Mechanism of impaired microtubule-dependent peroxisome trafficking and oxidative stress in *SPAST*-mutated cells from patients with Hereditary Spastic Paraplegia

Gautam Wali

Gautam Wali[1], Ratneswary Sutharsan[2], Yongjun Fan[2], Ariadna Recasens[1], Nicholas F. Blair[1], Carolyn M Sue[1] & Alan Mackay-Sim[2]

[1]Kolling Institute of Medical Research, University of Sydney, Sydney, Australia

[2]Eskitis Institute for Drug Discovery, Griffith University, Brisbane, Australia

Abstract

Hereditary spastic paraplegia is a neurological disorder characterised by degeneration of the axons along the corticospinal tract leading to lower limb spasticity. Mutations in the gene *SPAST* are the major cause of HSP. *SPAST* encodes Spastin, a protein involved in regulating microtubule dynamics.

We used olfactory-neurosphere derived (ONS) cells, a population of neural progenitor cells, derived from biopsies of the olfactory mucosa from HSP patients and from healthy controls, in order to identify cell functions altered in HSP. The patient cells have reduced Spastin, reduced stabilised microtubules (acetylated α tubulin), altered cellular distribution of cellular organelles mitochondria and peroxisomes and impaired peroxisome transport. When stabilised microtubule levels were restored in the patient cells using tubulin-binding drugs the peroxisome transport was also restored to control levels. Patient ONS cells were under oxidative stress and more sensitive to oxidative stress induced by hydrogen peroxide. These were restored to control levels by low doses of tubulin-binding drugs.

Based on our findings in patient ONS cells, we suggest a mechanism whereby *SPAST* mutations lead to reduced levels of stable microtubules which compromises peroxisome trafficking and leads to increased oxidative stress. To test if our findings in the patient ONS cells are relevant in patient cortical neurons those are degenerated in HSP patients, we reprogrammed *SPAST* patient skin fibroblast cells to induced pluripotent stem cells (iPSCs) and differentiated the iPSCs to cortical neurons. Patient derived iPSC-neurons showed amplified disease specific deficits previously observed in patient ONS cells. The tubulin-binding drugs rescued the disease-specific defects in *SPAST* iPSC-neurons. Our research establishes the use of multiple sources of patient-derived cells for disease modelling and drug discovery for HSP.