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1 Screening of candidate nuclear genes for modifying role in Leber's hereditary optic neuropathy penetrance: A signal from manganese superoxide dismutase

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Leber's hereditary optic neuropathy (LHON) is a maternally inherited blinding disorder due to three frequent mtDNA mutations affecting complex I and leading to retinal ganglion cells degeneration and optic atrophy. Penetrance is incomplete, even when the mtDNA mutation is homoplasmic. The modifying role of nuclear genes is assumed to play a role in expressing the pathology. Biochemical investigations in LHON established that bioenergetic inefficiency is combined with increased production of reactive oxygen species. In this study we explored the possible role of the antioxidant machinery as modifier for the expression of LHON. We screened functional polymorphisms in the manganese and copper/zinc superoxide dismutases (MnSOD, Cu/ZnSOD), in the glutathione peroxidase (GPx) and in the catalase (CAT). We first investigated a large LHON Brazilian/Italian pedigree, comparing affected individuals ($n = 26$) with unaffected mutation carriers ($n = 41$). The only significant association ($p = 0.036$) was found with the MnSOD Ala9Val variant, for which the Ala/Val genotype was most frequent in the affected subjects (65.4%), whereas the Val/Val genotype was most frequent in the carrier individuals (55.3%). Furthermore, we collected 111 Italian LHON unrelated probands and we found that the Ala9Val variant distribution overlapped that found in the Brazilian affected individuals, being significantly different from an equivalent control group of Italians matched for age and sex ($p = 0.05$). We conclude that only the MnSOD variant seems to modulate the risk of becoming affected. Contrary to the prediction the protective allele is Val, which affects the targeting sequence for mitochondrial import inducing a partial stalling of MnSOD transport, thus lowering the final amount of active enzyme in the matrix. We propose that high MnSOD activity in mitochondria of LHON subjects, which overproduce superoxide, may become deleterious if downstream GPx fails to buffer the excess H_2O_2.

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2 Reactive oxygen species mediated regulation of mitochondrial biogenesis in the yeast *Saccharomyces cerevisiae*

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Mitochondrial biogenesis is a complex process. It necessitates the participation of both the nuclear and the mitochondrial genomes. This process is highly regulated and mitochondrial content within a cell varies according to energy demand. In the yeast *Saccharomyces cerevisiae*, we have shown that the cAMP pathway is involved in the regulation of mitochondrial biogenesis. An overactivation of this pathway leads to an increase in mitochondrial enzymatic content. Out of the three yeast cAMP protein kinases (Tpk1p, Tpk2p and Tpk3p), we have previously shown that Tpk3p is the one involved in the regulation of mitochondrial biogenesis. We investigated the molecular mechanisms that govern this process. We show that in the absence of Tpk3p, mitochondria produce large amounts of ROS and that the ROS produced in the intermembranal space signal to the HAP2/3/4/5 nuclear transcription factors involved in mitochondrial biogenesis. We clearly establish that an increase in mitochondrial ROS production down-regulates mitochondrial biogenesis. It is the first time that a redox sensitivity of the transcription factors involved in yeast mitochondrial biogenesis is shown. Such a process could be seen as a mitochondria quality-control process.

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3 The unexpected capacity of melanin to dissociate water molecule is a new way to improve mitochondrial cytopathies

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Mitochondrial cytopathies—disorders of the energy-producing organelles of the cells—are an increasingly recognized cause of human illness Mitochondria, contained in all human cells except mature erythrocytes, perform the vital task of generating adenosine triphosphate (ATP), the molecule the cell uses for the bulk of its energy needs. ATP is continuously recycled in organisms, with the human body turning over its own weight in ATP each day. But the quantity of food that each day we consume, could not explain the energy need to recycled 70 kg ATP daily.

Melanin is a heteropolymer, with amazing properties. We found, after studied during more than 12 years the three main causes of blindness, that melanin is to the animal kingdom what chlorophyll is to the plant kingdom.[i] Both compounds separate the hydrogen from water. The unsuspected capability of the eukaryotic cell of take energy directly from water opens novel therapeutic pathways in many diseases.[ii] This energy, only observed in vegetables[iii] before we detected it in human retina, is essential for the human body, because comprise more than the 34% of the whole amount of the daily energetic requirements of our organism. Melanin delivers hydrogen from water to the cell and the hydrogen is the carrier of energy that nature uses most. In other words, melanin captures photonic energy and transforms it into chemical energy. We could define photosynthesis as the absorption of photons from electromagnetic radiation, which brings about an ionic event.

