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**Article**

# OPA1 in cardiovascular health and disease

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## ABSTRACT

Mitochondria are known to play crucial roles in normal cellular physiology and in more recent years they have been implicated in a wide range of pathologies. Central to both these roles is their ability to alter their shape interchangeably between two different morphologies: an elongated interconnected network and a fragmented discrete phenotype – processes which are under the regulation of the mitochondrial fusion and fission proteins, respectively. In this review article, we focus on the mitochondrial fusion protein optic atrophy protein 1 (OPA1) in cardiovascular health and disease and we explore its role as a potential therapeutic target for treating cardiovascular and metabolic disease.

## KEYWORDS

Mitochondrial morphology, fusion, fission, OPA1, cardiovascular disease

## Introduction

Cardiovascular disease (CVD) remains one of the leading causes of death and disability worldwide. Understanding the pathophysiology of CVD will help in the identification of novel therapeutic targets for treating CVD. The human heart beats on average 100,000 per day, a function which requires a constant energy supply. This is provided for by mitochondria, which are densely packed into, and occupy almost one third the volume of a cardiomyocyte. The role of mitochondria in cellular physiology extends far beyond the production of ATP required for normal contractile function, as they also play a variety of other essential roles including calcium signalling (1), reactive oxygen species (ROS) production (2-5), and the initiation of cell death (6). Central to these roles is the ability of mitochondria to alter their shape interchangeably between two different morphologies: an elongated interconnected network (mitochondrial fusion) and a fragmented discrete phenotype (mitochondrial fission). In this review article, we focus on the mitochondrial fusion protein optic atrophy protein 1 (OPA1) in cardiovascular health and disease and we explore its role as a potential therapeutic target for treating cardiovascular and metabolic disease.

## Mitochondrial morphology in cardiovascular health and disease

Mitochondria are dynamic organelles which are able to change their shape and number according to the cell type, metabolic requirements, and extracellular stimuli. This phenomenon has been termed ‘mitochondrial morphology’ and is essential for the maintenance of a healthy mitochondrial network and normal cellular physiology. Mitochondria fusion occurs when neighbouring organelles fuse with each other enabling them to elongate and form interconnected networks. As well as this, mitochondria are able to fragment and separate from each other to form discrete units, a process known as mitochondrial fission. The changes in mitochondrial morphology are mediated by the mitochondrial fusion proteins (Mitofusins 1 and 2 [Mfn1 and 2] and optic atrophy protein 1 [OPA1]) and the mitochondrial fission proteins (dynamin related peptide 1 [Drp1], human fission protein 1 [hFis1], mitochondrial fission factor [MFF], and mitochondrial dynamics proteins of 49kDa and 51kDa [MiD49/51]). The role of these mitochondrial shaping proteins has been comprehensively reviewed elsewhere (7-11). In this review article we will focus specifically on the role of OPA1 in cardiovascular health and disease.

## The discovery of OPA1

In 1959, Kjer, a Danish ophthalmologist first described the condition now known as dominant optic atrophy (DOA), also called Kjer’s optic neuropathy (12). This condition is characterized by mitochondrial dysfunction and degeneration of the optic nerves causing impaired colour vision, reduced visual acuity, and in many cases blindness (12). It has an estimated prevalence of 1:50 000 in most countries and a prevalence as high as 1:10 000 in Denmark (13). It is an autosomal neurodegenerative disease which was later discovered in 2000 to be due to a mutation in the OPA1 protein (14, 15). OPA1 expression is tissue-specific with some of the highest levels of expression found in the retina, brain, liver, heart and skeletal muscle (14, 15). Its best known role is as a mediator of mitochondrial fusion, but it is clear that many of its effects extend beyond this and rely upon its pleiotropic non-fusion roles. Alternative splicing and proteolysis of the OPA1 protein allows these diverse functions and is reviewed next.

**Proteolytic regulation of OPA1 function**

OPA1 function is determined by alternative splicing and post-translational modification, making the cellular processing of the OPA1 protein rather complex. OPA1 shares a number of common structural features with dynamins, including a GTPase domain, a middle domain, and GTPase effector domain (GED)(9). Alternative splicing of OPA1 generates eight OPA1 isoforms, the expression of which varies according to the tissue (16). Human and murine tissues mainly express the OPA1 isoforms 1 and 7. The mitochondrial import sequence located at the $NH_2$-terminal region of the OPA1 isoforms targets OPA1 to the mitochondria and is removed in the inter-membranous space (IMS) by the mitochondrial processing peptidase. This generates insoluble long forms of OPA1 (termed l-OPA1) anchored to the IMM (17, 18). The various forms of l-OPA1 possess either one or two cleavage sites: S1 (which is present in all forms of l-OPA1) and S2 (which is only present in some forms of l-OPA1).

Following the mitochondrial import of the OPA1 isoforms, the constitutively active IMS AAA (i-AAA) protease, YME1L, cleaves those forms of l-OPA1 containing the S2 cleavage site generating soluble short forms of OPA1 (termed s-OPA1) within the IMS and loosely attached to the IMM. Further cleavage of s-OPA1 by presenilin associated rhomboid-like (PARL) results in small quantities of s-OPA1 in the IMS. Under conditions of cellular stress (such as $H_2O_2$ (19), valinomycin and oligomycin (20)), the zinc metalloprotease OMA1 mediates the proteolysis of OPA1 at the S1 cleavage site resulting in the degradation of all forms of l-OPA1 to s-OPA1. The consequence of this is the inhibition of mitochondrial fusion (as this process requires the presence of both l-OPA1 and s-OPA1) resulting in unopposed mitochondrial fission and fragmented mitochondria (21, 22). The regulation of OPA1 function by these proteases and their interaction with each other is rather complex and has been reviewed in detail elsewhere (10, 23-25) – only an overview is provided here (see Figure 1).

