Leber hereditary optic neuropathy and oxidative stress

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Relatively little progress has been made in developing therapies for mitochondrial diseases in modern medicine as a result of the exquisite complexity of the structural proteins and pathways associated with mitochondrial functions and our incomplete understanding of pathophysiology (1). Leber hereditary optic neuropathy (LHON), in particular, provides a unique model for understanding molecular mechanism and testing promising treatments as a result of its characteristic sequential bilateral involvement and accessibility of retina as the target tissue within the eye. Lin et al. report the establishment of an Leber hereditary optic neuropathy (LHON) mouse model by introducing a mitochondrial DNA (mtDNA) mutation (2).

LHON is a mitochondrial disorder with a maternal inheritance. It is characterized by degeneration of retinal ganglion cells and the optic nerve with sudden onset and usually severe bilateral loss of central vision, predominantly in young men (3). LHON has been associated with three primary mtDNA mutations: G3640A (4), G11778A (5), and T14484C (6), leading to missense mutations in NADH dehydrogenase. NADH dehydrogenase is a part of a large multienzyme complex I that generates ATP as a cellular energy source through electron transfer and oxidative phosphorylation (OXPHOS). The pathological feature of LHON is the small-caliber axonal demyelination and loss, fiber swelling, and abnormal mitochondria. How these mutations in complex I elicit degeneration in the optic nerve is unclear. Furthermore, the relatively late onset, specific vulnerability in retinal ganglion cells (RGCs), and gender bias of LHON are not understood.

Many pathogenetic mechanisms have been proposed, including complex I dysfunction with decreased ATP synthesis, elevated levels of oxidative stress, and impaired glutamate transport, all leading to RGC dysfunction and ultimately to apoptotic cell death. However, these hypotheses are based on research in cybrid cell lines using transmitochondrial technology (7–11). An animal model with a mutated complex I gene in the mitochondria genome is much needed for the validation of in vitro experiments and study of the pathophysiological mechanism in vivo. Lin et al. describe the creation of a mouse model of LHON (2). In particular, the authors introduced the P25L mutation in ND6, which causes LHON in humans, into mice, and show that oxidative stress rather than energy deficiency appears to be an important factor contributing to LHON in this animal model.

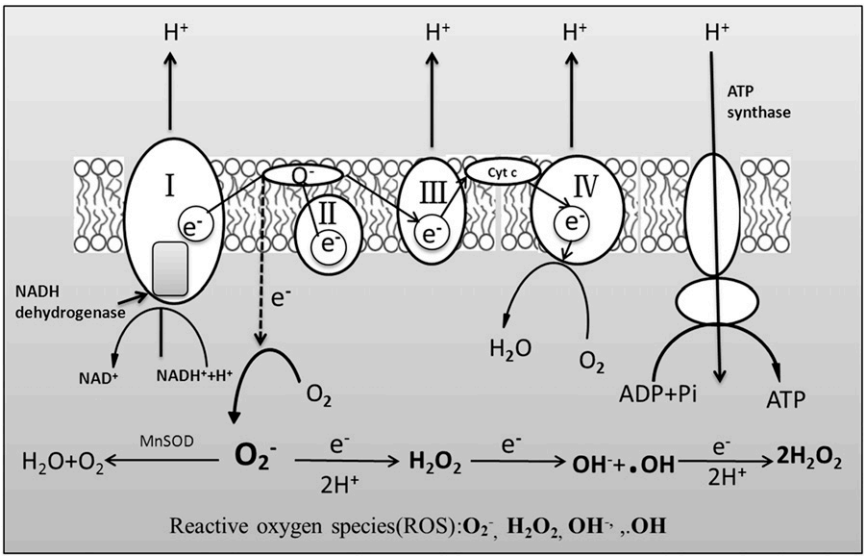

Fig. 1. ROS produced through the process of oxidative phosphorylation by the electron transport chain in mitochondria. NADH dehydrogenase mutations of complex I may increase the leaking of electrons from the chain, leading to increased accumulation of ROS, oxidative stress, and RGC death.

