CHAPTER I

CHARACTERISTICS AND MOLECULAR MECHANISMS OF A LEIGH SYNDROME MOUSE MODEL: AN APPROACH TO STUDY INTERVENTIONS IN MITOCHONDRIAL AND NEURODEGENERATIVE DISEASES

CARACTERÍSTICAS E MECANISMOS MOLECULARES DE UM MODELO ANIMAL DE LEIGH SÍNDROME: UMA ABORDAGEM PARA ESTUDAR INTERVENÇÕES EM DOENÇAS MITOCONDRIAIS E NEURODEGENERATIVAS

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ABSTRACT

Nicotinamide adenine dinucleotide (NAD) hydrogen (NADH) dehydrogenase (ubiquinone) Fe-S protein 4 (Ndufs4) is an essential subunit of mitochondrial complex I (MCI) electron transport chain. Mutation of this gene in humans is associated with the development of Leigh syndrome. In Ndufs4 knockout (KO) mice, a rodent model that mimics human Leigh syndrome, it has been shown that MCI deficiency can induce the reduction of NAD+ levels and NAD+ redox imbalance. However, partial inhibition of complex I can also be benefic in some mitochondrial and neurodegenerative diseases, through pharmacological and non-pharmacological approaches, such as physical exercise and nutrition. Here, we review studies that targeted the Ndufs4KO mice as a model to study possible interventions in mitochondrial and neurodegenerative diseases. We also show some of the cellular and molecular mechanisms involved in the MCI dysfunction and suggest possible targets using this mouse model that could lead to the development of new therapies to individuals with mitochondrial and neurodegenerative diseases.

Keywords: Mitochondria. Mutation. Health. Physical Exercise. Nutrition.

RESUMO

Nicotinamida adenina dinucleotídeo (NAD) hidrogênio (NADH) desidrogenase (ubiquinona) proteína Fe-S 4 (Ndufs4) é uma subunidade essencial da cadeia de transporte de elétrons do complexo mitocondrial I (MCI). A mutação deste gene em humanos está associada ao desenvolvimento da síndrome de Leigh. Em camundongos knockout para Ndufs4 (KO), um modelo de roedor que imita a síndrome de Leigh humana, foi demonstrado que a deficiência de MCI pode induzir níveis limitados de NAD+ e desequilíbrio redox de NAD+. Contudo, também um inibidor parcial do complexo I pode ser benéfico em algumas doenças mitocondriais e neurodegenerativas, através de abordagens farmacológicas e não farmacológicas, como exercício físico e nutrição. Aqui, revisamos estudos que tiveram como alvo camundongos Ndufs4KO como modelo para estudar intervenções potenciais em doenças mitocondriais e neurodegenerativas. Também mostramos alguns dos mecanismos celulares e moleculares envolvidos no mau funcionamento do MCI e sugerimos possíveis alvos utilizando este modelo de camundongo que poderia levar ao desenvolvimento de novas terapias para indivíduos com doenças mitocondriais e neurodegenerativas.

Palavras-chave: Mitocôndria. Mutação. Saúde. Exercício Físico. Nutrição.

1 INTRODUÇÃO

Nicotinamide adenine dinucleotide (NAD) hydrogen (NADH) dehydrogenase (ubiquinone) Fe-S protein 4 (Ndufs4) is considered to be one of the most important subunits of mitochondrial complex I (MCI) electron transport chain and its mutation when occurs in humansis associated with the development of Leigh syndrome (Shil et al., 2021). It has been shown that mutation in the Ndufs4 gene is able to abolish cAMP-dependent phosphorylation in patient with neurological fatal syndrome (Papa et al., 2001). The cellular, molecular and physiological consequences of such mutations are not entirely understood. Nevertheless, many different pathological conditions can emerge from these mutations, such as poor feeding, weight loss, elevated liquor lactate, muscle weakness, cardiovascular alterations, metabolic and respiratory problems (Finsterer & Zarrouk-Mahjoub, 2017). These characteristics of MCI are related to a vast number of different clinical disorders of energy metabolism of which Leigh syndrome is the most commonly diagnosed(Ugalde et al., 2004).