Yme1l mediates the proteolytic cleavage OPA1 at the S2 site (21). Genetic ablation of Yme1L result in mitochondrial fragmentation in a manner which is dependent on OPA1 (22). PARL (a mitochondrial rhomboid protease) has been reported to proteolyse the s-OPA1 constitutively produced by Yme1L, generating a minor fraction of soluble s-OPA1 in the IMS that is required for the anti-apoptotic function of OPA1 (26, 27). OMA1 is an IMM protease which is constitutively active, although its activity is enhanced under the conditions of cellular stress such as mitochondrial membrane depolarization, thermal stress, oxidative stress and Bax/Bak initiated apoptosis (28). The activation of OMA1 is regulated by an amino terminal stress-sensor domain and is associated with its autocatalyic degradation, thereby limiting its activation to the period of cellular stress and allowing OPA1-mediated mitochondrial fusion to continue once the stress has subsided (20). A recent experimental study has shown that OMA1 cleaves itself to a short form of OMA1 (s-OMA1), which is degraded quickly, but is stabilized by mitochondrial membrane depolarization and during apoptosis, thereby allowing the cleavage of OPA1 under conditions of stress (29). Recent data suggests that an interaction between the mitochondrial proteases Yme1l and OMA1 may facilitate OPA1 proteolysis in stressed mitochondria allowing the selected removal of damaged mitochondria by mitophagy. In response to oxidative stress Yme1l has been shown to be degraded in a manner which shown to be dependent on OMA1, thereby augmenting OPA1 proteolysis (30). Prohibitin, a IMM protein, has also been shown to regulate OPA1 processing (31). Loss of prohibitin subunit 2 (Phb2) resulted in the loss of l-OPA1, the result of which was abnormal cristae, reduced cell proliferation and increased sensitivity to apoptosis. The actual mechanism through which Phb2 regulates OPA1 processing remains unclear. Recently, Sood *et al* (32) have described a novel form of OPA1 processing in response to nutrient limitation, in which two novel regulatory C-terminal fragments of OPA1 are generated by a cysteine protease in a manner which is independent of OMA1 and PARL. Interestingly this novel type of cellular processing of OPA1 was shown to be dependent on the association of OPA1 with MFN2 present at contact sites between mitochondria and endoplasmic reticulum. The potential significance of this form of OPA1 processing is not clear and is discussed in a later section (32).

## Other regulators of OPA1 function

In addition to proteolytic cleavage, OPA1 has been found to be regulated by several other post-translational modifications, thereby complicating further the regulation of OPA1 function. Makino *et al* (33) have shown in neonatal cardiomyocytes that in the presence of high glucose, O-GlcNAcylation of OPA1 suppressed OPA1 activity resulting in mitochondrial fission. The potential implications of this for the diabetic heart are discussed in a later section. OPA1 activity has recently been reported to be modified by acetylation (34). In response to cellular stress, the class III histone mitochondrial deacetylase, Sirtuin 3, has been shown to deacetylate OPA1 at the lysine 926 and 931 residues, increasing its GTPase activity and promoting mitochondrial fusion and protection against cell death (34). The relevance of this has been explored in the context of cardiac disease and diabetes in a later section (34). Using a genome-wide RNA interference (RNAi) screen, Norton *et al* (35) have recently identified reactive oxygen species modulator 1 (ROMO1) to be a novel regulator of mitochondrial fusion and normal cristae morphology. They found that the oligomerization of OPA1 required for its anti-apoptotic effect against oxidative stress was dependent on the formation of mitochondrial ROMO1 complexes. The exact interaction between ROMO1 and OPA1 is unclear, but it has been proposed that by attenuating oxidative stress, ROMO1 acts to reduce the activation of OMA1 thereby preventing the proteolytic cleavage of OPA1 (36). Finally, Hypoxia-induced gene domain protein 1 (Higd-1a), an IMM protein which has been reported to suppress mitochondrial cytochrome *c* release and inhibit apoptotic cell death under conditions of hypoxia, has been recently linked to OPA1 function (37, 38). It has been shown to bind to and prevent the cleavage of l-OPA1 into s-OPA1, thereby attenuating cytochrome C release and inhibiting carbonyl cyanide-*4*-phenylhydrazone (FCCP)-induced mitochondrial fission and cell death (38). The mechanism through which Higd-1a prevents the proteolysis of OPA1 remains to be determined.

## OPA1 as a mitochondrial fusion protein

OPA1 is best known for its role as a mitochondrial fusion protein in which it hydrolyses GTP to fuse IMMs of adjacent mitochondria (39). Song *et al* have shown that functional OPA1 is maintained by constitutive processing and that both the long and short OPA1 forms need to be present for mitochondrial fusion to occur (21). Experimental studies have shown that ablating or reducing OPA1 protein levels results in the breakdown of integrated mitochondrial networks resulting in mitochondrial fragmentation (40, 41). Interestingly, it has been demonstrated that the OMM fusion protein, Mfn1 but not Mfn2, is required for OPA1 to mediate mitochondrial fusion, although the reasons for this are not clear (42). In this pro-fusion role, OPA1 is required to maintain a healthy mitochondrial network. Depolarization of the mitochondrial membrane potential in damaged mitochondria was found to increase the activity of OMA1 which in turn cleaved all forms of l-OPA1 into s-OPA1 thereby inhibiting mitochondrial fusion and permitting mitochondrial fragmentation, allowing the selective removal of damaged mitochondria by mitophagy (43, 44).

A recent study by Mishra *et al* (45) has investigated the link between mitochondrial fusion and mitochondrial function as measured by the level of oxidative phosphorylation (OXPHOS). They found that when oxidative respiration was stimulated, Yme1l mediated proteolysis of l-OPA1 was enhanced. The result of this was increased IMM fusion, providing further support for the dependency of mitochondrial fusion on s-OPA1. Another study has shown that OXPHOS is able to suppress stress-induced mitophagy and maintain mitochondrial fusion by attenuating OMA1-dependent cleavage of OPA1 and reducing Drp1 fission activity (44). In contrast to these findings, a recent experimental study has questioned this paradigm reporting that l-OPA1 alone is sufficient to mediate IMM fusion and that s-OPA1 may actually associate with the OMM and induce mitochondrial fission (19).

## Pleiotropic non-fusion roles of OPA1

In addition to its role as a pro-fusion mitochondrial protein, OPA1 is known to have several pleiotropic, non-fusion roles which have been recently shown to impact on cristae morphology and mitochondrial respiratory efficiency.

*Cristae remodelling and mitochondrial apoptosis*

The down-regulation of OPA1 and the over-expression of OPA1 have been reported to induce or prevent apoptosis respectively, suggesting that OPA1 has an anti-apoptotic effect (41, 46). In this regard, and independent of its role as a mediator of IMM fusion, OPA1 has been shown to regulate mitochondrial cristae morphology, cytochrome C distribution, and apoptotic cell death. It is well-established that cristae remodelling (cristae fusion and widening of the cristae junction), by tBID is required for the redistribution of cytochrome C from the intra-cristal space into the IMS and the initiation of apoptosis (47). By 'stapling' these cristae junctions closed, oligomers formed between PARL-proteolysed s-OPA1 in the IMS and insoluble l-OPA1 isoforms anchored to the IMM, have been shown to prevent the redistribution of cytochrome C and inhibit apoptotic cell death (46).

*Cristae remodelling and mitochondrial respiratory efficiency*

The respiratory complexes of the electron transport chain (ETC) are not arranged at random within the IMM, but are instead assembled into respiratory chain supercomplexes (RCS), the arrangement which facilitates the transfer of electrons between the respiratory complexes, thereby improving mitochondrial respiratory efficiency (reviewed in (48)). The regulation of cristae morphology by OPA1 has been recently shown to impact on the formation of RCS and mitochondrial energy production. Using genetic manipulation of OPA1, Cogliati *et al* (49) have demonstrated that the stability and assembly of RCS, mitochondrial respiratory efficiency, and mitochondria-dependent cell growth, were critically dependent on cristae morphology. These findings implicate OPA1 as a critical regulator of mitochondrial respiration and therefore a therapeutic target for modulating mitochondrial energy production.

