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New roles for OMA1 metalloprotease
From mitochondrial proteostasis to metabolic homeostasis

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The mitochondrial quality control system is essential for the preservation and regulation of mitochondrial function. This system is formed by a complex machinery that controls and maintains protein function and regulates mitochondrial morphology through a coordinated system of continual fusion and fission events. Impairments in the mitochondrial quality control system through either mutation or deficiency in any of its components, can lead to mitochondrial dysfunction. However, the physiological consequences of these deficiencies remain unknown in most cases. Here, we briefly review the role of the OPA1-OMA1 system in mitochondrial biology, and summarize our recent report on the generation and phenotypic characterization of a model deficient in OMA1, an ATP-independent mitochondrial metalloprotease that participates in mitochondrial quality control. Interestingly, *Oma1*-deficient mice display an obesity phenotype, characterized by hepatic steatosis, decrease in energy expenditure and defective thermogenic regulation. In addition, our study has provided in vivo evidence of OMA1 function as a mitochondrial quality control protease, inactivating OPA1 under stress conditions and inhibiting mitochondrial fusion. Further, we have demonstrated the essential role of the OMA1-OPA1 system for brown adipose function and how this system regulates metabolic homeostasis in mice.

Mitochondria are the central core of energy metabolism within the cell, producing ATP through oxidative phosphorylation, as well as participating in many pathways of intermediate metabolism, calcium regulation and other processes such as apoptosis.[1] For this reason, mitochondria have developed a complex quality control system, comprised of a network of proteases and chaperones, which regulate the assembly, folding and turnover of mitochondrial proteins to maintain proper function.[2,3] Mitochondria are highly dynamic organelles that continually undergo a process of fusion and division. As such, depending on the cell type, tissue or moment, mitochondria adopt morphologies ranging from small punctuate organelles to a highly connected network.[4] Linked to the quality control mechanism exerted by proteases and chaperones, the regulation of mitochondrial dynamics generates another point of control. Thus, mitochondrial dynamics can result in more efficient organelles through induction of mitochondrial fusion, or can protect the mitochondrial network under some stress conditions by coordinating mitochondrial fission. Due to the range and importance of the functions in which mitochondria participate, a highly evolved process of cellular safeguarding has developed. As an illustrative example, if mitochondrial damage continues unabated and its function cannot be rescued, small mitochondria generated by fission can be removed by mitophagy, or apoptotic signals can be orchestrated to remove damaged cells and protect organismal viability.[5,6] Given the importance of the quality control machinery, it is plausible that mutations in any of its protein components could induce mitochondrial dysfunction and underlie human diseases.

The study of mitochondrial dynamics during recent years has permitted the identification of several components of the fusion and fission machinery, as well as some components that participate in the regulation of these processes. In mammals, the fusion process is principally performed by three large GTPases, mitofusin 1 and 2 (MFN1 and MFN2) in the outer membrane and OPA1 in the inner membrane.[7,8] The fission process is largely controlled by the dynamin-related GTPase DRP1, located in the cytoplasm, which is recruited to mitochondria and recognized by specific mitochondrial receptors in the outer membrane, MFF or FIS1.[9,10] Both dynamic processes have to be highly controlled to maintain the balance between fusion and fission events, as well as to enable a rapid response to a wide variety of stimuli in the cell. Thus, a decrease in ATP levels, loss of mitochondrial membrane potential or apoptotic stimuli, induce fragmentation of the mitochondrial network due to inhibition of the fusion process.[11] Conversely, the fission process is inhibited in response to starvation, increasing the connection of the mitochondrial tubular network to protect mitochondria from degradation and increase cell viability.[12] Deregulation of fusion or fission events is implicated in several human diseases, most of them related to neurological disorders, as neurons appear to be particularly susceptible to mitochondrial defects.[13] Thus, Charcot-Marie-Tooth type 2A, autosomal dominant optic atrophy or postnatal death with neurodevelopmental disorder, are due to mutation in *MFN2*, *OPA1* and *DRP1*, respectively.[14-17] Moreover, *OPA1* mutations are also associated with different forms of optic atrophy with or without deafness, ophthalmoplegia, myopathy, ataxia and neuropathy,[18,19] as well as with susceptibility to normal tension glaucoma[20,21] Indeed, mutations in other proteases of the

