provide potential therapeutic drugs for attenuating pathological cardiac remodeling.

## CONFLICT OF INTEREST

None

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