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Review

Eight human OPA1 isoforms, long and short: What are they for?

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ABSTRACT

OPA1 is a dynamin-related GTPase that controls mitochondrial dynamics, *cristae* integrity, energetics and mtDNA maintenance. The exceptional complexity of this protein is determined by the presence, in humans, of eight different isoforms that, in turn, are proteolytically cleaved into combinations of membrane-anchored long forms and soluble short forms. Recent advances highlight how each OPA1 isoform is able to fulfill "essential" mitochondrial functions, whereas only some variants carry out "specialized" features. Long forms determine fusion, long or short forms alone build *cristae*, whereas long and short forms together tune mitochondrial morphology. These findings offer novel challenging therapeutic potential to gene therapy.

1. Introduction

The mitochondrial network morphology is the result of a constant dynamic balance between organelles fusion and fission, adapting the cell to the energetic requests and preserving the homeostasis and the quality of mitochondria. The protein machinery deputed to mitochondrial dynamics includes Mitofusins (MFN1 and MFN2) and DRP1, respectively involved in fusion and fission of the outer mitochondrial membrane (OMM), and the conserved dynamin-like GTPase OPA1 that plays a crucial role in the inner mitochondrial membrane (IMM) fusion [1].

Growing evidence indicates that, in addition to fusion, OPA1 is implicated also in other important mitochondrial functions, such as mitochondrial DNA (mtDNA) maintenance, probably by anchoring this genome to the IMM [2], the respiratory chain supercomplexes (RCS) assembly and the energetic efficiency [3]. OPA1 is also required for *cristae* structure organization [3] and apoptosis regulation through the compartmentalization of soluble cytochrome *c* within the *cristae* [4,5]. Recently, OPA1 has been shown to promote and regulate the mitochondrial pH flashes, which are bioenergetic responses to drops in mitochondrial membrane potential ($\Delta\psi_m$) [6,7]. OPA1 also plays a significant role in the control of mitochondrial Ca^{2+} homeostasis [8] (Fig. 1).

In the year 2000, OPA1 mutations were for the first time reported as causative for dominant optic atrophy (DOA), a blinding disease characterized by selective degeneration of the retinal ganglion cells (RGCs) and optic nerve atrophy [9]. About 50% of the pathogenic mutations are predicted to produce a truncated protein, indicating haploinsufficiency as the molecular mechanism causing DOA. The missense mutations, frequently clustered in the GTPase domain and assumed to exert a dominant-negative effect, are in most cases associated with the severe multisystem disorder recognized as DOA "plus". This is characterized by a multi-systemic involvement, associated with a large spectrum of clinical features, including Parkinsonism and dementia, as well as a disorder indistinguishable from multiple sclerosis [10].

The investigation of humans and mouse models identified further, more tissue-specific functional roles of OPA1. Retinal studies of an Opa1 haploinsufficient mouse model showed that the expression of the glutamate NMDA receptors was significantly increased [11] and OPA1 depleted RGCs were more susceptible to glutamate excitotoxicity [12]. OPA1 also seems to be somehow linked with aging, in fact selective loss of glutamatergic, but not GABAergic, synaptic sites, leading to dendritic degeneration was reported in aged $Opa1^{+/-}$ mice [13]. Sedentary but not active humans display an age-related decline of OPA1 protein levels associated with muscle loss [14] and OPA1, together with MFN1, regulates the metabolic shift from glycolysis to mitochondrial respiration in old human fibroblasts during chronological lifespan [15]. In adipocytes cellular triacylglycerol accumulation is regulated, at least in part, via mitochondrial fusion and fission processes, with mitochondrial morphology altered from filamentous to fragmented upon differentiation to adult adipocytes [16]. Furthermore, OPA1 has been proposed to anchor the A-kinase to lipid droplets to mediate the adrenergic control of lipolysis [17] (Fig. 1). Consistently perturbation of OPA1 processing causes obesity and defective thermogenesis in mice [18].

All together these findings support a multiplicity of cellular

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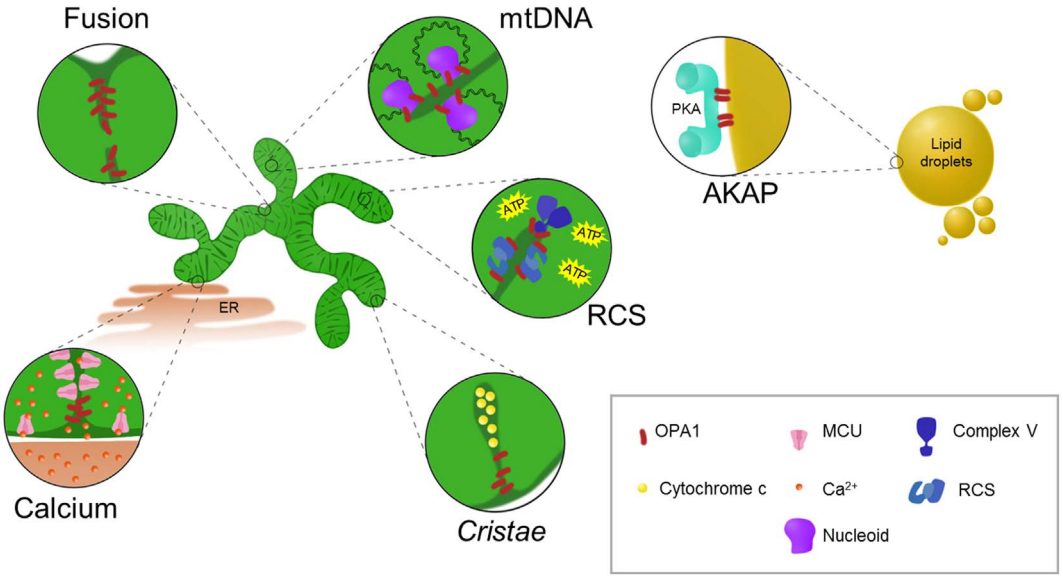

