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Analysis of opa1 isoforms expression and apoptosis regulation in autosomal dominant optic atrophy (ADOA) patients with mutations in the opa1 gene

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ABSTRACT

Autosomal dominant optic atrophy (ADOA) is a hereditary optic neuropathy characterized by bilateral symmetrical visual loss, decrease in retinal ganglion cells and a loss of myelin within the optic nerve. ADOA is associated to mutations in *Optic atrophy 1* gene (*OPA1*), which encodes a mitochondrial protein involved in cristae remodeling, maintenance of mitochondrial membrane integrity, mitochondrial fusion and apoptosis regulation. We thus evaluated the rate of apoptosis and the expression levels of OPA1 isoforms in ADOA and control cells.
Peripheral blood lymphocytes from eight patients with *OPA1* mutation and age matched controls were cultivated both in basal conditions or with 2-deoxy-D-ribose, a reducing sugar that induces apoptosis through oxidative stress. Apoptosis was analyzed by flow cytometry, phosphatidylserine translocation, mitochondrial membrane depolarization and caspase 3 activation. We also analyzed the expression levels of OPA1 isoforms in ADOA and control cells cultured with and without 2-deoxy-D-ribose.
We showed an increased percentage of apoptotic cells in ADOA patients compared to controls, both in basal culture conditions and after 2-deoxy-D-ribose treatment. This suggested a great susceptibility of ADOA cells to oxidative stress and a strong correlation between OPA1 protein dysfunctions and morphological–functional alterations to mitochondria. Moreover OPA1 protein expression was significantly decreased in lymphocytes from the ADOA patients after 2-deoxy-D-ribose treatment, implying a great sensitivity of the mutated protein to free radical damage.
Concluding, we could confirm that oxidative stress-induced apoptosis may play a key role in the pathophysiological process bringing to retinal ganglion cells degeneration in ADOA.

1. Introduction

Autosomal dominant optic atrophy (ADOA) is the most frequent form of hereditary optic neuropathy, generally characterized by juvenile onset and with a prevalence of 1:12,000 to 1:50,000 [11]. The majority of ADOA patients are mono-symptomatic, showing a moderate to severe decrease in visual acuity, color vision disturbance, central visual field defects and temporal optic nerve pallor. Visual loss is usually progressive, although there is considerable variability among patients [37]. ADOA has incomplete penetrance and its clinical expressivity is heterogeneous even within families: asymptomatic carriers have been identified only following diagnosis of their symptomatic family members [3]. A subset of patients (up to 20%) exhibit additional multi-system neurological disease known as ADOA plus or OPA1-plus syndrome, with a combination of optic atrophy, sensorineural hearing loss, peripheral neuropathy, ataxia and myopathy [3].

The basic pathological characteristics correlating with visual symptoms are a decreased number of retinal ganglion cells (RGCs), especially in the central retina, and a loss of myelin and nerve tissue within the optic nerve, optic chiasm, and optic tracts, suggesting that the disorder is a primary degeneration of RGCs with ascending optic atrophy [22,23].

Most ADOA cases are caused by mutations in the *optic atrophy 1* (*OPA1*) gene (3q28–29), a nuclear gene that spans approximately 100 kbp. The *OPA1* gene is composed of 30 coding exons of which exons 4, 4b, and 5b are alternatively spliced, generating 8 mRNA variants (isoforms) [10]. The OPA1 protein is a dynamin-related GTPase whose mature structure includes a transmembrane domain, a GTPase domain, a central dynamin region and a C-terminal coiled-coil GTPase

Abbreviations: AU, arbitrary units; ADOA, autosomal dominant optic atrophy; cyt c, cytochrome c; dRib, 2-deoxy-D-ribose; GED, GTPase effector domain; IMM, inner mitochondrial membrane; $\Delta\Psi m$, mitochondrial membrane potential; MTS, mitochondrial targeting signal; OMM, outer mitochondrial membrane; PBLs, peripheral blood lymphocytes; PS, phosphatidylserine; RGCs, retinal ganglion cells.

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