Editorial

Stem Cell INVESTIGATION

Human induced pluripotent stem cell-derived neuronal progenitors are a suitable and effective drug discovery model for neurological mtDNA disorders

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Mitochondria are double-membraned organelles of endosymbiotic origin (1). Mitochondria are critically involved in cellular homeostasis and need to be tightly controlled to maintain normal cellular function (2). Malfunctioning mitochondria can interfere with cellular homeostasis in multiple ways (3). Consequently, mitochondria have been associated with diverse diseases, including neurological and neurodegenerative disorders (4). Among those diseases, a group of severe disorders is directly associated with specific mutagenesis of the mitochondrial DNA (mtDNA) (5,6). Thus, despite the fact that most mitochondrial genes are encoded in the nucleus, mutagenesis of the mtDNA can have severe outcomes. Hence, the status of the mtDNA is of crucial relevance for normal cellular function (7). Several studies revealed that modeling and targeting mtDNA related diseases is experimentally extremely challenging. Therefore, the modeling of human mtDNA disorders is often based on so-called cybrid models (cytoplasmic hybrids), combining immortalized cells and the particular patient's mitochondria. These models were able to recapitulate the influence of patient specific mitochondria on cellular physiology, but unfortunately, cybrid models have limited physiological relevance. Cybrid models lack the patient-specific genetic background, are not suitable for modeling disease relevant tissues (8), and possess incompatible metabolic and bioenergetic states (1). Different cell types and subtypes strongly differ in their bioenergetic demands. Simplified, stem cells rather rely on glycolysis more than on mitochondrial oxidative phosphorylation (OXPHOS). In contrast, during commitment and differentiation especially cells from the neuronal lineage increasingly address their bioenergetic demands by using OXPHOS for energy generation (9).

Motivated by these limitations, Prigione and colleagues reached out to find a new, more advanced, and highly relevant human model for mtDNA-associated diseases (10). With the advent of induced pluripotent stem cells (iPSCs) it became possible to derive patient-specific cell types, which can be used for *in vitro* disease modeling (11). iPSCs based *in vitro* disease modeling aims at revealing the disease underlying molecular mechanisms in order to gain a deeper understanding of the pathophysiological aspects of disorders, finally resulting in the emergence of potential drug targets. Consequently, iPSC-based models bear great potential for the discovery of novel treatment strategies (12). In particular, for neural diseases iPSC-technologies revolutionized the availability and accessibility of the disease relevant material.

Here, Prigione and colleagues, applied for the first time

iPSC techniques for modeling a mtDNA associated disease and derived patient-specific iPSCs from Leigh syndrome patients (13). Since iPSC-based models were never used for modeling mtDNA disease before, the authors performed thoroughly validation steps. Importantly, inducing pluripotency in somatic cells bears pitfalls for modeling mtDNA diseases, the cellular reprogramming is not only resetting the potency of the cells but also extensively remodels the cells' metabolism and mitochondria (14,15). In addition, the conventional reprogramming process starts from somatic cells and includes clonal expansion. Clonal expansion is bearing the likelihood of an enrichment of highly mutagenic mtDNAs during mitochondrial inheritance due to the presence of heteroplasmy of mtDNA. This bottleneck could potentially result in the expansion of a pool of cells carrying a variety of additional, not disease-relevant mtDNA mutations, aggravating the identification of pathophysiological mechanisms (6). Another question that arises during an iPSC-based disease modeling is the selection of the most relevant cell type available for modeling the particular disease. Prigione and colleagues decided to utilize neural progenitor cells (NPCs) for modeling the impact of Leigh syndrome on normal neurodevelopment (16). The use of iPSC derived neural stem cells for modeling neurological diseases has been extensively reviewed before (17,18). In this study, the authors demonstrate that NPCs represent a physiologically relevant cellular model for neurological mtDNA disorders.

Accordingly, Prigione and colleagues dedicate the first part of their manuscript to the characterization and validation of the applicability of iPSC-based NPCs for mtDNA disease modeling. The authors describe the derivation of the pool of iPSCs used throughout the study. The authors further characterized and validated the neural identity of their neural precursor cell model. NPCs were derived from iPSCs using a small molecules based derivation protocol, which represents a robust, cost-efficient and highly defined patterning strategy (16,19). The NPCs are reminiscent of the early neural tube development and retain the differentiation potential into all cell types of the central nervous system (16). Apart from their disease specific relevance NPCs in comparison to iPSCs are easier to handle, cheaper in their maintenance, and already committed to the neural lineage. Specifically, the neural commitment is important for studying the recapitulation of the disease relevant neural alterations. The advantage of neural progenitor cultures over neuronal differentiation cultures is clearly their high definition and purity, while the outcome of a differentiation is bearing certain variability. Prigione and colleagues first characterized the derived iPSCs used in this study and further demonstrated that these are able to give rise to NPCs. The commitment of NPCs to the neural lineage was confirmed by immunofluorescence staining and calcium signaling analyses. At the same time, NPCs remained electrophysiological inactive and consequently non-neuronal. Further, the authors performed transcriptional analyses and demonstrate the high similarity of the generated NPCs to other published NPC variants. In this context the authors confirmed that the NPC transcriptome, indeed, overlaps the most with the transcriptional state reported for the early embryonic neurodevelopment (20) emphasizing the physiological validity of the NPC model.