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mitochondrial inner membrane that belong to quality control systems and are associated with OPA1 processing, have a clear neurological phenotype, such as spastic paraplegia and a dominant form of spinocerebellar ataxia caused by mutations in *SPG7* and *AFG3L2*, respectively.[22,23]

Despite recent advances in our understanding of mitochondrial dynamics, the generation of animal models is essential to understand the entirety of this molecular process and its functional relevance in pathophysiology. In this regard, we have recently reported that OMA1, a mitochondrial metalloprotease that processes the GTPase OPA1 in response to stress stimuli, has a new key role in metabolic homeostasis.[24] OMA1 was identified in yeast as a mitochondrial protease of overlapping activity with the m-AAA protease,[25] a mitochondrial quality control protease located in the inner membrane.[26] Subsequent in vitro studies in mammalian cells determined that OMA1 proteolytically inactivates OPA1, inhibiting the fusion process and catalyzing mitochondrial fragmentation.[27,28] However, the physiological role of this protease remained unknown. Thus, we generated a knockout mouse model of *Oma1* in order to delineate the in vivo roles and relevance in mitochondrial function and quality control of this protease. Surprisingly, *Oma1*-deficient mice showed an unexpected metabolic phenotype, characterized by increase in body weight and hepatic steatosis, together with defective energy balance and thermogenic regulation. Under control conditions, *Oma1*-deficient mice displayed a slight body weight increase and hepatic steatosis. However, on a high-fat diet, *Oma1* knockout mice developed a marked obesity phenotype with a significant increase in body weight and fat mass compared with controls. Further, gonadal and skin adipocytes showed a clear hypertrophy, and hepatic steatosis was enhanced together with an increase in triglyceride levels in blood. In addition, *Oma1*$^{-/-}$ mice displayed reduced energy expenditure, which is in accordance with previous studies in mouse models as a possible cause of an obesity phenotype.[29,30] Further, the metabolic alterations observed in *Oma1*-deficient mice were accompanied by a decrease in the expression of genes controlling mitochondrial dynamics and oxidative phosphorylation, metabolic regulation, β-oxidation and an increase in lipogenic genes in both liver and brown adipose tissues. These gene changes were similarly consistent with previous observations in other murine models in which an obesity phenotype was related to mitochondrial dysfunction.[31] Moreover, thermogenic control and adaptive thermogenic response to cold-stress were both altered, indicating a possible dysfunction in brown adipose tissue function regulating heat production.

Interest in brown adipose tissue has gained significant momentum over the last few years due to recent studies correlating its function to, not only the generation of heat in animals and newborns, but also as an integral tissue for regulating metabolic homeostasis and as a possible control point for obesity.[32,33] In our studies of *Oma1*-deficient mice, brown adipose tissue showed reduced β-oxidation rates in both normal and high-fat diets. These results indicated a dysfunction in brown adipose tissue due to deficiency of the OMA1 protease, under both normal and stress conditions. We analyzed mitochondrial structure by electron microscopy, and found that *Oma1*-deficient mice displayed an increase in mitochondrial size without changes in the number of mitochondria. It has been previously described that under cold-stress conditions, mitochondria elongate, increasing fusion in an attempt to protecting the mitochondrial network.[34] However, analysis of mitochondrial structure after cold-stress of brown adipose tissue from *Oma1*-deficient mice did no display an increase in mitochondrial size in contrast to control mice, indicating a dysfunction in the response to cold-induced stress by the mitochondrial dynamic machinery. In addition, using in vitro experiments with brown adipocytes, we confirmed that both OMA1 and OPA1 are required for β-oxidation, and that this function was dependent on OPA1 isoforms generated by OMA1 activity. Further, analysis of mitochondrial respiratory rates and ATP production were not altered in the liver under normal or high-fat diets; however, β-oxidation rates were significantly reduced in liver under high-fat diet treatment. These results confirmed that the OMA1-OPA1 system is required for the proper function of brown adipose tissue and for keeping metabolic homeostasis in mice.

