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Review

Investigating Leber's hereditary optic neuropathy: Cell models and future perspectives

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ABSTRACT

Leber's hereditary optic neuropathy (LHON) was the first human disease found to be associated with a mitochondrial DNA (mtDNA) point mutation. The most common LHON mutations are 11778G>A, 3460G>A or 14484T>C. The most common clinical features of LHON are optic nerve and retina atrophy. The affected tissue is not available for studies, therefore a variety of other cell types are used. However, all models face difficulties and limitations in mitochondrial disease research. The advantages and disadvantages of different cell models used to study LHON, recent advances in animal model generation and novel approaches in this field are discussed.

Contents

1. Introduction

Leber's hereditary optic neuropathy (LHON) is the most common mitochondrial disease (Chinnery et al., 2001; Carelli et al., 2004). The clinical phenotype of LHON is the degeneration of retinal ganglion cells (RGCs) and a progressive degeneration of the optic nerve. It typically presents in males during adolescence or young adulthood with painless loss of central vision in one eye, followed by loss of vision in the second eye within weeks or months (Hirano, 2014). LHON shows

Abbreviations: CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats; EBV, Epstein Barr virus; LHON, Leber's hereditary optic neuropathy; RGC, retinal ganglion cells; EtBr, ethidium bromide; mitoTALENs, mitochondria targeted transcription-activator-like effector nucleases.

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incomplete penetrance with only 50% of male and approximately 10% of female mutation carriers developing the symptoms in their lifetime (Kirkman et al., 2009a). LHON affects about 1 in 30,000 to 50,000 people in northeast England and Finland, with approximately 1 in 200 individuals being at risk mutation carriers in the United Kingdom (Yu-Wai-Man et al., 2011; Man et al., 2003). However, the prevalence of LHON in most populations is unknown.

The main function of mitochondria is to generate energy which is used by the cell. Mitochondria contain their own DNA (mtDNA) molecule of 16,569 bp. It encodes only 37 genes, 13 for respiratory complex subunits, 22 for mitochondrial tRNA and 2 for rRNA. Most cases of LHON are caused by single nucleotide point mutations resulting in a dysfunctional protein subunit of complex I of the electron transport chain (ETC). About 70% of all LHON cases are caused by the 11778G>A mutation (Cwerman-Thibault et al., 2014).

Battisti et al. (2004) reported that lymphocytes from patients were more prone to undergo apoptosis compared to age matched healthy controls. Evidence of mitochondrial involvement in activation of the apoptotic cascade in this study suggests that the changes in mitochondrial respiratory chain or in oxidative stress could play a role in the induction of apoptosis in LHON (Battisti et al., 2004).

LHON is generally perceived as a disease of young men, reflecting its male preponderance (80%) and its early disease onset between 15 and 30 years. While genetic causes of the disease are well known, pathogenesis still remains poorly understood. Therefore, cell models are needed to obtain better insight into the pathomechanism, the genotype-phenotype correlations and various factors affecting the susceptibility to this disease as RGCs from patients are not available.

Lymphoblasts, fibroblasts and transmitochondrial cytoplasmic hybrids, called cybrids, are the most commonly and widely used cell models in LHON investigation. Cybrid cell lines have made important contributions in revealing mitochondrial factors influencing LHON expression. Due to progress in science and technology, new promising models are currently being developed: induced pluripotent stem cells and animal models.

2. Cell line models most commonly used to study LHON

2.1. Lymphoblasts

Advances in biomedical research have been spurred in part by the availability of cell lines from biological material which offer a long lasting supply of cells with matching genotypes and phenotypes (Hussain and Mulherkar, 2012). Lymphoblast cell cultures are established by immortalization of the cells from peripheral blood samples by Epstein Barr virus (EBV) serving as rapidly growing and permanent cultures for studies. Moreover, it is possible to obtain higher quantities of material, which allows combination of quantitative and qualitative analysis of the mitochondrial OXPHOS system using a single aliquot of blood (Pecina et al., 2014).