## OPA1 and cardiovascular and metabolic diseases

OPA1 has recently been investigated in a number of cardiovascular and metabolic diseases. In general, it appears to play a beneficial role in these settings, positioning OPA1 as a potential therapeutic target in the novel treatment of these medical conditions (see Figure 2).

*OPA1 and heart failure*

One of the first experimental studies to investigate the role of OPA1 in the heart was by Chen *et al* (50). These authors observed that myocardial levels of OPA1 were reduced in ischemic heart failure patients and in a rat model of ischemic heart failure (50). These findings were associated with presence of small fragmented mitochondria and increased apoptotic cell death (50). The reduction of myocardial OPA1 was shown to affect all forms of l-OPA1 and s-OPA1 and was thought to be due to post-translational modification as the levels of OPA1 mRNA expression were not altered (50). Importantly, the protein levels of the other mitochondrial fusion proteins Mfn1 and Mfn2 and the fission proteins Drp1 and hFis1 were unchanged in the presence of ischemic heart failure (50). In contrast, in myocardial tissue harvested from patients with dilated cardiomyopathy, levels of OPA1 were unchanged with increased myocardial levels of Mfn1, Mfn2 and Drp1 (50).

The endogenous role of OPA1 in the heart has been recently investigated in mice partially deficient in OPA1 and resulted in a late onset cardiomyopathy at 12 months with a decrease

in cardiac output, reduced fractional shortening, and a blunted response to a β-adrenergic stimulus - findings which were associated with mitochondrial fragmentation, impaired mitochondrial respiration due to decreased complex I and IV activity, increased oxidative stress (down-regulation of the antioxidant enzymes Gpx3 and Gstk1), attenuated calcium transients, and a reduction in mitochondrial DNA copy number (51).

The mechanism for the down-regulation of OPA1 in heart failure is unclear and the role this plays in the pathophysiology of ischemic heart failure is not known. Whether the reduction in myocardial OPA1 contributes to the metabolic abnormalities and enhanced apoptotic cell death characteristic of heart failure remains to be determined.

*OPA1 and ischemia/reperfusion injury*

Several experimental studies have demonstrated that cardiac ischemia induces mitochondrial fission and that genetic or pharmacological inhibition of the mitochondrial fission protein, Drp-1, limited MI size (52-54). The role of OPA1 in the cellular response to I/R injury has also been investigated. Chen et al (50) demonstrated in H9C2 cells that simulated ischemia reduced OPA1 protein levels, increased mitochondrial fragmentation, and worsened apoptotic cell death. The siRNA knockdown of OPA1 exacerbated these effects of simulated ischemia (50). However, the over-expression of OPA1 did not appear to protect against ischemia-induced apoptosis and actually worsened it (50). In contrast to these findings, An *et al* (38) observed that OPA1 overexpression was protective against a hypoxic insult in HEK293T cells. Furthermore, Chen *et al* (51) noted that murine cardiomyocytes isolated from adult mice were more susceptible to cell death induced by simulated I/R injury suggesting a cardio-protective role for OPA1.

A recent experimental study investigating the role of OPA1 in I/R injury in the murine kidney has implicated OMA1 as a potential therapeutic target. Xiao et al (55) demonstrated that the genetic ablation of OMA1 protected renal proximal tubular cells against the detrimental effects of I/R injury in terms of preventing OPA1 proteolysis, inhibition of mitochondrial fragmentation, less cytochrome c release, and attenuated apoptotic cell death. Whether targeting OMA1 plays a similar protective role in hearts subjected to I/R injury remains to be determined.

Sanjuán Szklarz & Scorrano (26) have recently suggested that mild heat stress induced the PARL-mediated proteolysis of OPA1 and the accumulation of soluble s-OPA1 within the IMS, which then protected cells against a subsequent lethal heat shock, suggesting that OPA1 may be involved in mediating a 'conditioning' form of protection. Whether OPA1 plays a role as a mediator of conditioning the heart to resist lethal I/R injury is an interesting possibility which needs to be investigated.

*OPA1 and left ventricular hypertrophy*

OPA1 has been recently investigated in the setting of left ventricular hypertrophy (LVH) using adult mice partially deficient in OPA1 (56). Although these mice appeared to have no significant cardiac phenotype at baseline, these mice were shown to be more susceptible to total aortic constriction, developing twice the extent of LVH, when compared to wild-type mice (56). These findings were associated with clustering of large mitochondria with abnormal cristae morphology which were demonstrated to be resistant to calcium-induced mitochondrial permeability transition pore (MPTP) opening. Overall this data appears to suggest that OPA1 is protective against LVH but the mechanism for this beneficial effect remains unknown.

Samant *et al* (34) have found that myocardial OPA1 was hyper-acetylated in LVH induced by angiotensin II or total aortic constriction, findings which were associated with reduced OPA1

activity and mitochondrial fragmentation. In that study it was suggested that under basal conditions mitochondrial Sirtuin 3 deacetylates OPA1, thereby preserving the latter's function and maintaining mitochondrial fusion. Contrasting findings were, however, reported in a separate study which showed that total OPA1 expression was significantly increased in the compensatory LVH characteristic of the spontaneous hypertensive rat model (57).

*OPA1 in cardiac development and cardiomyocyte differentiation*

Mice completely deficient in OPA1 die *in utero*, suggesting that OPA1 plays a critical role in embryonic development and maintenance of mitochondrial function (58). Cardiac-specific RNA- knockdown of OPA1 in the heart tube of *Drosophila* had the following detrimental effects; mitochondrial clustering, fragmentation, a 30% decrease in mitochondrial size - features which were associated with severe contractile impairment, cardiac dilatation and death, underscoring the requirement of OPA1 in cardiac development (59). Genetic ablation of OPA1 in Zebrafish was shown to reduce both cardiac contractions and heart rate (60). The accumulated evidence suggests that OPA1 is a critical regulator of cardiac development, presumably as it is required to mediate the organisation of the mitochondrial network needed to provide the increased energy requirements required to support this critical process. OPA1 has also been shown to play a critical role in the differentiation of embryonic stem cells into cardiomyocytes. Kasahara *et al* (61) demonstrated that the genetic inhibition of Mfn2 and OPA1 interrupted cardiomyocyte differentiation by inhibiting mitochondrial fusion through a complex pathway involving calcium-induced activation of calcineurin and enhanced Notch signalling.

*OPA1 and cardio-metabolic disease*

Diabetes is one of the major risk factors for developing CVD and its prevalence is increasing worldwide. In the presence of high glucose, abnormalities in mitochondrial shape and function have been shown to occur. These abnormalities coincide with changes in mitochondrial morphology, characterized by Drp-1 dependent mitochondrial fission which results in mitochondrial dysfunction, the mitochondrial production of ROS, MPTP opening and apoptotic cell death (62). A propensity to apoptosis and increased susceptibility to MPTP opening has also been described in adult cardiac mitochondria isolated from the diabetic murine heart (63). Makino *et al* (64) has demonstrated that mouse coronary endothelial cells isolated from the diabetic murine heart had reduced levels of OPA1 and evidence of mitochondrial fragmentation. Furthermore, OPA1 expression and fusion were both found to be impaired in diabetic patients (65).