The deficiency in OMA1 alters the balance between fusion and fission, and can be the origin of the metabolic alterations observed in our mouse model. Thus, we have demonstrated in vivo that OMA1 has a non-redundant role in the proteolytic inactivation of OPA1 under stress conditions. OPA1 functions are controlled by alternative splicing and proteolysis of the different isoforms. Thereby, OPA1 is characterized by at least five different isoforms, two long isoforms (L-OPA1; a and b), and three short isoforms (S-OPA1; c–e).[11,35] Under stress conditions, such as loss of mitochondrial membrane potential or apoptotic stimuli, L-OPA1 isoforms are cleaved to S-OPA1 isoforms by OMA1 activity, inhibiting the fusion event.[36,37] This inhibition is due to the fact that, for an inner membrane fusion event to occur, OPA1 requires at least one of each of the long and short isoforms, forming a complex structure to induce the fusion of membranes. At present, several mitochondrial proteases have been implicated in the processing and regulation of OPA1 levels, such as PARL1, m-AAA and YME1L1.[27,35,38] Analysis of *Oma1*-deficient cells demonstrated the absence of one of the short isoforms of OPA1 and a decrease in the expression of the other under basal conditions (**Fig. 1**; c and e, respectively). Moreover, under stress conditions, *Oma1*-deficient cells displayed no proteolysis of OPA1 to generate the shorter isoforms. This observation reinforces that under normal conditions, several inner membrane proteases could regulate the generation of some S-OPA1 isoforms, although in response to stress conditions the inactivation of OPA1 is exclusively due to OMA1 function.

As fusion and fission are interdependent, inhibition of one of the two creates disequilibrium in the balance of these two events. Under normal conditions mitochondria adopt a tubular morphology that fragments in response to fission predominance. Conversely, inhibition of fission increases the tubular connection of mitochondria due to increased fusion. Analysis of mitochondria from *Oma1*-deficient MEFs showed, predominantly, an archetypal tubular morphology with a significant increase in the