Leigh syndrome is a type of a progressive mitochondrial encephalomyopathy disease, which presents neurological disturbances, metabolic dysfunction and premature death, which together will contribute to memory deficits and changes in behavior(Lee et al., 2019). The mitochondrial dysfunction that occurs in this disease contributes to a vast number of health problems, such as neurological degeneration (Johnson et al., 2013). The most common treatment for Leigh's disease is thiamine because it would help to maintain the pyruvate dehydrogenase complex in its active form, which can’t stop the progression of the disease or allow to the patients to have a normal life(Hommes et al., 1973). There are studies that evaluated the survival rate in Leigh syndrome, but they do not have a common sense and present ranges that flow from 20% to 39% of survival by the age of 20 years (Sofou et al., 2014).

In Ndufs4 knockout (KO) mice, a rodent model that mimics human Leigh syndrome, the death occurs around sixty days of life (P60) (Ferrari et al., 2017). It has been shown that MCI deficiency can induce the reduction of NAD+ levels and NAD+ redox imbalance (Lee et al., 2019). The Leigh syndrome is known as a subacute necrotizing encephalomyelopathy and it is the most common type of mitochondrial disease in infants(de Haas et al., 2016). The dysfunction of MCI in mice leads to failure to thrive, growth retardation, ataxia (unsteady gait, and abnormal body posture when suspended by the tail), hypotonia, optic atrophy, breathing problems, heart failure,

increased lactate levels in several areas of the brain, neuronal disturbances, and an extremely significant diminished life span resembling those patients with MCI disease (Gospe et al., 2019; Kagawa et al., 2020; Schleifer et al., 2019; Zhang et al., 2019). Thus, the Ndufs4KO mice closely mimics the aspects of the human disease (Johnson et al., 2021).

Due to the difficulty of performing cellular and molecular studies in human brain and the need of developing new research on this field (De Sousa, 2021), studies using animal models are an useful tool to investigate and suggest possible targets to avoid or inhibit MCI dysfunction. Here, we review the usage of different substances and methods of treating mitochondrial and neurodegenerative diseases through the usage of the Ndufs4KO model and the possibilities of translating these findings to applicable interventions in humans.

2 NDUFS4 MOUSE MODEL MAIN FEATURES

Definitely, the remarkable characteristic of Ndufs4KO mice is a reduction in the capacity of respiration at the cellular level (Ito et al., 2017; M. Wang et al., 2017). For example, even a partial absence of MCI is capable to sensitize the myocardium generating reactive oxygen species (ROS) that might influence respiration (Kuksal et al., 2018). These respiratory problems due to MCI dysfunction can be seen in different tissues, such as lungs (Schleifer et al., 2019), skeletal muscle (Alam et al., 2015), heart (Zhang et al., 2019), and brain (Piroli et al., 2016). Interestingly, the respiratory capacity is progressively worsen at synaptic connections(Kayser et al., 2016). A few different approaches have been tested to improve mitochondrial and neurodegenerative diseases, such as the usage of anesthetics(Ramadasan-Nair et al., 2017), medicines (Emmerzaal et al., 2020), and other substances (Ho, 2017; Woods et al., 2021).

Neurological symptoms and differences in the body weight start to become apparent at P35 (Kayser et al., 2016). Major retinal ganglion cell functional loss occurs at P32, retinal ganglion cell loss at P42, and preceding this loss there is the loss of starburst amacrine cells, reduced post-synaptic activity and significant reduction in bipolar cells(Song et al., 2017; Yu et al., 2015). Neurological lesions (retinal cell loss and optic atrophy, increased lipid peroxidation and lactate levels, reduced dopamine levels, and pre-synaptic changes) (De Haas et al., 2017; Ingraham, Christopher A. et al., 2010; Kim et al., 2015; Shil et al., 2021; L. Wang et al., 2020; Yu et al., 2017), neurological symptoms (muscle weakness, balance loss and locomotor impairment,

seizures, cognitive decline, changes in behavior, and vision loss) (Emmerzaal et al., 2020; McElroy et al., 2020), and weight loss (Jain et al., 2020) are present in 100% of the animals at P52 (Johnson et al., 2021). Ndufs4KO mice usually live until P60 (Silva-Pinheiro et al., 2020). However, there are a few articles showing survival curves ending at P55 or P65 (Di Meo et al., 2017; Johnson et al., 2015). Altered mitochondrial oxidative phosphorylation is obviously reported at all periods of life (Ito et al., 2017; Miller et al., 2021) (**Figure 1**).