The aetiology for the observed mitochondrial fission which occurs in response to the high glucose remains unclear. A number of potential mechanisms have been proposed: (1) In neonatal cardiomyocytes it has been shown that in the presence of high glucose, *O*-GlcNAcylation of OPA1 occurs, the consequence of which was suppression of OPA1 activity resulting in mitochondrial fission and dysfunction (33). Overexpression of GlcNAcase decreased OPA1 protein O-GlcNAcylation and significantly increased mitochondrial elongation (33). Moreover, OPA1 overexpression was able to prevent the changes in mitochondrial morphology induced by the hyperglycemia; (2) In the diabetic heart, OPA1 has been shown to be hyper-acetylated, reducing its activity and resulting in mitochondrial fragmentation (34).

Parra *et al* (66) have reported in neonatal rat cardiomyocytes that treatment with insulin increased OPA1 protein levels, induced mitochondrial fusion, increased mitochondrial membrane potential, and augmented both intracellular ATP levels and oxygen consumption. The effect of OPA1 on mitochondrial function was shown to be mediated by the activation of the Akt-mTOR-NFκB pathway in a manner which was shown to be dependent on OPA1 (66). The physiological significance of this effect of insulin on mitochondrial morphology and

metabolism is unclear and remains to be investigated. The role for OMA1 has been investigated by Quirós et al (67), who found that glucose homeostasis was altered in the OMA1 mutant mice with the development of insulin resistance, suggesting that in the presence of diabetes, changes in OMA1 and OPA1 activity may contribute to the acquisition of insulin resistance in this condition.

Whether the down-regulation of OPA1 in the presence of diabetes contributes to the mitochondrial fragmentation observed in the diabetic heart remains to be determined. The overexpression of OPA1 may be a therapeutic strategy for preventing the mitochondrial fission and dysfunction which occurs in the diabetic heart, thereby preventing the cardiac dysfunction observed in diabetic cardiomyopathy. In this regard, the over-expression of OPA1 has been shown to improve mitochondrial morphology in coronary endothelial cells isolated from a diabetic heart supporting the notion that OPA1 may be a potential therapeutic target to improve cardiac dysfunction in diabetes (33, 64).

**Pharmacological inhibition of OMA1 as a therapeutic strategy**

The accumulated data outlined previously suggests that the down-regulation of OPA1 may contribute to the pathogenesis of a number of different cardiovascular and metabolic diseases. In terms of a viable therapeutic approach experimental studies suggest that the genetic inhibition of OMA1 can increase levels of OPA1 and protect the heart against cellular injury, supporting pharmacological inhibition of OMA1 as a therapeutic strategy for treating cardiovascular and metabolic disease. While non-specific zinc chelators, such as *o*-phenanthroline, have been used in various studies to inhibit OMA1 activity and upregulate OPA1 (68-70), a more specific inhibitor of OMA1 needs to be discovered in order to make this therapeutic approach viable for the treatment of cardiovascular disease. The other issue to consider will be the possible detrimental effects of chronically upregulating OPA1 using long-term therapy with OMA1 inhibitors, and this limitation may restrict this therapeutic approach to conditions in which short-term OPA1 upregulation is beneficial.

**Summary and conclusions**

Cardiovascular and diabetes are the leading causes of death and disability today and new therapeutic targets are therefore required to discover novel therapies for treating these medical conditions and improving clinical outcomes in this patient group. Abnormalities in mitochondrial function are known to contribute to a number of cardiovascular conditions. In this regard, changes in mitochondrial morphology have been demonstrated to impact on mitochondrial function in cardiovascular health and disease. OPA1, a mitochondrial fusion protein in the IMM, is known to have a role which extends beyond its pro-fusion function. It has pleiotropic effects on cristae morphology which are critical to its effects on preventing apoptotic cell death and maintaining mitochondrial respiratory efficiency. Maintaining physiological levels of OPA1 protein has been shown to be important in cardiovascular health (cardiac development and cardiomyocytes differentiation), while down-regulation of OPA1 protein has been reported in cardiovascular disease (heart failure, ischemia/reperfusion injury, left ventricular hypertrophy and diabetes). Therefore, preserving OPA1 protein provides a novel therapeutic strategy for treating patients with cardiovascular disease, and this may potentially be achieved by pharmacological targeting of OMA1.

**Abbreviations**

| | |
|---|---|
| CVD | cardiovascular disease |
| OPA1 | optic atrophy protein 1 |
| ATP | adenosine triphosphate |
| ETC | electron transport chain |
| OXPHOS | oxidative phosphorylation |
| ROS | reactive oxygen species |
| MFN | mitofusin |
| DRP | dynamin-related protein |
| MFF | mitochondrial fission factor |
| I/R | ischemia/reperfusion injury |
| MPTP | mitochondrial permeability transition pore |
| TAC | trans-aortic constriction |
| DOA | dominant optic atrophy |
| MIS | mitochondrial import sequence |
| MPP | mitochondrial processing peptidase |
| RCC | respiratory chain complexes |
| RCS | respiratory chain supercomplexes |
| LVH | left ventricular hypertrophy |
| ROMO1 | reactive oxygen species modulator 1 |
| FCCP | carbonyl cyanide-*4*-phenylhydrazone |
| IMM | inner mitochondrial membrane |
| OMM | outer mitochondrial membrane |
| PARL | presenilin associated rhomboid like |
| I/R | ischemia/reperfusion |

**References**


1. Rizzuto R, De Stefani D, Raffaello A, Mammucari C. Mitochondria as sensors and regulators of calcium signalling. Nat Rev Mol Cell Biol. 2012;13(9):566-78.

2. Balaban RS, Nemoto S, Finkel T. Mitochondria, oxidants, and aging. Cell. 2005;120(4):483-95.

3. Muller F. The nature and mechanism of superoxide production by the electron transport chain: Its relevance to aging. J Am Aging Assoc. 2000;23(4):227-53.

4. Antico Arciuch VG, Elguero ME, Poderoso JJ, Carreras MC. Mitochondrial regulation of cell cycle and proliferation. Antioxid Redox Signal. 2012;16(10):1150-80.

5. Mandal S, Guptan P, Owusu-Ansah E, Banerjee U. Mitochondrial regulation of cell cycle progression during development as revealed by the tenured mutation in Drosophila. Dev Cell. 2005;9(6):843-54.

6. Yang J, Liu X, Bhalla K, Kim CN, Ibrado AM, Cai J, et al. Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked. Science. 1997;275(5303):1129-32.