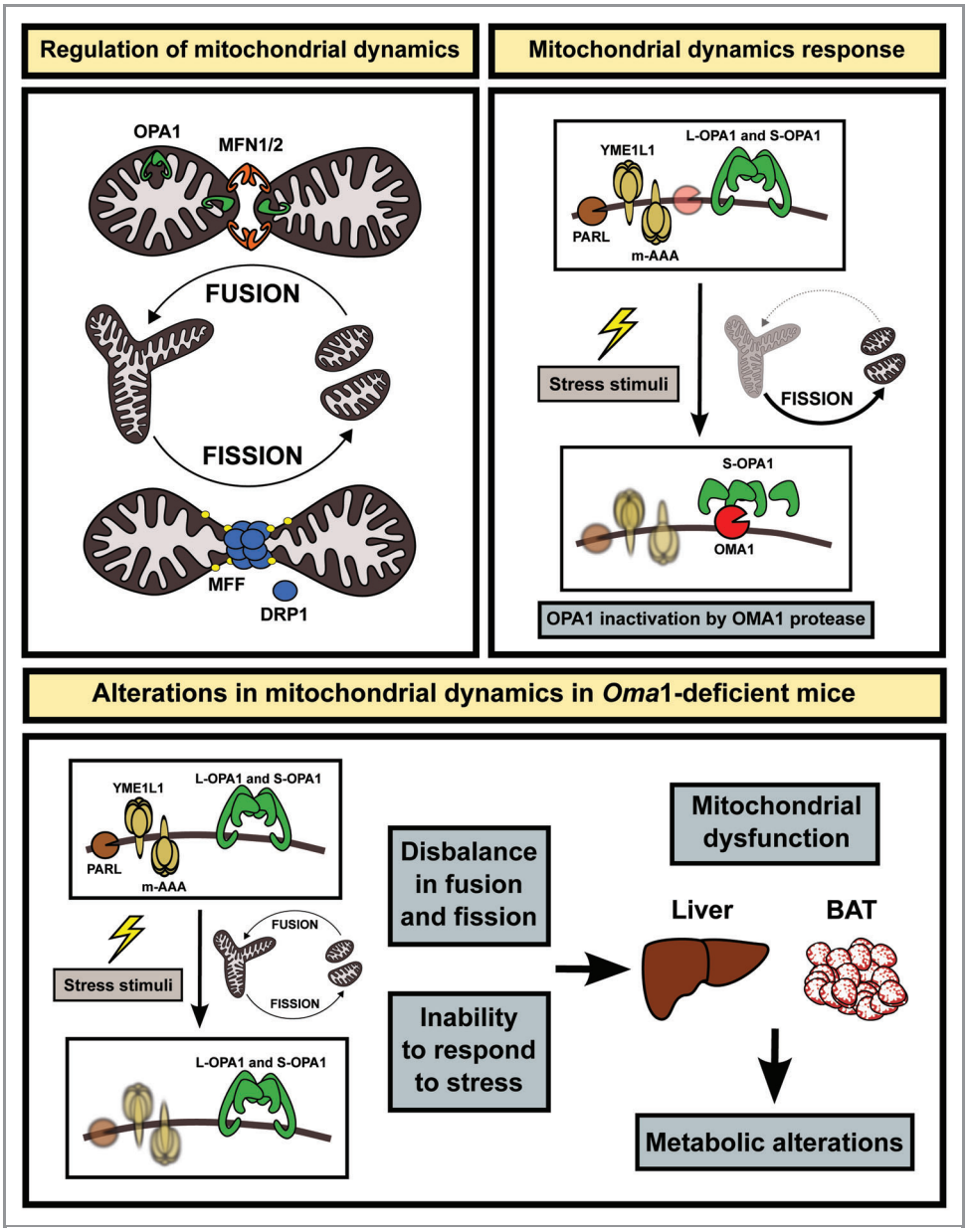

Figure 1. Schematic representation of mitochondrial dynamics regulation, the mitochondrial dynamics response mediated by OMA1 and the alterations observed in *Oma1*-deficient mice due to alterations in mitochondrial dynamics equilibrium under stress conditions.

elongated mitochondrial morphologies. Further, under stress conditions, *Oma1*$^{-/-}$ MEFs maintain their tubular morphology due to absent fusion. Intriguingly, the stabilization of L-OPA1 isoforms in *Oma1*-deficient MEFs protected cells from apoptotic stimuli, due to maintenance of cristae morphology and prevention of cytochrome c release.[38,39] Thus, the OMA1-OPA1 system is critical to maintain mitochondrial function and has an important role in mitochondrial quality control, exerting its function on mitochondrial dynamics and apoptosis levels. The alterations in this control system due to *Oma1* ablation induce mitochondrial dysfunction under stress conditions due to inability to adequately respond to stress stimuli, such as metabolic stress induced by high-fat diet or cold-stress.

Future Perspectives

Oma1-deficient mice have provided the first in vivo evidence linking dysfunction in the mitochondrial quality control system with an obesity phenotype and deregulated thermogenic control. Interestingly, recent publications have shown that OPA1 function is necessary for mitochondrial glucose-stimulated ATP production in pancreatic β cells,[40] and for mediating adrenergic control of lipolysis, as a dual-specificity A-kinase anchoring protein in lipid droplets.[41] Collectively, these findings further emphasize the importance of the OMA1-OPA1 system for metabolic homeostasis. Looking toward the future, the possibility must be entertained that OMA1 has other additional substrates and interaction partners that can contribute to

the quality control system or to other mitochondrial pathways. The potential identification of novel regulatory proteins belonging to the OMA1 proteostasis network would provide further insights into mitochondrial function and could also shed new light on human pathologies. Accordingly, *Oma1* knockout generation has allowed the discovery of an unexpected role of this mitochondrial quality control protease as a new key regulator of metabolic homeostasis. Hopefully, the use of this and other mouse models of mitochondrial quality control components will let us further understand this complex system and its associated human diseases.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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