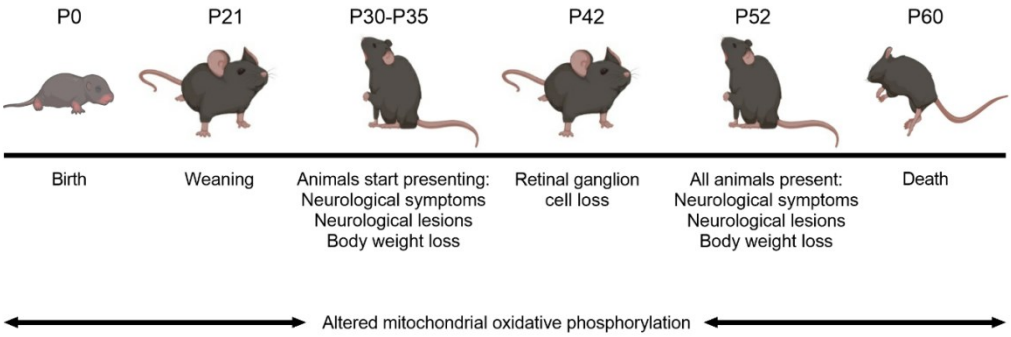

Figure 1. Timeline of the main characteristics developed during life by Ndufs4KO mice.

The animals start presenting neurological symptoms, neurological lesions and body weight loss that may be significant from P30 to P35. Ndufs4KO mice present significant differences to wild-type or control mice regarding neurological symptoms, neurological lesions and body weigh at P52 with death usually happening around P60.

3 THE USAGE OF ANESTHETICS AND MEDICINES IN THE NDUFS4KO MOUSE

The mechanisms that might lead to changes in synaptic function are not completely understood (Zimin et al., 2018). A recent study made by Woods et al. evaluated if synaptic or neuronal function could be hypersensitive to isoflurane when using spinal cord slices extracted from Ndufs4KO mice (Woods et al., 2021). It was found that non-cholinergic neurons regulated the increased holding current sensitivity. Isoflurane increased potassium current in ventral horn neurons at a diminished concentration when compared to controls. Intringuily, the presynaptic channels were not hypersensitive to isoflurane. Therefore, it was suggested thatisoflurane sensitivity, mitochondria, and postsynaptic channel activity are related.

Another recent study showed that isoflurane can disrupt excitatory neurotransmitter dynamics via inhibition of MCI in the hippocampus *cornuammonis* 1 (CA1) region (Zimin et al., 2018) and this sensitivity to volatile anesthetics might be

related to glutamatergic neurotransmission (Zimin et al., 2016). It has been reported significant differences between the electrocorticography of Ndufs4KO and control mice at equipotent doses for volatile anesthetics and ketamine, which was previously described as resistant to KO at MCI (Carspecken et al., 2018). It seems that the inhibitory effects of isoflurane and other anesthetics on mitochondria plays a crucial role for disrupting the maintenance of consciousness through thalamo cortical circuit (Ramadasan-Nair et al., 2017).

An interesting way to enhance the levels of these proteins is through non-pharmacological therapies like physical exercise and dietary interventions, which have been provided great information about possible molecular mechanisms that can be targeted to attenuate mitochondrial dysfunction and the development of neurodegenerative diseases.

4 THE POSSIBILITIES OF NON-PHARMACOLOGICAL INTERVENTIONS IN THE NDUFS4KO MOUSE

A recent study showed that mitochondrial SIRT3 mediates adaptive responses of neurons to physical exercise, and also plays a fundamental role in adaptive responses of neurons to exercise and resistance to degeneration(Cheng et al., 2016). It has been suggested that NAD+ precursor is capable to modulate post-ischemic mitochondrial fragmentation and the productions of ROS via SIRT3 dependent mechanisms (Klimova et al., 2019). SIRT1 is also enhanced through the regular practice of physical exercise and contributes to the production of peroxisome proliferator-activated receptor-gamma co-activator 1 alpha (PGC-1α) (Casuso et al., 2014). Thus, we believe that SIRT1 and SIRT3 play crucial roles in the MCI stability via enhancing neuroprotection mechanisms through the activation and/or enhancement of PGC-1α/ FNDC5/Irisin pathway, which is only activated by physical exercise (De Sousa, Rocha-Dias, et al., 2021).Higher activation of PGC-1α/ FNDC5/Irisin pathway also contributes to the enhancement of cAMPK-PKA-CREB pathway, an essential mechanism to avoid cognitive decline and dementia, and to the improvement of brain-derived neuro factor (BDNF), which is associated to the reduction of ROS and memory improvement (De Sousa, Improta-caria, et al., 2021). Physical exercise lead also to the improvement in PI3K pathway (De Sousa, 2018).

