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REVIEW ARTICLE

The pathological mechanisms and novel therapeutics for Leber's hereditary optic neuropathy

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Abstract: Optic neuropathies were estimated to affect 115 in 100,000 population in 2018. Leber's Hereditary Optic Neuropathy (LHON) as one of such optic neuropathy diseases that was first identified in 1871 and can be defined as a hereditary mitochondrial disease. LHON is associated with three mtDNA point mutations which are G11778A, T14484, and G3460A that affect the NADH dehydrogenase subunits of 4, 6, and 1, respectively. However, in most cases, only one point mutation is involved. Generally, in manifestation of the disease, there are no symptoms until the terminal dysfunction in the optic nerve is observed. Due to the mutations, nicotinamide adenine dinucleotide (NADH) dehydrogenase or complex I is absent and thus ATP production is stopped. This further causes the generation of reactive oxygen species and retina ganglion cells apoptosis. Aside from the mutations, there are several environmental factors such as smoking and alcohol consumption that can be pointed out as the risk factors of LHON. Nowadays, gene therapy has been intensively studied for LHON treatment. Disease models using human induced pluripotent stem cells (hiPSCs) have been utilized for LHON research.

Keywords: Induced pluripotent stem cells; Leber's Hereditary Optic Neuropathy (LHON); Optic neuropathy; Retina ganglion cell

1. INTRODUCTION

Optic neuropathies constitute a class of neurological disorders and are the common cause of vision loss.[1] The incidence of optic neuropathies is estimated to be around 115 per 100,000 population in 2018 and is expected to increase over the years.[2,3] Optic neuropathies present with different signs and symptoms; however, there are various causes, and they may be unique to each neurological disease.[2] After the signs and symptoms are known, the cause should be directly determined to determine the most suitable treatment to prevent other neurological diseases.[2] One of the clinical case of optic neuropathy presentation was not alike from the infectious neuroretinitis related to cat-scratch disease.[4] There are some causes of optic neuropathy that were identified such as an increase in intracranial pressure, inflammatory, vascular, compressive, infiltrative, paraneoplastic, toxic, nutritional, hereditary, traumatic, and congenital.[1]

Diagnosis of optic neuropathy is usually dependent on the identification of the location of damage that is responsible for the visual loss.[4] The mechanism of optic neuropathy varies and typically is manifested with decreased visual acuity, change in color vision, abnormal visual field.[4] All diagnosis is based on the patient's clinical history and examination that includes the onset of visual loss, presence of pain in the eye, visual acuity, and retention of color vision. Diagnosis of optic neuropathy can also be done using optic nerve imaging such as retinal digital photography, optical coherence tomography, and magnetic resonance imaging.[4] However, disease history and clinical examination are still necessary to narrow down the various causes and diagnoses of optic neuropathy.[5] Several diseases relate to optic neuropathy such as Leber's Hereditary Optic Neuropathy (LHON), traumatic optic neuropathy, ischemic optic neuropathy, and brain tumor-caused optic neuropathy.[5] This article is only going to focus on LHON.

2. LEBER'S HEREDITARY OPTIC NEUROPATHY

Leber's Hereditary Optic Neuropathy (LHON) was first identified in 1871 by Theodor Leber, whereby 55 patients from 16 families were identified, comprising mostly young adult males.[6] As was investigated from the patient's descendants, the mutation was inherited from the mother to the affected son or a carrier

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daughter. Furthermore, it was initially classified as an X-linked recessive disorder, however, because of the greater occurrence in women than in maternal grandfathers affected males this suggests otherwise.[6]