7. Ikeda Y, Shirakabe A, Brady C, Zablocki D, Ohishi M, Sadoshima J. Molecular mechanisms mediating mitochondrial dynamics and mitophagy and their functional roles in the cardiovascular system. J Mol Cell Cardiol. 2014.

8. Hall AR, Burke N, Dongworth RK, Hausenloy DJ. Mitochondrial fusion and fission proteins: novel therapeutic targets for combating cardiovascular disease. Br J Pharmacol. 2014;171(8):1890-906.

9. Belenguer P, Pellegrini L. The dynamin GTPase OPA1: more than mitochondria? Biochim Biophys Acta. 2013;1833(1):176-83.

10. Liesa M, Palacín M, Zorzano A. Mitochondrial dynamics in mammalian health and disease. Physiol Rev. 2009;89(3):799-845.

11. Alavi MV, Fuhrmann N. Dominant optic atrophy, OPA1, and mitochondrial quality control: understanding mitochondrial network dynamics. Mol Neurodegener. 2013;8:32.

12. KJER P. Infantile optic atrophy with dominant mode of inheritance: a clinical and genetic study of 19 Danish families. Acta Ophthalmol Suppl. 1959;164(Supp 54):1-147.

13. Kjer B, Eiberg H, Kjer P, Rosenberg T. Dominant optic atrophy mapped to chromosome 3q region. II. Clinical and epidemiological aspects. Acta Ophthalmol Scand. 1996;74(1):3-7.

14. Delettre C, Lenaers G, Griffoin JM, Gigarel N, Lorenzo C, Belenguer P, et al. Nuclear gene OPA1, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy. Nat Genet. 2000;26(2):207-10.

15. Alexander C, Votruba M, Pesch UE, Thiselton DL, Mayer S, Moore A, et al. OPA1, encoding a dynamin-related GTPase, is mutated in autosomal dominant optic atrophy linked to chromosome 3q28. Nat Genet. 2000;26(2):211-5.

16. Delettre C, Griffoin JM, Kaplan J, Dollfus H, Lorenz B, Faivre L, et al. Mutation spectrum and splicing variants in the OPA1 gene. Hum Genet. 2001;109(6):584-91.

17. Olichon A, Emorine LJ, Descoins E, Pelloquin L, Brichese L, Gas N, et al. The human dynamin-related protein OPA1 is anchored to the mitochondrial inner membrane facing the inter-membrane space. FEBS Lett. 2002;523(1-3):171-6.

18. Satoh M, Hamamoto T, Seo N, Kagawa Y, Endo H. Differential sublocalization of the dynamin-related protein OPA1 isoforms in mitochondria. Biochem Biophys Res Commun. 2003;300(2):482-93.

19. Anand R, Wai T, Baker MJ, Kladt N, Schauss AC, Rugarli E, et al. The i-AAA protease YME1L and OMA1 cleave OPA1 to balance mitochondrial fusion and fission. J Cell Biol. 2014;204(6):919-29.

20. Baker MJ, Lampe PA, Stojanovski D, Korwitz A, Anand R, Tatsuta T, et al. Stress-induced OMA1 activation and autocatalytic turnover regulate OPA1-dependent mitochondrial dynamics. EMBO J. 2014;33(6):578-93.

21. Song Z, Chen H, Fiket M, Alexander C, Chan DC. OPA1 processing controls mitochondrial fusion and is regulated by mRNA splicing, membrane potential, and Yme1L. J Cell Biol. 2007;178(5):749-55.

22. Griparic L, Kanazawa T, van der Bliek AM. Regulation of the mitochondrial dynamin-like protein Opa1 by proteolytic cleavage. J Cell Biol. 2007;178(5):757-64.

23. McBride H, Soubannier V. Mitochondrial function: OMA1 and OPA1, the grandmasters of mitochondrial health. Curr Biol. 2010;20(6):R274-6.

24. Chan DC. Fusion and fission: interlinked processes critical for mitochondrial health. Annu Rev Genet. 2012;46:265-87.

25. Pellegrini L, Scorrano L. A cut short to death: Parl and Opa1 in the regulation of mitochondrial morphology and apoptosis. Cell Death Differ. 2007;14(7):1275-84.

26. Sanjuán Szklarz LK, Scorrano L. The antiapoptotic OPA1/Parl couple participates in mitochondrial adaptation to heat shock. Biochim Biophys Acta. 2012;1817(10):1886-93.

27. Cipolat S, Rudka T, Hartmann D, Costa V, Serneels L, Craessaerts K, et al. Mitochondrial rhomboid PARL regulates cytochrome c release during apoptosis via OPA1-dependent cristae remodeling. Cell. 2006;126(1):163-75.

28. Jiang X, Jiang H, Shen Z, Wang X. Activation of mitochondrial protease OMA1 by Bax and Bak promotes cytochrome c release during apoptosis. Proc Natl Acad Sci U S A. 2014;111(41):14782-7.

29. Zhang K, Li H, Song Z. Membrane depolarization activates the mitochondrial protease OMA1 by stimulating self-cleavage. EMBO Rep. 2014;15(5):576-85.

30. Rainbolt TK, Saunders JM, Wiseman RL. YME1L degradation reduces mitochondrial proteolytic capacity during oxidative stress. EMBO Rep. 2014.

31. Merkwirth C, Dargazanli S, Tatsuta T, Geimer S, Löwer B, Wunderlich FT, et al. Prohibitins control cell proliferation and apoptosis by regulating OPA1-dependent cristae morphogenesis in mitochondria. Genes Dev. 2008;22(4):476-88.

32. Sood A, Jeyaraju DV, Prudent J, Caron A, Lemieux P, McBride HM, et al. A Mitofusin-2-dependent inactivating cleavage of Opa1 links changes in mitochondria cristae and ER contacts in the postprandial liver. Proc Natl Acad Sci U S A. 2014.

33. Makino A, Suarez J, Gawlowski T, Han W, Wang H, Scott BT, et al. Regulation of mitochondrial morphology and function by O-GlcNAcylation in neonatal cardiac myocytes. Am J Physiol Regul Integr Comp Physiol. 2011;300(6):R1296-302.

34. Samant SA, Zhang HJ, Hong Z, Pillai VB, Sundaresan NR, Wolfgeher D, et al. SIRT3 deacetylates and activates OPA1 to regulate mitochondrial dynamics during stress. Mol Cell Biol. 2014;34(5):807-19.

35. Norton M, Ng AC, Baird S, Dumoulin A, Shutt T, Mah N, et al. ROMO1 is an essential redox-dependent regulator of mitochondrial dynamics. Sci Signal. 2014;7(310):ra10.

36. Semenzato M, Scorrano L. O ROM(e)O1, ROM(e)O1, wherefore art thou ROM(e)O1? Sci Signal. 2014;7(310):pe2.

37. An HJ, Shin H, Jo SG, Kim YJ, Lee JO, Paik SG, et al. The survival effect of mitochondrial Higd-1a is associated with suppression of cytochrome C release and prevention of caspase activation. Biochim Biophys Acta. 2011;1813(12):2088-98.