Another possible non-pharmacological interventions that has been shown to be effective fighting neurodegenerative diseases and mitochondrial diseases are caloric

restriction (Dias et al., 2020) and intermittent fasting (Liu et al., 2019). Caloric restriction has shown to improve cardiovascular mechanisms (Melo et al., 2016), and one of the main hallmarks among the caloric restriction effects is the reduction of oxidative damage and modulation of mitochondrial activity (Picca et al., 2017). Intringuily, caloric restriction has shown to increase the reward-related and mood behaviors (Q. Wang et al., 2021). Therefore, these non-pharmacological and pharmacological interventions shall improve mitochondrial functioning in Ndufs4KO mice through several molecular and cellular mechanisms (**Figure 2**).

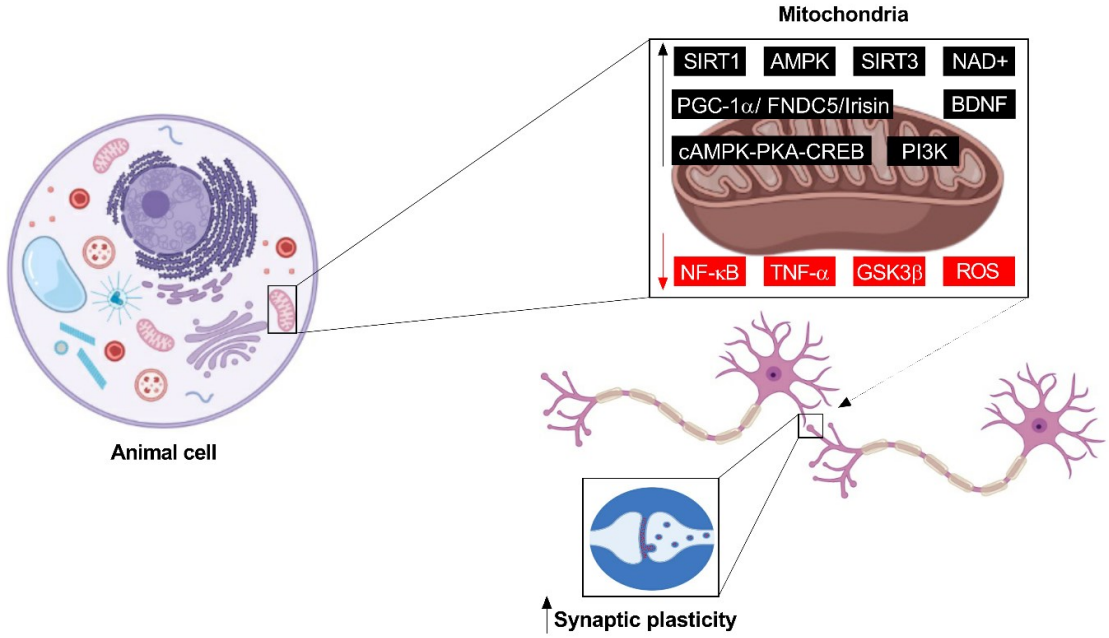

Figure 2. Molecular mitochondrial mechanisms influence synaptic plasticity in Ndufs4KO mice.

Improvement in mitochondrial function occurs mainly mediated by the enhancement of SIRT1, AMPK, SIRT3, NAD+, BDNF, with simultaneous greater activation of different signaling pathways like PGC-1α/ FNDC5/Irisin, cAMPK-PKA-CREB and PI3K in different animal cells (neurons, muscle cells, etc). The better mitochondrial functioning also inhibits the activation of NF-κB, TNF-α, GSK3β, and ROS. Changes on these molecular mechanisms will favor greater synaptic plasticity with better functioning of pre- and post-synaptic activities. These molecular and cellular mechanisms together can effectively fight mitochondrial and neurodegenerative diseases.

5 CONCLUSIONS

Mutations in the Ndufs4 gene can lead to respiratory problems, an abnormal feeding, significant weight loss, higher lactate levels, muscle weakness, changes in the metabolism and in the cardiovascular system. The usage of Ndufs4KO mice is a reasonable approach to evaluate mitochondrial dysfunction and to target different mechanisms that could enhance the life span of these animals giving them a better quality of life. These new discoveries could lead to the development of new therapies that could improve not only the life span, but also the quality of life of individuals with mitochondrial and neurodegenerative diseases, especially in Leigh Syndrome.

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