LHON can be defined as an inherited mitochondrial disease that can cause blindness and is more common in males. LHON specifically damages the retinal ganglion cells (RGCs) by decreasing the ability to produce energy.[7] LHON is the most common mitochondrial hereditary disease, for example it is estimated to affect 1 in 25,000 in the North-East of England.[7] Signs and symptoms of LHON may show as subacute central scotoma, painless and progressive visual disturbance including blind spot and central fixation, and may affect both eyes at once or consecutively within a short time.[8] There are three mtDNA point mutations implicated in LHON which affect the genes MT-ND4, MT-ND1, and MT-ND6; however, in 90% of LHON cases, blindness is caused by only one point mutation.[7,8] The mutations do not cause any signs and symptoms until an unknown trigger induces the dysfunction of the RGCs, which causes the decreased vision in both eyes.[7,8] All three mutations are involved in gene encoding complex I subunits (NADH dehydrogenase) of the mitochondria.[7] Complex I dysfunction can further lead to RGC apoptosis related to cytochrome c release, Fas-induced apoptosis, or caspase-independent apoptosis caused by the lack of ATP.[8] Nevertheless, there are some cases of patients carrying mtDNA mutations that are not affected by the disease. Recently circular RNAs (circRNAs) appear to be the potential regulators of cellular processes.[9,10] It was suspected that other factors can cause LHON patients to have vision loss such as environmental factors.[11–13]

3. PATHOLOGICAL MECHANISMS

RGCs in LHON are characterized by the impaired energy production of the mitochondria. Previous research focused on repairing the ATP production in the complex I dysfunction mitochondria also to find out the cause of this dysfunction.[14,15] RGCs are the most affected neuron cells in LHON, it causes injury and even death of RGC. RGC uses adenosine triphosphate (ATP) as an energy that is produced by the mitochondria from oxidative phosphorylation (OXPHOS).[14,16] ATP production using OXPHOS uses several kinds of enzymes such as nicotinamide adenine dinucleotide (NADH) ubiquinone oxidoreductase also known as NADH dehydrogenase or complex I which is made of several subunits.[14] In 90% of LHON patients, it is found that there are three primary points mitochondrial DNA (mtDNA) mutations, furthermore, these mutations can affect different kinds of subunits of the NADH dehydrogenase and cause dysfunction of the electron transport chain, decrease in ATP production, and generation of reactive oxygen species (ROS).[14] Low ATP production and ROS can eventually cause zero energy production and cell death and result in a visual loss in LHON patients.[14] The most common three point mutations G11778A, T14484, and G3460A affect the NADH dehydrogenase subunits of 4, 6, and 1, respectively.[14,17] Clinically it is hard to differentiate these mutations, especially during the acute phase of LHON. Previous studies showed that the ND4 mutation has the worst prognosis with only 23 out of 204 patients who show visual recovery.[14]

Apart from having the point mutation, there are also some other risk factors and causes of this disease. Environmental factors such as smoking and alcohol are pointed out as the possible risk factors of LHON. Previous studies showed the cyanogenic substances resultant from tobacco smoke may trigger LHON.[11,18] There are also associations between smoking and the decreased activity of complex I that may lead to mitochondrial dysfunction in LHON.[11,18] Furthermore, the risk of male LHON patients mutation carriers increased by 43% in smokers.[11] Tobacco toxicity reduces mtDNA copy number in cells and can also directly affect the mitochondrial functions in mutant cells.[12] It is suggested that tobacco directly inhibits the single respiratory complex I and IV in normal cells.[12] On the other hand, mutant cells are unaffected and can deal with the toxicity and activate mitochondrial biogenesis.[12] However, the exact mechanism and signaling pathways are still unclear. Additionally, heavy alcohol intake has also been associated with LHON disease.[13] Previous studies have shown that there is a significantly higher proportion of male LHON patients with excessive alcohol consumption compared with the male general population.[11]

