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REVIEW ARTICLE

Minireview: Cell biological consequences of Leigh syndrome

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Abstract:
Mitochondria are double membrane-enveloped organelles that produce ATP in eukaryotic cells through the oxidative phosphorylation (OXPHOS) process. Accordingly, mitochondrial dysfunction is implicated in a broad range of human diseases, such as Parkinson's disease, Alzheimer's disease, cancer and diabetes. From a pediatric perspective, isolated malfunction of the first OXPHOS complex (complex I or NADH:ubiquinone oxidoreductase), is the most frequently observed defect. Complex I dysfunction may manifest itself as Leigh syndrome, which is an early-onset neurodegenerative disorder with a very poor prognosis. In addition to ATP generation, complex I dysfunction can also affect various other key cellular processes, like the generation of reactive oxygen species, maintenance of a sufficiently negative mitochondrial membrane potential, mitochondrial dynamics and calcium homeostasis. In the recent past, we performed a comprehensive live-cell analysis with skin fibroblasts from Leigh syndrome patients. These cells harbored nuclear-DNA encoded mutations in complex I subunits and displayed an isolated complex I deficiency. Here, we provide a brief overview of our key findings and directions for future research.

Keywords: *Mitochondria, oxidative phosphorylation, Leigh syndrome, treatment*
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Introduction

Mitochondria are semiautonomous, double membrane-bound organelles, which produce cellular energy via aerobic respiration. This task is executed by the protein machinery of the oxidative phosphorylation (OXPHOS) system, located at the inner mitochondrial membrane (IMM). In this system, four large multi-protein complexes transport electrons from reduced coenzymes (NADH and $FADH_2$) to molecular oxygen (O_2) via sequential redox reactions. This transport is coupled to the vectorial outward translocation of protons (H^+) across the IMM at complex I, III and IV. The H^+ gradient generated by this translocation, and the ensuing inward-negative mitochondrial membrane potential across the IMM ($\Delta\Psi$), provide the driving force for ATP synthesis at complex V [1]. Importantly, dysfunction of the OXPHOS system is generally associated with severe progressive disorders that can display their first onset at any age.

Mitochondrial diseases in children constitute a diagnostic and therapeutic challenge for the clinician.

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The majority of the early onset forms of these diseases are caused by an isolated malfunction of mitochondrial complex I (NADH:ubiquinone oxidoreductase) [2, 3, 4]. This respiratory chain complex represents the main entry point of electrons into the OXPHOS system. It is composed of 45 subunits of which seven are encoded by the mitochondrial DNA (mtDNA) and 38 by the nuclear DNA (nDNA). Disease-causing mutations have been identified in all of the mtDNA genes and several of the nDNA genes encoding structural subunits of complex I [4]. In addition to the 45 structural components, several assembly factors are needed to allow proper biogenesis of complex I [5]. During the last years, disease-causing mutations have also been identified in complex I assembly factors.

Isolated, nDNA-encoded complex I deficiency generally causes Leigh syndrome (also known as subacute necrotizing encephalomyelopathy), which is one of the mitochondrial diseases with a very poor prognosis [4]. The disease mainly manifests in the central nervous system, typically causing symmetrical lesions in the basal ganglia or brain stem. However, also cases with extensive leukodystrophy have been reported [4]. Clinical symptoms include loss of previously acquired motor skills, disturbed eye movements and/or loss of visual contact, episodic vomiting, seizures and irritability. In most cases, Leigh syndrome is inherited as an autosomal recessive trait. However, X-linked recessive and mitochondrial inheritance have also been reported [6].

Although research is ongoing, there is no cure yet for Leigh syndrome but supportive treatment may help to reduce symptoms or delay the progression of the disease. Ideally, this treatment should be individualized for each patient, as every child is clinically and genetically different. Therefore, detailed insights into the consequences of Leigh syndrome at the cellular level are required.

