Published in final edited form as:

J Cardiovasc Pharmacol. 2017 April ; 69(4): 212–214. doi:10.1097/FJC.0000000000000471.

G α_q SIGNALING IN THE REGULATION OF AUTOPHAGY AND HEART FAILURE

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REVIEW

The adult heart undergoes distinct remodeling processes in response to either acute or chronic insults, which can lead to myocyte hypertrophy and ventricular wall thickening or myocyte elongation, death and ventricular dilatation [1–3]. While an increase in cardiac tissue mass diminishes systolic wall stress to improve contractile performance in the short term, prolonged hypertrophy in response to pathological signaling is associated with a progression toward decompensated heart failure (HF) [1–3]. Accumulating evidence from studies in human patients and animal models suggests that in most instances hypertrophy is not a compensatory response to the change in mechanical load, but rather is a maladaptive process [1, 2, 4]. On the other hand, physiological hypertrophy, as occurs during normal postnatal development or in highly trained athletes, represents a beneficial form of cardiac growth [5].

Numerous neuroendocrine and autocrine factors such as phenylephrine, angiotensin II and endothelin-1 are well-known inducers of cardiomyocyte hypertrophy, and inhibition of their actions can be beneficial for the treatment of chronic HF following cardiac hypertrophy [2, 3]. Many such agonists act through G protein-coupled receptors (GPCR) that couple with G α_q , which canonically activate phospholipase C β (PLC β), generating the second messengers inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG), which then act to mobilize intracellular calcium and activate numerous downstream kinases, respectively. Chronic GqPCR activation results in cardiac hypertrophy and subsequently leads to cardiomyopathy with depressed contractile function [2, 3]. Although a low level of G α_q overexpression in mice does not significantly affect the heart, mild G α_q overexpression results in increased hypertrophic markers with minimal cardiac dysfunction [6, 7] and high G α_q overexpression results in marked hypertrophy, HF, and increased mortality [6]. Further, G α_q -overexpressing hearts that are exposed to pressure overload show increased apoptosis

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DISCLOSURES

None

[8], while overexpression of an inhibitory peptide that interferes with $G\alpha_q$ coupling prevents the development of cardiac hypertrophy and dysfunction in pressure-overloaded mice [9, 10]. Altogether, these data have highlighted the critical role of $G\alpha_q$ -signaling pathways in mediating the hypertrophic signaling and the transition to HF.

Several studies have investigated the potential contributions of cardiomyocyte autophagy on protein turnover and cardiac hypertrophy. Autophagy is an evolutionally conserved mechanism for the degradation of cellular components and organelles by lysosomes. Autophagy also plays an important role in the maintenance of cellular energetics by recycling amino acids and fatty acids for ATP production [11]. In eukaryotic cells, autophagy occurs constitutively at low levels to facilitate homeostatic functions such as protein and organelle turnover. However, autophagy is rapidly upregulated in conditions requiring the generation of high intracellular nutrients and energy, such as during starvation, growth factor withdrawal, or removal of damaging cytoplasmic components [11, 12]. Consistent with these functions, autophagy appears to play a protective role during normal conditions and in response to mild stress [13, 14]. However, excessive autophagy has been associated to cardiomyocyte death and the development of HF [13, 14]. Autophagy was observed in failing human hearts caused by dilated cardiomyopathy [15], by valvular disease [16], and by ischemic heart disease [17]. Moreover, analysis of hearts from patients with end-stage HF indicated that cardiomyocytes died by a variety of mechanisms including necrosis, apoptosis, and autophagy [18]. However, despite considerable evidence linking autophagic activity to HF progression, it is still debated whether autophagy is an adaptive response to protect cardiomyocyte from stress conditions or a maladaptive response that causes HF.

