Exploring the dysregulation of RNA-binding proteins, including matrin-3, in MJD

A Potapenko[1], JM Davidson[1], M Watchon[1], KJ Robinson[1], S An[1], A Lee[1], and AS Laird[1]

[1]Motor Neuron Disease Research Centre, Macquarie Medical School, Faculty of Medicine, Health and Human Sciences, Macquarie University, Australia

ABSTRACT:

Machado-Joseph disease (MJD) is caused by CAG repeat expansion in the *ATXN3* gene. This results in polyglutamine (polyQ) expansion in the ataxin-3 protein, which causes the protein to misfold and aggregate in the brain of people living with MJD. An important yet under-researched function of ataxin-3 is its deubiquitinase function, meaning that ataxin-3 edits ubiquitin chains on proteins and can therefore regulate the degradation, protein-protein interactions, and subcellular localisation of proteins. Emerging evidence suggests that polyQ expansion may alter ataxin-3's deubiquitinase function, however, if polyQ expansion causes a gain or loss of ataxin-3's deubiquitinating function, the impact of this on the downstream targets of ataxin-3, and how this contributes to MJD pathophysiology, remains unclear. We have used a combination of immunoblot, co-immunoprecipitation, LC-MS/MS proteomic analysis, and immunofluorescent staining, in cell, zebrafish, and mouse models of MJD to investigate whether polyQ expansion within ataxin-3 alters its deubiquitinase function and whether this contributes to MJD pathophysiology.

Ubiquitination via K48-linked chains is the most common linkage type sequestering proteins to proteasomal degradation, and dysfunction of the ubiquitin-proteasome system is observed in MJD. We therefore sought to identify proteins that may be differentially K48-ubiquitinated in cells expressing polyQ-expanded compared to wild-type ataxin-3. We hypothesised that these may be proteins differentially deubiquitinated by polyQ-expanded ataxin-3, and therefore important to MJD pathophysiology. We immunoprecipitated K48-ubiquitinated proteins in cells expressing either wild-type or polyQ-expanded human ataxin-3, or no human ataxin-3, and performed LC-MS/MS proteomics. Several RNA metabolism proteins were suggested to be differentially K48-ubiquitinated in cells expressing polyQ-

expanded compared to wild-type ataxin-3, including matrin-3, which is implicated in motor neuron disease, however, is studied in MJD for the first time here. We subsequently identified: 1) expression of matrin-3 protein is increased in the cerebellum and cerebrum of MJD mice compared to wild-type mice, and in cells expressing polyQ-expanded or wild-type human ataxin-3 compared to deubiquitinase-inactive ataxin-3; 2) ataxin-3 and matrin-3 proteins co-localise and co-immunoprecipitate in cells; 3) matrin-3 is degraded predominantly by autophagy and its degradation by autophagy appears to be impaired in cells expressing polyQ-expanded ataxin-3. Collectively, this suggests that ataxin-3 can regulate matrin-3 and that there is dysregulation of matrin-3 in MJD. We are continuing to investigate how changes in ataxin-3's deubiquitinase function due to polyQ expansion may underpin dysregulation of matrin-3 in MJD, with the hope that understanding how polyQ expansion changes ataxin-3's deubiquitinase function will unravel new avenues for MJD treatment.