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Methods for assessing mitochondrial quality control mechanisms and cellular consequences in cell culture

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ABSTRACT

Mitochondrial quality is under surveillance by autophagy, the cell recycling process which degrades and removes damaged mitochondria. Inadequate autophagy results in deterioration in mitochondrial quality, bioenergetic dysfunction, and metabolic stress. Here we describe in an integrated work-flow to assess parameters of mitochondrial morphology, function, mtDNA and protein damage, metabolism and autophagy regulation to provide the framework for a practical assessment of mitochondrial quality. This protocol has been tested with cell cultures, is highly reproducible, and is adaptable to studies when cell numbers are limited, and thus will be of interest to researchers studying diverse physiological and pathological phenomena in which decreased mitochondrial quality is a contributory factor.

1. Introduction

Mitochondria are complex cellular organelles responsible for maintaining cellular energy status and homeostasis through production of ATP, cell signaling and generation of intermediary metabolites. Mitochondria are highly dynamic and in a constant state of structural flux through fission and fusion processes and have important roles in cellular signaling cascades. During the physiological process of metabolism, the components of the organelle including proteins, lipids and DNA become damaged and must be repaired to maintain normal function. This maintenance process requires the integrated participation of a number of key pathways and is generally termed mitochondrial quality control [1].

An important contributor to mitochondrial quality control is autophagy, which is an indispensable cellular process responsible for the clearance of cellular debris, organelles, and damaged proteins [2,3]. This complex process is regulated by nutrient availability and response to stress. Specific recycling of mitochondria by autophagy, termed mitophagy, is important for maintaining optimal mitochondrial function [4–6].

Mitochondrial dysfunction is associated with aging and chronic pathologies including neurodegenerative diseases, cardiovascular disease and diabetes [7,8]. Mitochondrial dysfunction is a hallmark of pathologies in tissues that are highly energetic, in particular the heart and brain but also in a broad range of cells including platelets and those controlling immunity. In the brain, mitochondrial dysfunction has been frequently implicated in a range of age-dependent neurodegenerative diseases, including Parkinson's disease (PD) and Alzheimer's disease (AD). In these diseases, protein aggregation and mitochondrial dysfunction interplay and ultimately both contribute to neurodegeneration [9–15]. Notable examples of the importance of mitochondria at the center of this disease are evidenced by toxin-induced models of PD through administration of rotenone, paraquat, and MPTP [16]. These compounds have been reported to act through either complex I inhibition or through redox cycling [17,18]. Furthermore, the lipid peroxidation product, 4-hydroxynonenal (HNE) has been shown to accumulate in both PD and AD brains, and to significantly alter mitochondrial and autophagic function in diverse cell types [19–24]. We have proposed that autophagy can serve as an essential antioxidant pathway to attenuate oxidative damage [25].

Given the importance of mitochondria in health and disease, here we have outlined the initial approaches that are accessible to most

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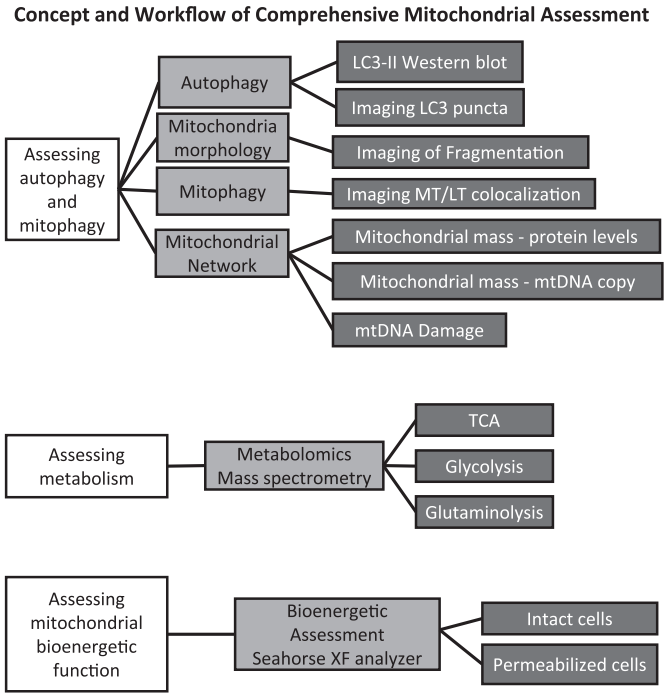

Fig. 1. Workflow of comprehensive mitochondrial assessment. Using primary neurons, assessment of mitochondrial function can be obtained by employing diverse techniques. Shown here is a list of experiments used to examine autophagy, mitophagy, mitochondrial damage, mitochondrial morphology, metabolism and mitochondrial bioenergetic function.

laboratories using existing technology to investigate different facets of mitochondrial health in a given disease paradigm (Fig. 1). We have grouped similar modalities together allowing investigators to select the appropriate methods and integrate them into a structured work flow. Noting the importance of mitochondria in chronic pathologies, we have used both primary cortical neurons and β-cells for the purposes of this manuscript.