In the current issue of the journal, Liu et al. provide the first analysis of the role of $G\alpha_q$ activation on autophagy in the heart [19]. Using a cardiomyocyte-specific transgenic mouse model of inducible $G\alpha_q$ Q209L, a constitutive $G\alpha_q$ mutant deficient in GTP hydrolysis [7, 20], the authors demonstrate that autophagic vacuoles, levels of proteins involved in autophagy, and autophagic activity were enhanced in $G\alpha_q$ Q209L hearts at 7 days after transgene induction by tamoxifen treatment. $G\alpha_q$ Q209L hearts also exhibit enhanced lysosomal degradation activity and elevated levels of the autophagy initiation complex containing the Class III phosphoinositide 3-kinase Vps34, which accounts for the higher abundance of autophagic vacuoles. This increase in autophagy in $G\alpha_q$ Q209L mice was associated with a significant decrease in cardiac contractile function at 7 days after transgene induction. The study not only contributes to our understanding of the role of $G\alpha_q$ on autophagy in the heart, but also opens new directions by raising important questions as outlined below.

1. How does $G\alpha_q$ activation regulate autophagy in the heart?

The study by Liu et al. demonstrates that expression of $G\alpha_q$ Q209L in the heart leads to increased assembly of the autophagy-initiation Beclin1/Vps34/Atg14 complex, Vps34 activity, and production of PI(3)P, culminating in autophagosome formation [19]. As discussed above, $G\alpha_q$ activation leads to stimulation of PLC β to generate IP3 and DAG production, but has also been shown to activate the Class1A phosphoinositide 3-kinase

PI3K α , which is inhibited by binding to G α_q Q209L [21]. Thus, to determine which of these pathways contributes to the increase in autophagy in G α_q Q209L hearts, Liu et al. used a second cardiomyocyte-specific transgenic mouse line (G α_q Q209L-AA) that expresses a tamoxifen-regulated G α_q Q209L mutant in which Arg256 and Thr257 are changed to Ala. This protein cannot activate PLC β but still inhibits PI3K α [20, 22]. In contrast to G α_q Q209L mice, the G α_q Q209L-AA mice did not develop a contractility defect after 7 days of tamoxifen treatment and there was no observable difference in the levels of p62, LC3-II, Vps34, Beclin1, Atg7 or Atg14 between WT and G α_q Q209L-AA mice, suggesting that enhanced autophagy requires G α_q activation of PLC β [19]. However, investigation of canonical G α_q signaling pathway molecules have reported mixed effects on autophagy. Knockdown of G $\alpha_q/11$ in HeLa cells using siRNAs have been associated with increased autophagy [23], while elevation of intracellular IP $_3$ in cardiac and non-cardiac cells decreased autophagy independently of mammalian target of rapamycin complex (mTORC) regulation [24]. The mechanisms by which IP $_3$ reduced autophagy are still debated with studies suggesting that elevated IP $_3$ receptor levels inhibit autophagy by either sequestering Beclin 1 away from other autophagic machinery [25], or by increasing intracellular Ca $^{2+}$ and activating calcium-dependent cysteine proteases (calpains) [26]. The increase in Ca $^{2+}$ release by IP $_3$ receptor activation can also maintain mTORC1 activity and therefore reduce autophagy [27]. However, it is noteworthy that elevation of cytosolic Ca $^{2+}$ can also increase autophagy through the Ca $^{2+}$ -dependent activation of PKC β , the Erk1/2 or the calcium/calmodulin-dependent protein kinase kinase β (CAMKK β) which can activate AMP-activated protein kinase (AMPK) and initiate autophagy by multiple mechanisms [28]. The role of other signaling pathways in mediating G α_q -induced autophagy cannot be ruled out as G α_q may also induce mitochondrial oxidative stress [29], which is closely related to mitochondrial dysfunction and autophagy [30]. Therefore, more detailed mechanistic information is needed to dissect the role of G α_q -induced PLC β signaling on autophagy, and specifically in the heart.