38. An HJ, Cho G, Lee JO, Paik SG, Kim YS, Lee H. Higd-1a interacts with Opa1 and is required for the morphological and functional integrity of mitochondria. Proc Natl Acad Sci U S A. 2013;110(32):13014-9.

39. Malka F, Guillery O, Cifuentes-Diaz C, Guillou E, Belenguer P, Lombès A, et al. Separate fusion of outer and inner mitochondrial membranes. EMBO Rep. 2005;6(9):853-9.

40. Griparic L, van der Wel NN, Orozco IJ, Peters PJ, van der Bliek AM. Loss of the intermembrane space protein Mgm1/OPA1 induces swelling and localized constrictions along the lengths of mitochondria. J Biol Chem. 2004;279(18):18792-8.

41. Olichon A, Baricault L, Gas N, Guillou E, Valette A, Belenguer P, et al. Loss of OPA1 perturbates the mitochondrial inner membrane structure and integrity, leading to cytochrome c release and apoptosis. J Biol Chem. 2003;278(10):7743-6.

42. Cipolat S, Martins de Brito O, Dal Zilio B, Scorrano L. OPA1 requires mitofusin 1 to promote mitochondrial fusion. Proc Natl Acad Sci U S A. 2004;101(45):15927-32.

43. Twig G, Elorza A, Molina AJ, Mohamed H, Wikstrom JD, Walzer G, et al. Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. EMBO J. 2008;27(2):433-46.

44. MacVicar TD, Lane JD. Impaired OMA1-dependent cleavage of OPA1 and reduced DRP1 fission activity combine to prevent mitophagy in cells that are dependent on oxidative phosphorylation. J Cell Sci. 2014;127(Pt 10):2313-25.

45. Mishra P, Carelli V, Manfredi G, Chan DC. Proteolytic cleavage of Opa1 stimulates mitochondrial inner membrane fusion and couples fusion to oxidative phosphorylation. Cell Metab. 2014;19(4):630-41.

46. Frezza C, Cipolat S, Martins de Brito O, Micaroni M, Beznoussenko GV, Rudka T, et al. OPA1 controls apoptotic cristae remodeling independently from mitochondrial fusion. Cell. 2006;126(1):177-89.

47. Scorrano L, Ashiya M, Buttle K, Weiler S, Oakes SA, Mannella CA, et al. A distinct pathway remodels mitochondrial cristae and mobilizes cytochrome c during apoptosis. Dev Cell. 2002;2(1):55-67.

48. Lenaz G, Genova ML. Kinetics of integrated electron transfer in the mitochondrial respiratory chain: random collisions vs. solid state electron channeling. Am J Physiol Cell Physiol. 2007;292(4):C1221-39.

49. Cogliati S, Frezza C, Soriano ME, Varanita T, Quintana-Cabrera R, Corrado M, et al. Mitochondrial cristae shape determines respiratory chain supercomplexes assembly and respiratory efficiency. Cell. 2013;155(1):160-71.

50. Chen L, Gong Q, Stice JP, Knowlton AA. Mitochondrial OPA1, apoptosis, and heart failure. Cardiovasc Res. 2009;84(1):91-9.

51. Chen L, Liu T, Tran A, Lu X, Tomilov AA, Davies V, et al. OPA1 mutation and late-onset cardiomyopathy: mitochondrial dysfunction and mtDNA instability. J Am Heart Assoc. 2012;1(5):e003012.

52. Ong SB, Subrayan S, Lim SY, Yellon DM, Davidson SM, Hausenloy DJ. Inhibiting mitochondrial fission protects the heart against ischemia/reperfusion injury. Circulation. 2010;121(18):2012-22.

53. Din S, Mason M, Völkers M, Johnson B, Cottage CT, Wang Z, et al. Pim-1 preserves mitochondrial morphology by inhibiting dynamin-related protein 1 translocation. Proc Natl Acad Sci U S A. 2013;110(15):5969-74.

54. Gao D, Zhang L, Dhillon R, Hong TT, Shaw RM, Zhu J. Dynasore protects mitochondria and improves cardiac lusitropy in Langendorff perfused mouse heart. PLoS One. 2013;8(4):e60967.

55. Xiao X, Hu Y, Quirós PM, Wei Q, Lopez-Otin C, Dong Z. OMA1 mediates OPA1 proteolysis and mitochondrial fragmentation in experimental models of ischemic kidney injury. Am J Physiol Renal Physiol. 2014.

56. Piquereau J, Caffin F, Novotova M, Prola A, Garnier A, Mateo P, et al. Down-regulation of OPA1 alters mouse mitochondrial morphology, PTP function, and cardiac adaptation to pressure overload. Cardiovasc Res. 2012;94(3):408-17.

57. Tang Y, Mi C, Liu J, Gao F, Long J. Compromised mitochondrial remodeling in compensatory hypertrophied myocardium of spontaneously hypertensive rat. Cardiovasc Pathol. 2014;23(2):101-6.

58. Davies VJ, Hollins AJ, Piechota MJ, Yip W, Davies JR, White KE, et al. Opa1 deficiency in a mouse model of autosomal dominant optic atrophy impairs mitochondrial morphology, optic nerve structure and visual function. Hum Mol Genet. 2007;16(11):1307-18.

59. Dorn GW, Clark CF, Eschenbacher WH, Kang MY, Engelhard JT, Warner SJ, et al. MARF and Opa1 control mitochondrial and cardiac function in Drosophila. Circ Res. 2011;108(1):12-7.

60. Rahn JJ, Stackley KD, Chan SS. Opa1 is required for proper mitochondrial metabolism in early development. PLoS One. 2013;8(3):e59218.

61. Kasahara A, Cipolat S, Chen Y, Dorn GW, Scorrano L. Mitochondrial fusion directs cardiomyocyte differentiation via calcineurin and Notch signaling. Science. 2013;342(6159):734-7.

62. Yu T, Robotham JL, Yoon Y. Increased production of reactive oxygen species in hyperglycemic conditions requires dynamic change of mitochondrial morphology. Proc Natl Acad Sci U S A. 2006;103(8):2653-8.

63. Williamson CL, Dabkowski ER, Baseler WA, Croston TL, Alway SE, Hollander JM. Enhanced apoptotic propensity in diabetic cardiac mitochondria: influence of subcellular spatial location. Am J Physiol Heart Circ Physiol. 2010;298(2):H633-42.

64. Makino A, Scott BT, Dillmann WH. Mitochondrial fragmentation and superoxide anion production in coronary endothelial cells from a mouse model of type 1 diabetes. Diabetologia. 2010;53(8):1783-94.

65. Zorzano A, Liesa M, Palacín M. Role of mitochondrial dynamics proteins in the pathophysiology of obesity and type 2 diabetes. Int J Biochem Cell Biol. 2009;41(10):1846-54.