After successful and thorough characterization of the iPSCs and the derived NPC model, Prigione and colleagues continued and assessed the metabolic status in the NPC model. In particular, the authors validated the mitochondrial microstructural remodeling taking place during the derivation of NPCs and confirmed a high similarity to neurons and dissimilarity to immature mitochondria in iPSCs. In addition to structural remodeling, the authors also confirmed a bioenergetic shift of NPCs towards neurons rather than iPSCs; this is specifically prominent in the downregulation of glycolytic activity. Next, and most importantly, Prigione and colleagues were able to validate that NPCs, despite potential pitfalls during the reprogramming and clonal expansion processes, retain the disease-specific mtDNA mutation pattern. In particular, the authors describe the preservation of the spectrum of the D310 tract hyperplasmy in the hypervariable region (57–372) of the D-loop. Due to the continuous advancement of reprogramming techniques, the authors used iPSCs that were generated by different techniques. However, in the context of the mtDNA analyses the authors were able to demonstrate that the preservation of mtDNA patterns is independent of the iPSC derivation technique.

After these model validation steps Prigione and colleagues continued with the derivation and characterization of a pool of Leigh syndrome specific NPCs. In a first step, the authors focus on ATP-related plasma membrane resting potential, proliferation, and mitochondria specific reactive oxygen phenotypes. They demonstrate that NPCs retain Leigh syndrome specific pathogenicity in comparison to specific cybrids and parental fibroblasts. Further, the authors based their mitochondria related phenotyping on the assessment of the mitochondrial membrane potential (MMP). Using

MMP analysis, the authors verify a hyperpolarization of mitochondria in association with the Leigh syndrome. A subsequent transcriptional analysis revealed calcium signaling pathway related genes as differently regulated, a phenotype that is conserved between fibroblasts, iPSCs, and NPCs. Among the differently regulated calcium signaling genes specifically *LETM1*, *VDAC3*, *ATP50* were strongly altered between control and Leigh syndrome NPCs. Consequently, the authors applied calcium-imaging analysis to assess the pathophysiological impact of the disease associated transcriptional changes. Thereby they verified the negative impact of the transcriptional changes on the time-resolved calcium signaling activity. Next, specific compound modulation of the calcium signalling revealed the mitochondrial specificity of the phenotype. In a final step, the authors transferred the image based MMP phenotyping to a high-throughput analysis pipeline and screened a compound library of 130 selected FDA approved compounds. After 24 h of treatment, the effect of the compounds on the MMP was analyzed. This screen revealed several potential rescue compounds. Among these hits Avanafil, a PDE 5 inhibitor, which is approved for treating pulmonary arterial hypertension was of particular interest. Using Avanafil the authors, in addition to the MMP rescue, also verified a rescue of ATP production and calcium signaling.

In summary, Prigione and colleagues reached several milestones advancing the field of mtDNA diseases. Most importantly, despite the potential pitfalls, the authors verified iPSC-based modeling approaches for studying mtDNA diseases. The authors demonstrate that iPSC-based models perfectly retain the characteristics of the mtDNA. A finding of outstanding importance for all iPSC-based studies involving mitochondria. The authors further verified that patient-specific iPSCs derived NPCs recapitulate the most important Leigh syndrome related alterations in mitochondria. They also demonstrate that NPCs represent a valuable tool for high throughput screening approaches and drug discovery strategies. Compared to neuron based screening this shortcut proves NPC models an ideal tool for the implementation of new treatment and prevention strategies in precision medicine. Conclusively, the study holds great promises not only for mtDNA related disease but also for other diseases involving mitochondrial dysfunctions such as seen in Parkinson's disease (21). Only further studies will be able to reveal the outcome of such alterations e.g., on neurodevelopment or the definition of an appropriate treatment window.

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None.

Footnote

Conflicts of Interest: JW is employee of the biotech company Braingineering Technologies SARL. JCS is co-founder and CSO of Braingineering Technologies SARL. The other author has no conflicts of interest to declare.

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