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Imbalance of the REDOX state in dominant Optic Atrophy: the way of mathematical modeling

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Abstract

Autosomal dominant optic atrophy (DOA) is a common cause of inherited visual failure affecting at least 1 in 50,000 of the general population. OPA1 mutations are the main genetic cause of DOA, causing bilateral optic atrophy due to specific loss of retinal ganglion cells. Although optic nerve degeneration remains the hallmark of DOA, syndromic form of the pathology, including deafness, ataxia, myopathy, peripheral neuropathy and progressive external ophtalmoplegia, was reported to affect up to 20% of all mutations carriers. More than 300 mutations have been listed, the major mutations being truncations, therefore supporting haploinsufficiency as the major pathogenic mechanism of DOA. To assess the consequences of OPA1 depletion on mitochondrial energetic metabolism and redox state, we mimicked haploinsufficiency phenomenon, using a silencing RNA strategy to down-regulate OPA1 expression in rat cortical neurons in primary culture.

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Introduction

DOA, also known as Kjer's disease, is characterized by moderate to severe loss of visual acuity with insidious onset in early childhood (Amati-Bonneau et al., 2009) associated with optic nerve atrophy. The disease affects primary the retinal ganglion cells (RGC) and their axons forming the optic nerve, which transfer the visual information from the photoreceptors to the lateral geniculus in the brain. Estimated disease prevalence is between 1:10,000 in Denmark and 1:50,000 worldwide. There is a considerable inter- and intra-familial variation in visual acuity, and the penetrance may be as low as about 40%. This complex pathology remains without effective treatment to date. The majority of patients (about 75%) with DOA harbors mutation in the OPA1 gene (Delettre et al., 2000). 358 different OPA1 mutations have been reported to date (http://mitodyn.org), the majority of which results in premature termination codons and lead to haploinsufficiency by the reduction in OPA1 protein levels. Although, there is a marked variability in the rate of disease progression, a significant proportion of patients (50-75%) will experience further worsening of their visual function in later life. Interestingly, recent studies evidenced a severe multi-systemic disorder associated with some OPA1 mutations, named 'DOA plus' syndrome (OMIM#125250) (Amati-Bonneau et al., 2008). Patients with DOA plus syndrome present additional neurological complications such as ataxia, sensorineural deafness, chronic progressive external ophtalmoplegia (CPEO) and sensory-motor neuropathy and myopathy in adult life. Although these syndromal DOA variants show significant phenotypic variability even within the same family, a consistent finding is a worse visual prognosis among this patient subgroup. These observations are of major pathophysiological importance, highlighting the widespread deleterious consequences of OPA1 mutations, not only for RGCs, but also for other neuronal populations and skeletal muscle.

The OPA1 gene encodes a mitochondrial protein localized in the inter-membrane space (IMS) and anchored to mitochondrial inner membrane. Using common genetically modified cell lines (HeLa, Cos, MEF), we and others have shown that OPA1 has various functions, which include inner membrane fusion, cristae structuration, mtDNA maintenance, mitochondrial energetics modulation and protection from apoptosis (Landes et al., 2010). To what extent inactivation of OPA1 function contribute to DOA and DOA+ pathogenesis still has to be elucidated. Data on skin-fibroblasts, lymphoblasts or muscles from patients, suggest that impairment of mitochondrial morphology, respiration and energetics, loss of mtDNA integrity, and an increased sensitivity to apoptosis could be involved. However, there were a lot of contradictory reports regarding the existence and nature of energetic defects in DOA patients (Landes et al., 2010). Mouse DOA or DOA plus models evidenced mitochondrial fragmentation, cristae disorganisation, increased mitophagy and cytochrome c oxidase deficiency (Bertholet et al., 2016). Two DOA invertebrate models underlined the critical generation of ROS associated to OPA1 dysfunction, as it was proposed for Leber Hereditary Optic Neuropathy (LHON) and other neurodegenerative pathologies such as Alzheimer and Parkinson diseases (Chao de la Barca et al., 2016). We thus addressed the question of the impact of OPA1 inactivation on redox state as well as considering the controversy on oxidative phosphorylation and respiration. Because haploinsufficency is primarily responsible for DOA and as the effects of OPA1 inactivation are not restricted to retinal ganglionic cells, we considered the general impact of OPA1 inactivation on oxidative metabolism by downregulating OPA1 in rat cortical neurons in primary culture, in which OPA1 was silenced by RNA interference, in OPA1enu/+ mice as well as in fibroblasts from DOA or DOA+ patients.

To further circumvent the problem of variability between patients, we aimed to build a mathematical model which is able to predict ROS production by complex I of the mitochondrial respiratory chain, one of the two major sites of ROS production by mitochondria.

Materials and methods

Chemicals and reagents

The different immunoblots were realized as described in (Millet et al., 2016) with anti-aconitase (1/500, Abcam, Cambridge, USA), anti-SOD1 and anti-SOD2 (1/2000, Epitomics, Abcam, Cambridge, USA), anti-

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catalase (1/3000, Abcam, Cambridge, USA), anti-actin (1/25000, Chemicon, Merck Millipore, UK).
Aconitase and catalase activities measurements were performed as mentioned in (Millet et al., 2016)
Statictical analysis are detailed in (Millet et al., 2016)

Neurons materials

Cortical neurons from rat embryos in primary culture ($1x10^6$) were obtained and described in (Bertholet et al., 2013) and electroporated using the Rat Neuron Nucleofector Kit (Amaxa, Lonza) according to the manufacturers' optimized protocol as mentioned in (Millet et al., 2016) as well as the validation of Si OPA1.