66. Parra V, Verdejo HE, Iglewski M, Del Campo A, Troncoso R, Jones D, et al. Insulin stimulates mitochondrial fusion and function in cardiomyocytes via the Akt-mTOR-NFκB-Opa-1 signaling pathway. Diabetes. 2014;63(1):75-88.

67. Quirós PM, Ramsay AJ, Sala D, Fernández-Vizarra E, Rodríguez F, Peinado JR, et al. Loss of mitochondrial protease OMA1 alters processing of the GTPase OPA1 and causes obesity and defective thermogenesis in mice. EMBO J. 2012;31(9):2117-33.

68. Duvezin-Caubet S, Jagasia R, Wagener J, Hofmann S, Trifunovic A, Hansson A, et al. Proteolytic processing of OPA1 links mitochondrial dysfunction to alterations in mitochondrial morphology. J Biol Chem. 2006;281(49):37972-9.

69. Ehses S, Raschke I, Mancuso G, Bernacchia A, Geimer S, Tondera D, et al. Regulation of OPA1 processing and mitochondrial fusion by m-AAA protease isoenzymes and OMA1. J Cell Biol. 2009;187(7):1023-36.

70. Ishihara N, Fujita Y, Oka T, Mihara K. Regulation of mitochondrial morphology through proteolytic cleavage of OPA1. EMBO J. 2006;25(13):2966-77.

**Figure 1**

**Proteolytic regulation of OPA1**
This simplified scheme illustrates the regulation of OPA1 function by proteolysis in mammalian tissue. Alternative splicing of the OPA1 protein yields eight OPA1 isoforms - human and murine tissue predominantly express the OPA1 isoforms 1 and 7. Both these OPA1 isoforms have a S1 cleavage site (exon 5) whereas only OPA1 isoform 2 has a S2 cleavage site (exon 5b). Under basal conditions, the constitutive proteolysis of the OPA1 isoform 2 at the S2 cleavage site results in un-cleaved long OPA1 (l-OPA1) from OPA1 isoform 1 and short OPA1 (s-OPA1) from OPA1 isoform 7, both of which are required for: (1) fusion of the inner mitochondrial membranes (IMM); (2) 'stapling' the cristae junctions closed to prevent cytochrome C redistribution from the cristae to the inter-membranous space (IMS), thereby inhibiting apoptosis; (3) maintaining cristae morphology so as to allow the formation of respiratory complex super-complexes (RCS) and efficient mitochondrial respiration. In contrast, under conditions of stress (mitochondrial membrane depolarization and ATP depletion, heat-stress, oxidative stress and apoptotic signals) the regulated proteolysis of the two OPA1 isoforms at the S1 cleavage site results in the proteolysis of all long OPA1 (l-OPA1) to short OPA1 (s-OPA1), the consequences of which include: (1) the inhibition of mitochondrial fusion thereby allowing un-opposed mitochondrial fission and the selected removal of damaged mitochondria by mitophagy; (2) the opening of the cristae junctions (black arrows) and the redistribution of cytochrome C from the cristae to the IMS, thereby initiating apoptosis; (3) abnormal cristae morphology thereby disrupting the formation of RCS and impairing mitochondrial respiratory efficiency.

**Figure 2**

**Major cardiovascular and metabolic effects of OPA1**
This figure highlights the major cardiovascular and metabolic conditions in which OPA1 has been investigated and implicated.

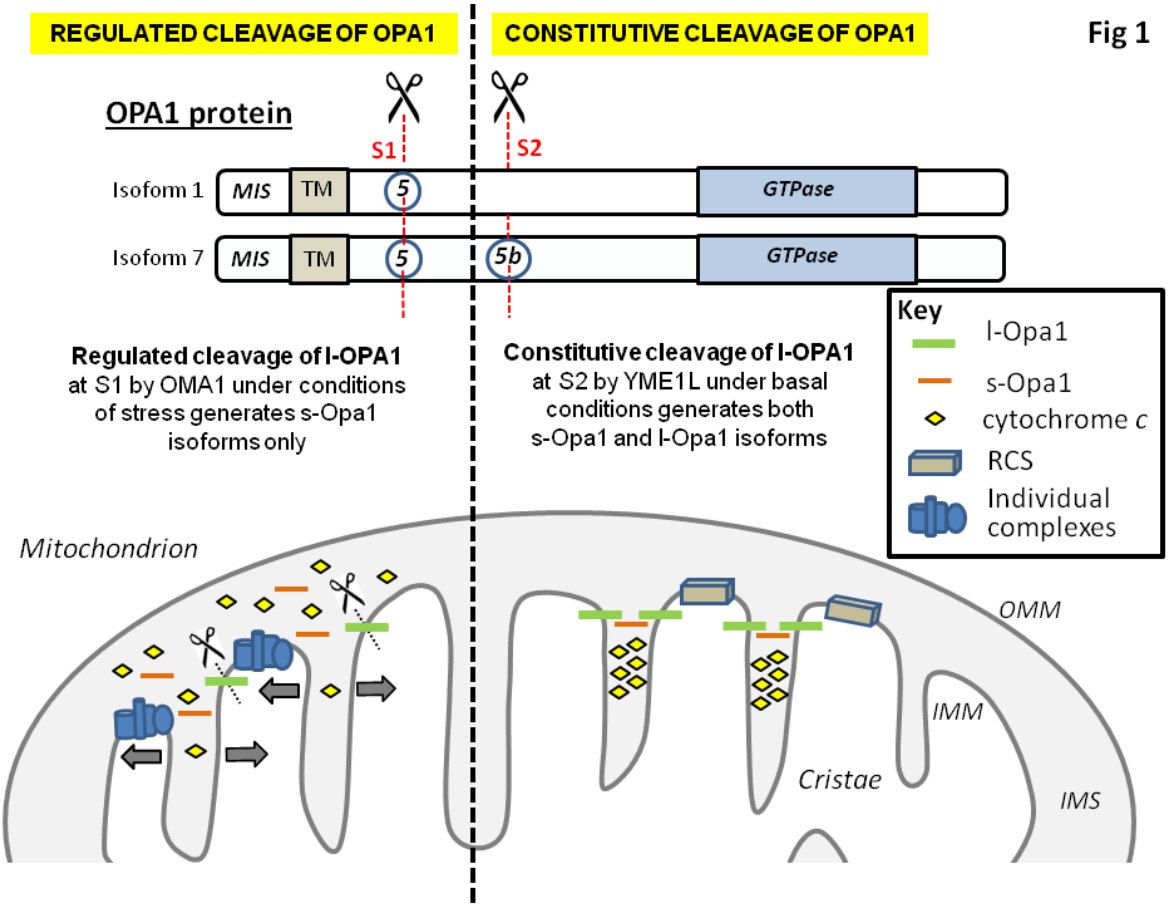
REGULATED CLEAVAGE OF OPA1
CONSTITUTIVE CLEAVAGE OF OPA1
Fig 1
OPA1 protein
S1
S2
Isoform 1
MIS
TM
5
GTPase
Isoform 7
MIS
TM
5
5b
GTPase
Regulated cleavage of l-OPA1
at S1 by OMA1 under conditions
of stress generates s-Opa1
isoforms only
Constitutive cleavage of l-OPA1
at S2 by YME1L under basal
conditions generates both
s-Opa1 and l-Opa1 isoforms
Key
l-Opa1
s-Opa1
cytochrome c
RCS
Individual
complexes
Mitochondrion
OMM
IMM
Cristae
IMS