During the last years, we have developed and applied live cell microscopy techniques to quantify key biochemical parameters in cells from Leigh syndrome patients. These approaches were primarily set up for patient skin fibroblasts, which are usually easy to obtain and therefore frequently used for diagnostic purposes in children with a suspected mitochondrial deficiency. Our patient cohort consisted of 15 children with isolated, nDNA-encoded complex I deficiency (for an extensive review see Distelmaier *et al.*, 2009) [4]. Mutations were established in several complex I subunits including the NDUFV1 subunit (two patients), the NDUFS1 subunit (one patient), the NDUFS2 subunit (four patients), the NDUFS4 subunit (five patients), the NDUFS7 subunit (one patient) and the NDUFS8 subunit (two patients). All patients displayed a complex I deficiency in skin fibroblasts and muscle tissue, suffered from Leigh/Leigh-like syndrome and died during the first years of live. Below we will briefly discuss the key findings that we obtained in the patient cells.

Reactive oxygen species levels in Leigh syndrome patient fibroblasts

Importantly, (dys)function of the mitochondrial respiratory chain is regarded as an important source of cellular reactive oxygen species (ROS) [7]. Therefore, mitochondrial constituents like proteins, lipids and mtDNA are exposed to oxidative stress and might be damaged/impaired over time. Although ROS also have important physiological functions within organisms (*e.g.* cellular signaling), sustained ROS-induced damage likely will negatively affect cell biological processes if not properly counterbalanced by repair mechanisms. During conditions of mitochondrial dysfunction this damage/repair balance may already be disturbed at an early stage. Using live cell imaging microscopy we demonstrated that the rate of oxidation of two ROS-sensitive chemical reporter molecules, hydroethidine (HEt) and CM-H_2DCF (5-(and-6)-chloromethyl-2',7'-dichlorodihydro fluorescein), was significantly increased in skin fibroblasts from Leigh syndrome patients [8, 9]. Importantly, an inverse relationship with the amount and residual activity of complex I was observed (*e.g.* higher cellular ROS levels were found in cells with a lower residual complex I activity and *vice versa*).

Mitochondrial membrane potential in Leigh syndrome patient fibroblasts

As explained above, the inward-negative potential across the IMM ($\Delta\psi$) is co-sustained by the action of complex I. This potential is regarded as a key-indicator of mitochondrial health because it is required to drive ATP synthesis and many other mitochondrial functions. Obviously, complex I

dysfunction may disturb its proton-translocating ability, leading to a less negative (depolarized) $\Delta\psi$. We developed a sensitive method to estimate $\Delta\psi$ in living skin fibroblasts [10, 11]. This revealed a depolarized membrane potential in all patient cell lines investigated [12]. Of note, controlled leak of protons via mitochondrial uncoupling proteins (UCPs) might function as a regulator of increased ROS production [13]. Compatible with this idea, regression analysis revealed a linear correlation between extent of $\Delta\psi$ depolarization and the levels superoxide-derived ROS (*e.g.* the higher the ROS levels the more depolarized $\Delta\psi$) [12].

Mitochondrial dynamics in Leigh syndrome patient fibroblasts

In living cells, mitochondria are often not bean-shaped but form a filamentous network that is constantly remodeled by fusion and fission events. It appears that these dynamics are crucial for proper mitochondrial function and are related to the ROS levels and/or metabolic state of the cell. Quantification of mitochondrial structure in fibroblasts of Leigh syndrome patients revealed that mitochondrial shape and number were normal in cells with a moderate complex I deficiency (Class II), whereas mitochondrial were fragmented in cells with a more severe complex I deficiency (Class I) [14]. Interestingly, cellular ROS levels were much higher in Class I patient cells suggesting that Leigh syndrome patient cell lines with a fragmented mitochondrial morphology have possibly surpassed a certain threshold of oxidative stress.

ATP homeostasis in Leigh syndrome patient fibroblasts

OXPHOS-mediated ATP synthesis in respiring cells clearly depends on the proper function of the involved protein complexes and the maintenance of $\Delta\psi$ (see above). Accordingly, we studied mitochondrial ATP production in Leigh syndrome patient fibroblasts, stimulated with the hormone bradykinin (Bk). To this end, we measured the ATP-dependent luminescence signal of a mitochondria targeted ATP-sensitive protein (luciferase) using a very sensitive photomultiplier tube (PMT) detection system. We observed that the Bk-stimulated increase in mitochondrial ATP concentration was reduced in most of the patient cell lines [15]. Regression analysis revealed a close relationship between this increase and cellular ROS levels as well as $\Delta\psi$ (details are reviewed in Valsecchi *et al*., 2009) [12, 16].