The chemical reactions in chlorophyll and melanin have similarities, and basically are:

$2H_2O \rightarrow 2H_2 + O_2$, but only in melanin are fully reversible and the direction of the reaction depend of the concentration of the reactants and/or their products.

Water molecule is a very important source of energy to any eukaryotic cell, and this energy is the best fitted to improve mitochondrial function, because the hydrogen it is the exactly compound that drives the function of the ATP synthase, and if this enzyme has not a well performance, then the function of mitochondria, eukaryotic cell, the tissue, organs and the system would be damaged.

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[iii]Dadachova, Ekaterina, Bryan Ruth, A., Howell Robertha, C., Schweitzer Andrew, D., Aisen Philip, Nosanchuk Joshua, D., Casadevall Arturo, 2007. The radioprotective properties of fungal melanin are a function of its chemical composition, stable radical presence and spatial arrangement. Pigment Cell Melanoma Res. 21, 192–199. doi:10.1111/j.1755-148X.2007.00430.x.

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4 Cytochrome *c*: A recurring nightmare in mitochondrial diagnostic assays

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A contaminant in commercial cytochrome *c*, identified as a quinol [Mitochondrion 8(155), 2008], resulted in a large antimycin A insensitive background rate in Complex III assays. This was circumvented by purchasing cytochrome *c* which did not contain the quinol from another vendor. In February 2008 a marked delay occurred in the delivery of cytochrome *c* from this later vendor. When the material did become available, high backgrounds were noted not only in the Complex III assay, but also in the linked Complex I/Complex III assay. Furthermore, and more distressing, there was a marked impairment in determining the first-order rate constants for cytochrome *c* oxidase. The previously described quinol contaminant was not the culprit as measured by FPLC of cytochrome *c*. In fact, only one main chromatographic peak was observed.

In discussions with Dr. Ferguson-Miller (Michigan State University) she described this problem as a well known issue of cytochrome *c* degradation uncovered by her laboratory, even for high-grade commercial preparations. Using a weak cation exchange chromatography protocol adapted from the Ferguson-Miller lab's method, we were able to separate intact cytochrome *c* from three earlier eluting (weaker binding) contaminants, previously characterized as deaminated forms of cytochrome *c*. These contaminants accounted for 25–35% of the total starting material. The purified cytochrome *c* exhibited markedly decreased inhibitor-insensitive background rates when used in Complex III and coupled Complex I/Complex III assays, and, upon reduction, the reduced cytochrome *c* produced well behaved first-order rate constants in Complex IV assays even with low-activity samples.

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6 Respiratory chain complex I deficiency in oncocytic tumours

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Many cancer cells are characterized by a shift in energy metabolism from aerobic oxidation in the mitochondria to anaerobic glycolysis.

Recently, we have demonstrated that mitochondria rich (oncocytic) tumours of the thyroid and kidney lack respiratory chain complex I, caused by disruptive mutations in mitochondrially encoded complex I subunits.

To elucidate if complex I deficiency is a general feature of oncocytic tumours, we immunohistochemically quantified respiratory chain enzymes and screened for mutations of the mitochondrial genome in oncocytomas of the parathyroid ($n = 6$), parotid gland ($n = 4$), pituitary gland ($n = 2$), the eyelid ($n = 1$), adrenal gland ($n = 1$) and salivary gland ($n = 1$).

We observed a lack of complex I in 14/15 oncocytomas, while other respiratory enzymes were considerably up-regulated. Pathogenic mutations have been found in mitochondrially encoded subunits of complex I.

In summary, oncocytomas, independent of their localization, seem to be characterized by a loss of complex I and a compensatory hyperproliferation of other respiratory chain enzymes.

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7 Mitochondrial haplogroups and control region polymorphisms are not associated with prostate cancer in Middle European caucasians

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Besides being responsible for energy production in the cell, mitochondria are central players in apoptosis as well as the main source of harmful reactive oxygen species. Therefore, it can be hypothesised that sequence variation in the mitochondrial genome is a contributing factor to the etiology of diseases related to these different cellular events, including cancer. The aim of the present study was to assess the frequency of haplogroups and polymorphisms in the control