A commonly used assay to screen or measure mitochondrial impairment is glucose-free galactose medium which forces generation of ATP through OXPHOS. A recent study showed that in glucose-free media LHON lymphoblast cultures grew approximately over 2 times more slowly than cells of age-matched healthy controls. In addition, the degree of complex I driven ATP synthesis decreased significantly by 17% in LHON lymphoblast cultures (Van Bergen et al., 2015). Lymphoblasts derived from a female LHON patient (affected in both eyes) were found to be more susceptible to oxidative and nitrosative stress. In this patient an abnormal increase of lactate in serum after exercising was observed, suggesting possible limited mitochondrial reserve capacity, which leads to the pathomechanism of the disease (Fallabela et al., 2016).

While the lymphoblast cell culture model unlike other cell models allows maintaining a higher amount of research material, there are some problems that have to be taken into account: sensitivity to various factors, such as temperature, pH, the medium or serum in which cells are being cultured, possible contamination and the length of time in continuous culture. It is worth noting that lymphoblasts are heterogeneous cell cultures therefore some scientists depending on study needs may reconsider their use. Moreover, an immortalization procedure is necessary to establish this cell line and this transformation affects cell metabolism. The above-mentioned issues make these cell lines less valuable for biochemical and cellular studies. However, lymphoblast cell cultures provide a cost-effective testing system in which environmental factors, such as medications, can be controlled (Welsh et al., 2009). Lymphoblast cell lines are useful when a single sample is required for a variety of experiments if the patient withdraws from the study (Hussain and Mulherkar, 2012).

2.2. Fibroblasts

Fibroblasts are another cell culture widely used for LHON research. Fibroblasts derived from skin biopsies may mirror some important features of the RGCs. Fibroblasts are also easy to culture, which makes them attractive as a cell culture. Moreover, they are primary cells that do not require transformation. Therefore fibroblasts as primary cells can represent what is occurring *in vivo*. Lymphoblasts and fibroblasts allow observing events in a cell with a LHON mutation and the nuclear background of the patient.

Fibroblast cell cultures have been used for mitochondrial proteome analysis and to study the effects of idebenone on respiratory chain activity. In one study a statistically significant increase by 42% of complex I enzymatic activity in fibroblasts derived from LHON affected patients was observed suggesting that idebenone might improve respiration in LHON cases (Angebault et al., 2011). Tun et al. (2014) have found that proteins responsible for bioenergetic pathways and mitochondrial protein quality control are significantly differently expressed in fibroblasts of the patients, 11778G>A mutation carriers and control individuals. Moreover, other proteins, such as catalase and mitofilin were also differently expressed. The results imply that bioenergetic perturbations and poor protein quality control systems, both incompatible with regular functioning of RGCs, may play a role in LHON pathogenesis.

Fibroblasts are broadly used in environmental studies. Such environmental factors as alcohol consumption or tobacco smoke were suspected of modifying susceptibility to LHON. Previously, several research groups published data indicating a correlation between tobacco smoke and high alcohol intake and LHON (Kirkman et al., 2009b). One study did not find any association between tobacco smoke or alcohol consumption and LHON (Kerrison et al., 2000). However, Giordano et al. (2015) exposed fibroblasts from affected individuals, unaffected mutation carriers and healthy controls to cigarette smoke condensate (CLS) and found that mtDNA content and ATP were lower in fibroblasts from affected individuals. In addition, the authors reported that the level of detoxifying enzymes was higher in mutation carriers, suggesting that some compensatory mechanism was activated.

Fibroblasts were also used in studies of ATP compensation systems. Fibroblasts obtained from unaffected LHON mutation carriers, grown under metabolic pressure driven by galactose medium or after ethidium-bromide driven mitochondrial depletion, activated mitochondrial biogenesis and mitochondrial replication more efficiently than those from LHON affected individuals (Giordano et al., 2014). These findings imply that mitochondrial biogenesis is highly efficient in unaffected carriers.

Use of fibroblast cells is quite a common procedure in LHON studies. However, a successfully established fibroblast cell culture from a patient's skin biopsy may take several weeks of cultivating to obtain a sufficient quantity of cells for the planned experiments.

2.3. Cybrids

Cytoplasmic hybrid cells (cybrids) are cells constructed by fusion of cytoplasts harboring mutant mtDNA (platelets or enucleated