Fig. 1. Mitochondrial and extra-mitochondrial OPA1 functions.
Schematic representation of OPA1 functions. OPA1 is responsible for IMM fusion and, as a component of nucleoids, is involved in mtDNA maintenance by its anchoring to the IMM. It is crucial for the proper assembly of respiratory chain supercomplexes (RCS) and Complex V. OPA1 participates to the *cristae* organization, keeping tight the *cristae* junctions, and to apoptosis, by cytochrome *c* compartmentalization. OPA1 is implicated also in mitochondrial calcium homeostasis at the mitochondrial associated membranes (MAMs), endoplasmic reticulum (ER) subdomains in close contact with mitochondria. In adipocites OPA1 acts, on lipid droplets surface as A-kinase anchoring protein (AKAP), thus regulating the lipid metabolism. MCU (mitochondrial calcium Unipoter).

functions for this dynamin related GTPase, in most cases strictly associated with the classical mitochondrial location but a few possibly occurring also in extra-mitochondrial compartments. Further studies are needed to better understand the relevance of these latter aspects within mitochondrial functions.

2. OPA1 protein: structure and isoforms

The OPA1 protein is localized in the mitochondrial intermembrane space (IMS), anchored to the IMM. In humans, OPA1 is present in eight mRNA variants, deriving from the alternative splicing of exons 4, 4b and 5b, encoding proteins of 924–1014 aminoacids, whose N-terminus includes a mitochondria targeting sequence, followed by a transmembrane domain (TM), embedded in the IMM, and a coiled coil domain. The next portion of the protein includes three highly conserved dynamin regions: the GTPase domain, the middle domain and the C-terminus GTPase effector domain (GED) [19] (Fig. 2). The eight different isoforms are ubiquitously expressed but present in different amount depending on the tissue considered [20]. After import of the precursor protein through the OMM and IMM translocases, cleavage of the mitochondrial targeting sequence generates the membrane-anchored OPA1 long forms (l-forms) that may be further proteolytically processed at the N terminus producing the short forms (s-forms) soluble in the IMS [21]. The four isoforms containing the exon 4b are totally processed into the soluble s-forms [22]. Two IMM peptidases are involved in the process, OMA1 operating at the cleavage site S1 in exon 5, and YME1L at site S2 in exon 5b (Fig. 2). Under normal conditions, YME1L is constitutively active, whereas OMA1 is inactive, but can be activated by stress conditions and mitochondrial dysfunction (dissipation of $\Delta\Psi_m$) [23,24], increasing the s-forms. Activated OMA1 was shown to undergo autocatalytic self-degradation, thus allowing for mitochondrial network recovery [23]. Interestingly, during mitochondrial depolarization the two proteases are differentially degraded through a mechanism controlled by cellular ATP availability. In fact treatments that drop both $\Delta\Psi_m$ and ATP levels stabilize active OMA1 and provoke YME1L degradation, strongly influencing OPA1 processing and the pattern of the s-forms [25]. The complex reciprocal degradation of the two proteases can therefore profoundly affect the balance of l-/s-forms as well as the network morphology.

The unbalance toward the OPA1 s-forms, fusion inhibition and unopposed network fragmentation have fundamental consequences within the cell, triggering the activation of a process referred to as "mitochondrial quality control" or MQC This marks the isolated mitochondrial fragments with reduced $\Delta\Psi_m$ toward their removal by autophagy, a process defined ad mitophagy [26,27]. Signaling of and marking dysfunctional mitochondria for mitophagy is driven by a complex machinery of factors, which includes the PINK1/Parkin axis, a hot topic for the pathogenesis of Parkinson disease [28]. Interestingly, the E3 ubiquitin ligase Parkin, through linear ubiquitination of NF-kB essential modulator (NEMO) may also regulate the expression of OPA1 [29]. On the contrary, mitochondrial network elongation, e.g. during starvation, was shown to hinder autophagic degradation [30]. There is therefore a tight homeostatic relationship between mitochondrial dynamics, energetic status and MQC. Remarkably, recent results reported in fibroblasts bearing different *OPA1* mutations, including those linked to Parkinsonism, demonstrate excessive mitochondrial network fragmentation which results into increased mitophagy [31,32]. However, a detailed understating of the mechanistic interplay between mitochondrial dynamics and MQC needs further studies on a larger number of patients and by refining reliable quantitative mitophagy assays, under different metabolic conditions.

3. Role of the eight OPA1 isoforms in mitochondrial functions

A great variability of the eight OPA1 isoforms was detected in different human tissues, suggesting a fine regulation of alternative splicing of OPA1 mRNA. To gain insights on the phenotypes associated with the expression of different isoforms, selective silencing of each of the three alternative exons was performed in HeLa cells, where the variants bearing exon 4 are the most abundant. Noticeably, the mitochondrial morphology of exon 4 silenced cells exhibits highly fragmented network and loss of $\Delta\Psi_m$, whereas both exon 4b and 5b silenced cells display typical hallmarks of apoptotic cell death [20]. Interestingly, only exon 4b silenced cells displayed mtDNA depletion and a marked