4. DISEASE MODELING

In the past, the rodent model system has been used as a disease model for LHON.[19,20] LHON mtDNA mutation was introduced into the mouse germline. Mouse with mutation shows a reduction of retinal function, age-related decline in central smaller caliber optic nerve fibers, accumulation of abnormal mitochondria, and demyelination.[20] The mitochondrial analysis also shows an increased production of ROS.[20] The rotenone-induced model has also been utilized for the LHON disease model. This model does not carry LHON disease-specific mutation, however, it is a chemically induced complex I inhibitor model where rotenone was intravitreally injected to cause ROS-mediated toxicity and apoptosis in RGCs to mimic LHON.[21,22] However, because these models did not provide disease-relevant tissue availability and the species-specific difference, the results are not accurate.[19] Nowadays, RGCs derived from human induced pluripotent stem cells (hiPSCs) have been utilized as an in vitro disease model for LHON. hiPSC is promising because it can directly reproduce the patient-specific conditions for research. Moreover, observation of pathological phenotypes of the mitochondrial disease will be more accurate because patient-specific iPSC-derived RGCs were directly used.[8] Previous studies showed iPSC-derived from patients have a normal karyotype, and it can still retain the mutation that caused the disease.[23] This model gives new potential therapeutic approaches and specific targets for better treatment of LHON.[19] hiPSC is reprogrammed from adult somatic cells and can be generated into different types of cells.[19] Compared with the two disease models mentioned, hiPSC has more advantages because it can be made to both patient and carrier specific. These models also showed survival and integration of RGC with no rejection at long-term usage.[19] RGC derived from hiPSC derived from patients was further researched as LHON disease model. Comparing the RGC from a healthy subject, it was shown that ROS production in patient and carrier RGC was increased.[24] Furthermore, apoptosis in patient RGC increased significantly compared to a healthy subject. On the other hand, there is no significant increase of apoptosis in carrier RGCs.[24] Our unpublished data further indicated that upregulation of hsa_cir0087207 may contribute to the differential manifestations of disease phenotypes between patient- and carrier-derived RGCs (Biomedicine, in press).

5. TREATMENTS

Currently, a lot of treatments have been utilized to treat LHON, but available treatments are not efficient yet. Gene therapy is one of the treatments utilized to replace the missing protein products. Gene therapy for LHON is challenging because targeting the double membranes of mitochondria is not easy.[25] Furthermore, gene therapy needs to sustain the gene expression and delivery into the mitochondrial matrix. Nowadays genetically modified adeno-associated viral vectors (AAV2) have been

used to compensate for the mutated gene MT-ND4 as one of the therapeutic strategies.[25] Preclinical data have shown promising results *in vitro* and the decrease of RGC loss and thus improve visual functions.[25] Nevertheless, this treatment strategy still needs to undergo clinical trials and tests on LHON patients.

Another treatment that can target the dysfunction of mitochondria in LHON disease is mitochondrial replacement techniques.[25] This technique is developed to prevent the transmission of mtDNA mutations from parents to children. The transfer of parental nuclear material into the mitochondrial donor zygote by carrying only the wild-type mtDNA is done in this technique. Experimental data have also shown that the technique is promising and does not carry over the mutant mtDNA.[25] However, this method is quite controversial because it may modify the child's genetic material from a third person. There is also a concern that there might be adverse effects from the new genetic admixture.[25]

Although both LHON patients and carriers have the same mutation, only patients undergo vision loss, suggesting that there might be another secondary cause for patients to lose vision. This matter can be further investigated to find out the cause and treatment. It has been reported that a high concentration of ROS contributes to the exacerbated apoptosis in RGCs in LHON patients.[24] However, in vitro data showed that both carrier and LHON patient iPSC-derived RGCs develop high ROS but only the RGCs from patients exhibited high apoptosis level.[24] This observation challenged the hypothesis that ROS serves as the pivotal factor in the pathogenesis of LHON, suggesting that other factors may contribute to the RGC apoptosis in LHON patients. Recently, our unpublished data indicated that hsa_circ0087207 was increased in the iPSC-derived RGCs from LHON patients but not in those from unaffected carriers. Manipulation of hsa_circ0087207 indicated that this circular RNA was a contributor to apoptosis in patient iPSC-derived RGCs. However, whether hsa_circ0087207 can be used as a diagnostic biomarker or a potential therapeutic target requires more efforts in preclinical studies in the future. Recent studies provided remarkable data demonstrating that transplanted pluripotent stem cell-derived RGCs can integrate and form synapses in a mouse model with RGC depletion.[26] These findings showed promising therapeutic potential and raised the possibility that pluripotent stem cell-derived RGCs can be used for in vivo transplantation and the rescue of vision loss in patients suffering from severe and irreversible optic neuropathy.

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