Calcium homeostasis in Leigh syndrome patient fibroblasts

A sufficient supply of ATP is crucial to maintain proper cell functioning. The latter includes the continuous action of ATPases that remove ionic calcium (Ca^{2+}), a key cellular signalling molecule, from the cytosol towards the endoplasmatic reticulum (ER) [17]. We investigated cellular Ca^{2+} homeostasis by measuring the cytosolic free Ca^{2+} concentration (using the fluorescent Ca^{2+} indicator fura-2) and the ER Ca^{2+} content under resting and Bk-stimulated conditions. Additionally, we quantified the Ca^{2+}-dependent luminescence signal of a mitochondria targeted Ca^{2+}-sensitive protein (aequorin) using a PMT detection system [15, 18, 19, 20]. It was found that the ER Ca^{2+} content was reduced in several patient cell lines under resting conditions. As a consequence, the amplitude of the cytosolic and mitochondrial Bk-induced Ca^{2+} peaks were reduced. In agreement with these results, the rate of ATP-dependent refilling of the ER with Ca^{2+} was also reduced. These observations support a model in which impaired ATP production by the OXPHOS system leads to a reduced fueling of the ATPases of the intracellular Ca^{2+} stores. The latter then leads to a disturbed Ca^{2+} signaling upon cell stimulation with Bk. Of note, evidence was provided that an increase in mitochondrial Ca^{2+} concentration stimulates OXPHOS-mediated ATP production. It is expected that complex I deficiency hampers this process and thereby leads to disturbances in ATP supply upon cell stimulation (details are reviewed in Willems *et al*., 2008 and Valsecchi *et al*., 2009) [16, 20].

Implications for future research

The data presented above illustrate some important cellular consequences of complex I dysfunction. Given their interdependence, it will be a challenge to mitigate or prevent these consequences using drug therapy. However, this task might be facilitated by studying recently developed animal models of Leigh syndrome [21]. Thus far, some potential treatment strategies for Leigh syndrome have been applied. These strategies include prescription of vitamins and food supplements, including coenzyme Q_{10}, thiamine, riboflavin, vitamin E and L-carnitine [22, 23].

Additionally, a ketogenic diet might also be useful. However, the therapeutic effects of these approaches are generally unsatisfactory, especially on the long term. In our cell experiments we tested the water-soluble vitamin E analogue Trolox, which is a potent antioxidant, and observed beneficial effects in certain patient cell lines [24]. Importantly, patient fibroblasts with a moderate complex I deficiency (*e.g.* considerable residual complex I activity, rather low reactive oxygen species levels, normal mitochondrial morphology, etc.) benefited from chronic Trolox treatment whether patient cell lines with a severe deficiency (very low residual complex I activity, high reactive oxygen species levels, etc.) responded less well to this treatment. These findings might have important implications for antioxidant treatment of Leigh syndrome patients and may explain apparent differences in clinical response to drug therapy. In addition, our live cell data suggest that modulators of cellular Ca^{2+} homeostasis might be of interest in the treatment of Leigh syndrome. For instance, the drug CGP-37157, which is a blocker of mitochondrial Ca^{2+} export, improved Bk-stimulated mitochondrial ATP levels in skin fibroblasts of Leigh syndrome patients [18].

Currently, supportive therapy is still a key element of the treatment for Leigh syndrome patients. General aspects include supplementation of sufficient calories, fluids and electrolytes. Because of feeding problems, percutaneous endoscopic gastrostomy is frequently required. It is crucial to avoid periods of fasting and catabolism. Accordingly, during episodes of infections (especially if they manifest with fever) intravenous supplementation of glucose and fat might be required. Patients frequently develop seizures and antiepileptic therapy is often necessary. Importantly, drugs that possibly affect mitochondrial function should be strictly avoided (*e.g.* sodium valproate, barbiturates, tetracyclines and chloramphenicol). Moreover, physical therapy is a crucial part of supportive therapy. Finally, for parents considering having other children, genetic counseling might be available, especially if the genetic cause of the disease is identified.

Future studies with patient material in combination with animal models of mitochondrial complex I deficiency will hopefully expand our understanding of mitochondrial genetics and function. This might lead to more specific and targeted therapies that will improve patient care and increase the quality of life for children affected with Leigh syndrome.

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