Fig 2

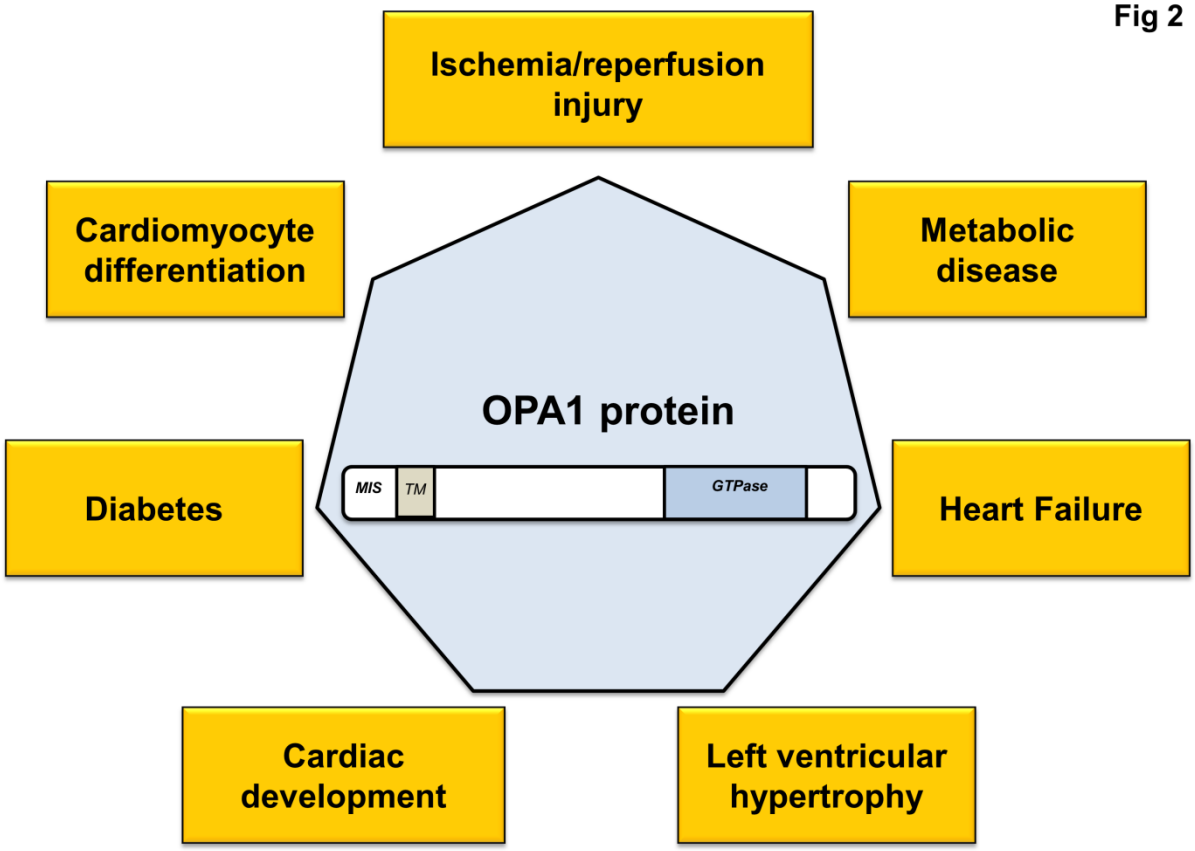
Ischemia/reperfusion injury
Cardiomyocyte differentiation
Metabolic disease
OPA1 protein
MIS
TM
GTPase
Diabetes
Heart Failure
Cardiac development
Left ventricular hypertrophy

**Table 1.** Overview of the main findings investigating the role of OPA1 in cardiovascular and metabolic disease.

| Setting | Study | Model | Outcome |
|---|---|---|---|
| Cardiac development and cardiomyocyte differentiation | Kasahara *et al* 2013 | Mouse ESCs | Cardiac development arrested upon gene trapping OPA1 |
| | Rahn *et al* 2013 | Zebrafish | OPA1 knockdown reduces cardiac contractions and heart rate |
| | Dorn *et al* 2011 | Drosophila | Impaired cardiac contraction + dilatation |
| Left ventricular hypertrophy | Piquereau *et al* 2012 | $OPA1^{+/-}$ knockout mice | Hypertrophy and reduced ejection fraction in $OPA1^{+/-}$ knockout mice |
| | Tang *et al* 2014 | Spontaneously hypertensive rat (SHR) | OPA1 down-regulated in SHR model |
| Heart Failure | Chen L *et al* 2009 | Murine and human heart failure | OPA1 protein levels reduced in these models. Reduced cardiac output, fractional shortening and contraction in $OPA1^{+/-}$ knockout mice |
| | Chen *et al* 2012 | $OPA1^{+/-}$ knockout mouse | These mice develop late-onset cardiomyopathy |
| Ischemia/reperfusion injury | Chen L *et al* 2009 | H9c2 cells | OPA1 levels reduced after simulated ischemic insult |
| | An *et al* 2011 | HEK293T cells | OPA1 over expression is protective simulated ischemic insult |
| | Chen L *et al* 2012 | $OPA1^{+/-}$ knockout mouse | Less cell death after IRI in $OPA1^{+/-}$ knockout cardiomyocytes |
| Diabetes and metabolic disease | Parra *et al* 2014 | Neonatal cardiomyocytes | Insulin-mediated fusion lost when OPA1 silenced |
| | Quirós *et al* 2012 | OMA1 KO mouse | Altered glucose homeostasis and insulin resistance in OMA1 KO |
| | Makino *et al* 2010 | Type I diabetic mouse | Over-expression of OPA1 improves morphology of mitochondria from diabetic murine coronary endothelial cells |

**Table 2.** Summary of the main post-translational modifications of OPA1 protein.

| Modification Type | Effector | Result |
|---|---|---|
| Proteolysis | PARL | Regulates cytochrome *c* release |
| | OMA1 | Decreases l-OPA1 upon ATP depletion/loss of $\Delta\Psi m$ |
| | YME1L | Cleaves to maintain balance of l-OPA1 and s-OPA1. Increased with higher levels of OXPHOS |
| Acetylation | SIRT3 | Improves OPA1's GTPase activity upon stress |
| Acylation | *O*-GlcNAcylation | Suppresses OPA1 activity in presence of high levels of glucose |
| Other | Higd1a | Preserves OPA1 function by binding to l-OPA1 under hypoxic conditions |
| | ROMO1 | Facilitates OPA1 oligomerization |