Results and discussion

Down regulation of OPA1 induces an imbalance of the redox state

In cortical neurons from rat embryos in primary culture, we found that cellular respiration is diminished when OPA1 is decreased. This is accompanied by an increase in mitochondrial ROS production (Millet et al., 2016)(table 1: decreased aconitase activity revealing an increase in mitochondrial ROS production) which is buffered by the activation of NRF2 pathway and increased levels and activity of catalase but no increase of SOD 1 and 2 (other major NRF2 targets involved in dismutation of superoxide anion) (table 1). Contrary to results obtained in DOA mice models in which we observed an increase of SOD1 and SOD2 expression between 4 and 10 month old mice (Millet et al., 2016). However, this situation leads to a pro-oxidative state, since further acute or chronic exogenous oxidative stress (rotenone treatment) challenged both the antioxidant response and the viability of OPA1 depleted neurons analysed by trypan blue exclusion and counting picnotic nuclei after DAPI staining (Millet et al., 2016).

Mathematical model of ROS production by the Complex I of the mitochondrial respiratory chain

Our objective is to create a mathematical model of the molecular mechanisms involved in the pathogenesis of Dominant Optic Atrophy in order to predict their evolution and give appropriated treatment based on in-silico analysis with physiological parameters from a specific patient. We present a stochastic model of catalytic activity and ROS production of respiratory complex I which is the first step towards a more complete mathematical model describing the different dysfunctions encountered in DOA or pathologies related to OPA1 gene disorders. We developed the model using a Petri net formalism and a continuous-time Markov chains theory. The simulations were realized on Matlab and compared to kinetic data from the literature. Our model is able to reproduce the dynamics of the complex I system and to simulate observed behaviours of this system regarding ROS production (Merabet et al., 2017).

Conclusion

Our study shows a link between OPA1 and oxidative metabolism as we demonstrated an inhibition of aconitase activity, a major activation of the NRF2 transcription factor and an increase of some NRF2 targets directly involved in antioxidant signaling. Indeed, we show that the mitochondrial respiratory chain is impaired by the loss of OPA1 and in neurons in primary culture. On the other hand, we observed a small decrease in the total ROS levels (Bertholet et al., 2013). We suggest that antioxidant defenses are activated to circumvent oxidative imbalance induced by OPA1 inactivation, which indicated that the cells are in a "pro-oxidative state". Retinal ganglion cells could present a modified oxidative metabolism as we observed the mechanism in different models (cortices from DOA mice model, cortical neurons in primary culture or patients fibroblasts). Furthermore, these cells are exposed to UV and hypoxia conditions. These exogenous stresses could induce degeneration of DOA patient retinal ganglion cells.
Moreover, while analyzing their fibroblasts, we found, that some patients showed altered expression of genes implicated in oxidative metabolism. On the one hand, these data reveal modifier genes of the severity of DOA and one explanation for the inter- and intra-familial variations and on the other hand, the results direct pharmacological approaches to prevent and treat DOA pathogenesis. Thus, we can propose that mutations or decreased expression of OPA1 induces an imbalance in the cellular redox state, weakening cells to exogenous pro-oxidative stresses. This phenomenon would be the major key of the molecular mechanisms involved in DOA pathogenesis and diseases related to OPA1 gene disorders (Figure 1).

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Table 1. Results obtained from siOPA1 or siControl treated Neurons in primary culture in immunoblot (relative quantities with actin contents) or enzyme activities.

Protein Name	Neurons treated with si control	Neurons treated with si OPA1
Superoxide dismutase 1 (SOD1)	1.10+/-0.10 AU	1.65 +/-0.19 AU (NS)
Superoxide dismutase 2 (SOD2)	1.19+/-0,17 AU	1.67 +/-0.25 AU (NS)
Catalase quantity	0.85+/-0.14 AU	1.67 +/-0.25 AU (p<0.01)
Catalase activity	100%	161% (p<0,05)
Aconitase quantity	0.83+/-0.16 AU	0.77 +/-0.11 AU (NS)
Aconitase activity	100%	73% (p<0.05)

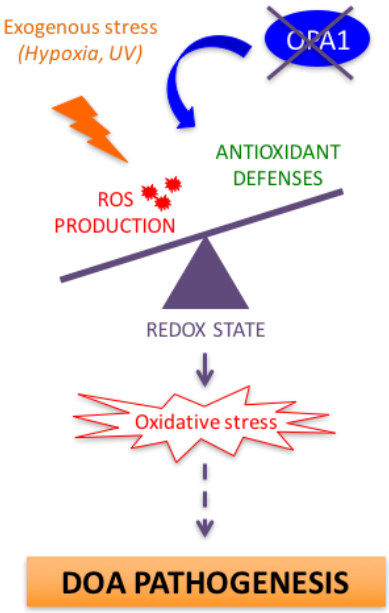

Figure 1. Cascade of events implying the oxidative metabolism in DOA and OPA1 gene deficit related diseases

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