Identification of genetic rescuer of mitochondria functions and morphology in SPG7 patients.

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INTRODUCTION

Mitochondria are essential for the cells. They have multiples function (ATP synthesis, calcium homeostasie, program cell death...) and there dys-function could lead to severe disease, which could occur in 1:5000 individuals. These mitochondrial disease (MD) are complex and heterogene even between individuals with the same mutations.
Hereditary Spastic Paraplegias (HSP) is the second most common type of motor neuron diseases, it belongs to a group of rare neurodegenerative diseases characterized by lower limb spasticity. Those inherited Mendelian disorders[1] show high genetic variability associated with wide clinical diversity.
Here we will focused on Spastic Paraplegia Type 7 (*SPG7*) which is an autosomal recessive disorder caused by mutations in the gene encoding the paraplegin protein, a subunit of AAAprotease, located at the inner mitochondrial membrane[2]. Moreover it's known that *SPG7* is involved in processing mitochondrial proteins and the assembly of the mitochondrial ribosome. However, the mechanism causing the disease is still not elucidated and there is no treatment.
In this study, we will try to find new mitochondrial players in the disease, to better decipher the mechanism of the disease and potentially find new target.

MODELS

HeLa cells knock-down for *SPG7*

Patients' fibroblasts

Caenorhabditis elegans KO for *spg7*

AIMS

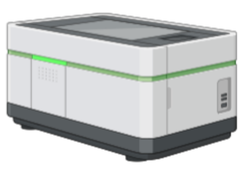
Investigate new players modulating mitochondrial activity by using different models.

DESIGN AND METHODOLOGY

1 HeLa cells KO for *SPG7*

mitome siRNA library

High-throughput imaging-based pipeline[3]



Proliferation, membrane potential and mitochondrial morphology

In HeLa cells KO for *SPG7*, we will down-regulate mitochondrial genes and look at the effects of this down-regulation : restoration or degradation of mitochondria morphology, membrane potential (using TMRE) and proliferation (with NucBlue).

2 Patients fibroblasts

potential genetic candidates

OXPHOS
Apoptosis
Mitophagy

We will use patients' fibroblasts obtained thanks to a collaboration with Pr. A Durr and SPATAX network. Using these fibroblast, we will be able to reflect patient biology. We will look at potential rescue or degradation of mitochondrial functions by using the genetics candidates identified in step 1.

Caenorhabditis elegans

siRNA library

Mitochondria morphology and functions
Behaviour assays

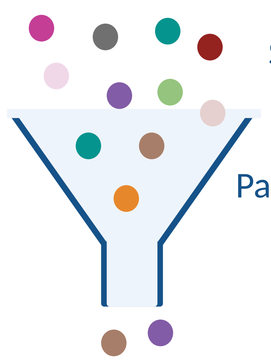
In *C.Elegans* KO for *spg7*, we will down-regulate mitochondrial genes found as interesting in step 1 and look at restoration or degradation of mitochondria morphology and functions, allowed by worm transparency. We will also measure time lifespan and assess behavior assays on worms : pharynx and rectum contraction.

At the same time...
WES of *SPG7* patients

WES

New variants

Sequence the whole exome of *SPG7* patients to investigate for other variants .



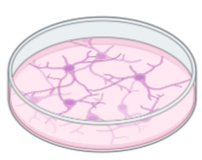
Screening and genetic analysis

Patients' fibroblast and *C.Elegans*

⇒ Players modulating mitochondrial activity

GOING FURTHER

Mechanism



Developed iPSCs - derived neurons from patients :
- Better understand molecular and cellular mechanisms
- Screening approach with drugs of therapeutic interest

REFERENCES

[1] Darios, F., Coarelli, G., and Durr, A. (2021). Genetics in hereditary spastic paraplegias: Essential but not enough. Current Opinion in Neurobiology *72*, 8–14.
[2] Wedding IM et Al. Spastic paraplegia type 7 is associated with multiple mitochondrial DNA deletions. PLoS One. 2014 Jan 22
[3] Cretin E et Al. High-throughput screening identifies suppressors of mitochondrial fragmentation in OPA1 fibroblasts. EMBO Mol Med. 2021 Jun 7;13