2. Protocol concept

The concept that autophagy, mitophagy, mitochondrial bioenergetics and mitochondrial quality control are linked has emerged over the last 10 years [26]. Although many studies have focused on individual elements of these processes, an integrated methodological approach to provide a comprehensive view of cellular and tissue-specific responses to physiological, pharmacological, and pathological perturbations has not been proposed. This is important since the assessment of mitochondrial quality requires standardized protocols that allow testing of key elements of this complex process using a broad range of biological read-outs. We provide here a streamlined comprehensive strategy to combine autophagy assessment with mitochondrial damage, mitochondrial dynamics and mitochondrial function, as well as cellular metabolism. Mitochondrial function can be assayed in real time to assess the consequence of exposures to nutrients, toxins and pharmacological agents. This approach will be of significant value to researchers over a broad area of biomedical research. The workflow is outlined in Fig. 1.

3. Applications and target audience

There are currently 4407 papers available in PubMed (surveyed on Mar 27th, 2018) discussing various aspects of autophagy and mitochondria. Of these, 1438 papers discussed mitochondrial bioenergetics, while 478 papers discussed mitochondrial quality control. Our protocols will be of use to researchers with established interest in a combined approach, as well as those who have been solely focused on one or two of these topics and wish to expand their repertoire of investigation, with the realization that these aspects of cellular function are fundamental to most biomedical problems. Researchers working on diverse biological challenges, including oxidative stress, mitochondrial biology, autophagy, mitophagy, metabolism and a broad range of pathologies are the target audience for the article.

4. Materials

Tables of needed chemicals, PCR primers, antibodies, consumables and equipment needed are listed in Supplemental Tables 1–5.

4.1. Protocol: steps and procedures

4.1.1. Neuron preparation

Primary neurons isolated from either rat or mice provide an excellent platform in which to test aspects of neuropathologies, especially before, during or after either neurotoxin or neuroprotective treatments administered through genetic and/or pharmacological means [27,28]. While there are certainly some alterations of metabolism due to being cultured, primary neurons provide more relevant read-outs than immortalized cells. This is particularly the case when measuring parameters of mitochondrial metabolism and function.

The isolation workflow is diagrammed in Fig. 2. The day before isolation, coat 24 well tissue plates for ≥1 h at room temperature in a

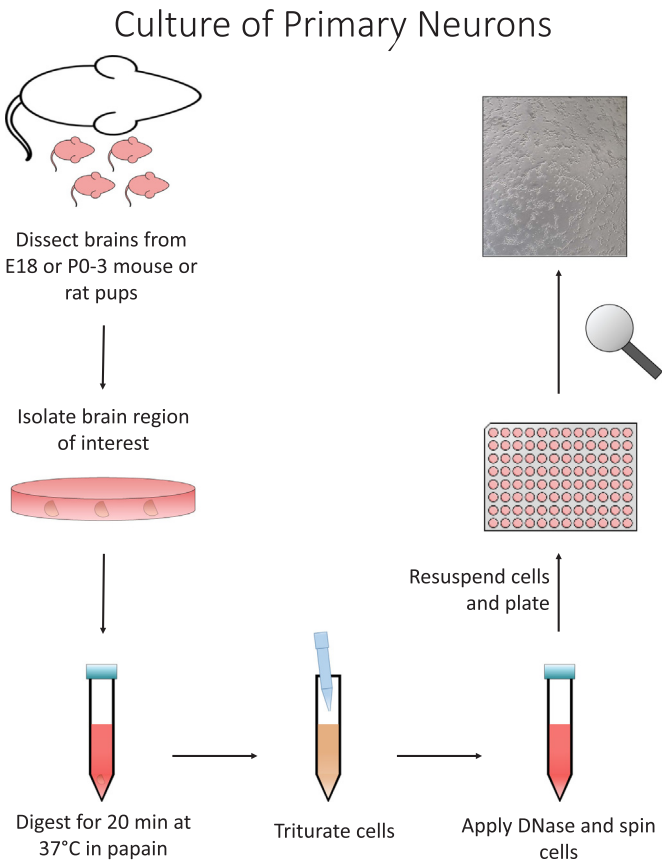

Fig. 2. Primary neuronal culture. Briefly, pups can be obtained from mice or rats ranging from E18 to p0–3 in age. Desired brain region can then be microdissected from the brain. Tissue is then digested using a papain solution for 20 min at 37 °C with gentle agitation every ~3 min. Cells are then triturated using 1 mL pipet tip with smooth edges. Remaining debris should be removed and DNase added. Cells are then centrifuged at ~250×*g* for 5 min. Cells are then to be resuspended, counted, and plated.