Two major approaches have been tried to transmit genetically modified mtDNAs into the mouse germ line: (*i*) fusion of cytoplasts of enucleated cell with mutant mtDNA to undifferentiated mouse female stem cells and injection of the stem cell cybrids into mouse blastocysts; and (*ii*) fusion of cytoplasts from mutant cells directly to mouse single-cell embryos (12). Lin et al. (2) report a multiple-step process to produce a mtDNA ND6 G13997A P25L transgenic mouse by first isolating the desired mouse mtDNA mutation, in homoplasmic form in cultured mouse cells following random chemical mutagenesis, then fusing cytoplasts derived from these cells with embryonic stem (ES) cells (from which the mitochondrial genomes had been acutely removed by rhodamine treatment) and, finally generating viable mice from these cybrid ES cells. The phenotypic, biochemical, and molecular analysis of the mice reveal many features seen in patients with LHON: including reduced elecroretinographic response, small-fiber axonal swelling and loss of RGCs, and abnormal mitochondrial morphology. Thus, this mouse model should provide valuable insights into the pathophysiological basis of LHON.

Little is known about progressive morphologic changes in LHON since postmortem eyes have been subjected to histopathologic examinations decades after the onset of disease. By this late stage, the optic nerves were found to be severely degenerated with demyelination and axonal loss in the retrobulbar space. The animal model established by Lin et al. (2) makes it possible to observe the progressive developmental process of this disease. The mutant mice exhibited age-related loss of small-caliber optic nerve fibers, giving rise to swollen, demyelinated fibers that harbored increased numbers of highly abnormal mitochondria (2). Thus it may be possible to identify early markers of disease progression that could aid in diagnosis and predictive treatment.

The mitochondria produce much of the cellular energy via a process of OXPHOS, in which ATP is coupled to protons across the inner mitochondrial membrane by means of the electron

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transport chain (Fig. 1). As a byproduct, OXPHOS produces most of the endogenous toxic reactive oxygen species (ROS; Fig. 1). It is intriguing that no other major neuronal cell loss has been observed in affected retinas except RGCs, even though photoreceptors also have very high energy requirement and a correspondingly high mitochondrial content. Moreover, patients with LHON rarely have other systemic symptoms except RGC loss. Therefore, the hypothesis of bioenergetic deficiency seems unlikely, suggesting the possibility that RGCs may be highly sensitive to ROS-induced apoptosis (13).

In LHON, the flow of electrons that normally pass along the electron transport chain may be disturbed by a result of the mutated complex I gene (Fig. 1). The freed electrons are then available to react with molecular oxygen, generating levels of superoxide beyond the capability of dismutation by endogenous manganese superoxide dismutase within mitochondria, thereby leading to oxidative stress in RGCs. This oxidative stress may damage proteins, lipids, and DNA, ultimately culminating in RGCs death and optic neuropathy. Lin et al. (2) conduct synaptosome analysis in LHON model mice and found that ATP production is maintained under various energetically demanding conditions coincident with an increase in ROS production and oxidative damage, providing experimental evidence that the ROS play a pivotal role in the pathogenesis of LHON.

Oxidative damage has been proposed to participate the pathophysiology of several neurodegenerative diseases, such as Parkinson disease, Alzheimer's disease, amyotrophic lateral sclerosis, and age-related macular degeneration. The ROS and oxidative stress hypotheses are also broadly consistent with epidemiological studies that have found that environmental factors such as smoking and ethanol intake (which can exacerbate oxidative stress) can worsen the disease course and vision loss.

Results of the study of Lin et al. suggest that treatments to reduce oxygen toxicity may be beneficial in slowing, preventing, or restoring vision loss.

At the present time, there is no effective treatment for LHON. Results of the study of Lin et al. (2) suggest that treatments to reduce oxygen toxicity may be beneficial in slowing, preventing, or restoring vision loss. Treatment with antioxidants such as vitamins (D and E), coenzyme Q10 and its analogues, Trolox, thiamine, and superoxide dismutase-catalase mimetics could be tested in this mouse model. We note, however, that results of previous epidemiological studies in which people were treated with antioxidants were inconclusive and controversial (14).

The method described by Lin et al. could be used to address additional questions related to LHON. For example, it is not understood why patients with LHON carrying the m.11778G>A or m.3460G>A mutation have a more severe clinical presentation and a much lower chance of spontaneous recovery from vision loss compared with patients with the m.14484T>C mutation. Interactions between nuclear genes and the mitochondrial genome, and the effects of heteroplasmy, both of which add a level of complexity to genotype/phenotype relationships in LHON, could also be studied (15).

In conclusion, Lin et al. (2) successfully establish a mouse model of LHON by introducing an mtDNA mutation into the female mouse germ line to be subsequently transmitted maternally through repeated generations. This animal model of LHON is available for detailed biochemical, physiological, and molecular analysis, and for testing candidate therapies.

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