2. What is the impact of G α_q activity on autophagy-induced HF progression?

The work by Liu et al. provides new insight into the role of G α_q -mediated autophagy in the heart [19]. However, whether autophagy is activated as part of a compensatory pathway to protect the heart against G α_q -induced cardiac decompensation or actually contributes to G α_q protein-mediated HF remains to be resolved. Liu et al. performed their experiments on mice injected with tamoxifen for 7 days, a timepoint at which the heart has been shown to display a mild contractile defect and abnormal sarcomere structure in cardiomyocytes [19, 20], but they did not assess whether the autophagy markers are still elevated in G α_q Q209L hearts after 14 to 28 days post-tamoxifen treatment, when pronounced cardiac contractile dysfunction and transition to HF would be detected [19]. Previous studies delineated sequential and ordered changes in autophagy and mitochondrial autophagy that occurred at different phases of development of hypertrophy and cardiac dysfunction, wherein mixed results were observed depending on the duration and the severity of the stress stimuli [31–33]. Autophagic activity was reduced during compensatory hypertrophic response induced by moderate thoracic transverse aortic constriction (TAC) at 1 week post-surgery in wild-type mice [33]. However, autophagy was upregulated at 4 weeks post-TAC when left ventricular dilation and cardiac dysfunction developed, during the transition from

hypertrophy to HF [33]. In contrast, autophagic activity increases from 24 h after severe TAC and persists for at least 2 weeks in wild-type or autophagy reporter mice [32]. This load-induced pathologic remodeling was significantly reduced in beclin 1 haplo-insufficient mice, although compensatory ventricular growth was not altered [32]. Conversely, cardiac-specific Beclin 1 transgenic mice exhibit cardiac dilatation and dysfunction 3 weeks after moderate TAC. These findings suggest that autophagy may increase in hypertrophied hearts under conditions of severe stress, such as occurs with $G\alpha_q$ overexpression, and that an excessive increase in autophagy may play a detrimental role in the transition from cardiac hypertrophy to HF.

3. Does $G\alpha_q$ -induced autophagy offer a potential target for treatment of chronic HF?

Although the study by Liu et al. suggests that $G\alpha_q$ -mediated autophagic flux may contribute to HF [19], caution is in order when considering translational implications. The view that $G\alpha_q$ -coupled receptor signaling is toxic is based in large part on a transgenic mouse model with $G\alpha_q$ overexpression that exceeds the 2-fold increase found in human HF [34–36] and thus cannot be considered to simulate human pathophysiology. In human HF, the maximal increase in $G\alpha_q$ abundance is 2-fold [35], and transgenic mice with 2-fold cardiomyocyte-specific $G\alpha_q$ overexpression have no discernible cardiac phenotype [34, 36]. Moreover, the study of Liu et al. used transgenic mice that express recombinant $G\alpha_q$ Q209L-hbER fusion proteins to activate $G\alpha_q$ signaling selectively in cardiac myocytes [19]. This inducible model, in contrast to the standard transgenic models, was found to cause a dilated cardiomyopathy and HF that does not appear to progress through a hypertrophic stage [20]. These discrepancies in $G\alpha_q$ -induced hypertrophy and failure could be related to the transgene being expressed (wild-type $G\alpha_q$ vs. $G\alpha_q$ Q209L mutant), their levels of expression or to the overexpression approaches that, even in transgenic mice with tissue-specific promoters, do not provide an ideal means of annotating specificity compared with loss-of-function experiments. We also lack a clear understanding of the role of autophagy in non-cardiac cells during $G\alpha_q$ -induced cardiac hypertrophy and failure and how inhibition of autophagy in the failing heart could affect the overall role of $G\alpha_q$ -dependent autophagy in maintaining cellular homeostasis during cardiac remodeling. Therefore understanding the characteristics of autophagy and the effects of $G\alpha_q$ signaling on its induction is important to translate this body of evidence derived from mouse models into pathological conditions.

Acknowledgments

SOURCE OF FUNDING

This work was supported by the National Institute of Health HL128446 (AS) and HL105414 (DGT).

ABBREVIATIONS

HF	Heart failure
GPCR	G protein-coupled receptors
PLCβ	Phospholipase C β
IP3	Inositol 1,4,5-trisphosphate

DAG	Diacylglycerol
mTORC	Mammalian target of rapamycin complex
CAMKKβ	Calcium/calmodulin-dependent protein kinase kinase β
AMPK	AMP-activated protein kinase
TAC	Transverse aortic constriction

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