Final Report

Author: Dr Michael Janitz
Qualification: Senior Lecturer
Institution: UNSW Australia

Title of Project:
The role of hemoglobin proteins in molecular pathology of multiple system atrophy.

Summary
Multiple system atrophy (MSA) is defined as a sporadic neurodegenerative disease, with an onset in adult life characterized by progressive development with etiology of an underdetermined nature. It is clinically characterized by varying degrees of the features of Parkinson's disease (PD) such as shaking, rigidity, slowness of movement, and difficulty with walking, and autonomic disorders of the genitourinary system and cortex. MSA equally affects both men and women, primarily in their 50s; however, disease onset as early as age 30 has been diagnosed. The progression of disease is rapid, and patients are confined to bed within 5 years of symptom onset with death resulting within an average of 9 years. With 3 cases per 100,000 individuals, MSA is considered rare; however, its prevalence is similar to multiple sclerosis (MS) (2.5 per 100,000) and motor neuron disease (1.5-2 per 100,000).

We recently discovered that haemoglobin (HB) genes are highly expressed in the MSA brain. Hemoglobin protein transports oxygen throughout our tissues. It is the largest source of peripheral iron in the human body and it may play a role in regulation of iron level in the brain. We hypothesised that the increased levels of hemoglobin cause oxidative stress, which leads to impairment of brain cells functionality. Moreover, oxidative stress, together with increased levels of hemoglobin proteins, might have a direct impact on α-synuclein aggregation.

To determine the influence of HB overexpression on oligodendrocyte maturation we generated HBA and HBB transgenic oligodendrocyte precursor cells (OPCs), which have then been induced to differentiate into mature oligodendrocytes. After 4 days of culture the cells were harvested and total RNA isolated. Next, using quantitative reverse transcription PCR (RT-qPCR), we assessed an impact of HB gene overexpression on oligodedrocyte maturation markers. We observed downregulation of MOBP, OMG, MAG, CNPase and MBP genes. Further, we examined phenotypic changes of the cells, such as proliferation rate and extension of processes, and again observed diminished growth of transgenic OPCs as compared to non-overexpressing cells.

In the next step of the study we evaluated an induction of oxidative stress in HB-transgenic OPCs using CellROX reagents, that measure generalized oxidative stress in

cells using conventional fluorescence microscopy. Interestingly, we observed a significant increase of reactive oxygen species upon overexpression of HB.

HB overexpression and α-synuclein aggregation is accompanied by a cellular response. In the next stage of the project we have characterised this physiologic response of transgenic OPCs to exposure to soluble and fibrillar α-synuclein using two-dimensional differential gel electrophoresis (2D-DIGE) analysis. Using mass spectrometry we evaluated changes upon HB overexpression and α-synuclein exposure as well as assessed post-translational modifications as a result of α-synuclein aggregation. Principal component analysis of selected proteins of interest revealed that the differences between untreated control cells and cells exposed to soluble oligomeric α-synuclein are not significant. In contrast analysis of the protein spots from cells exposed to fibrillar α-synuclein showed significant difference as compared to control. Proteins which underwent expression changes upon exposure to exogenous α-synuclein fibrils, were excised from 2D gels, digested with trypsin and identified using tandem MS with a nanoLC-LTQ-Orbitrap. Proteins were identified based on specific trypsin cleavage sites using Mascot software. We identified proteins involved in regulation of transcription, DNA replication, cell signalling and regulation of translation.

Taken together, the results of this project suggest that HB overexpression has a significant impact on alpha-synuclein aggregation and maturation of oligodendrocytes which is a primary target of MSA molecular pathology.

Unanswered Questions
In this project we focused on investigation of biological effects of hemoglobin overexpression on oligodendrocyte growth and maturation. Our study strongly suggests that HB proteins excess has a profound effect on oligodendrocyte physiology and also impacts cellular reaction on α-synuclein aggregation. Future studies should focus on assessment of transcriptome changes as result of HB overexpression using genome-wide approches such as RNA sequencing. Correlative analysis between global changes of gene expression and their protein products would provide further insights into unique MSA pathology and deliver greater molecular resolution to differentiate early onset of this neurodegeneration from other α-synucleinopathies.

What these research outcomes mean
The outcomes of this project are of fundamental significance in resolving the pathogenesis of the clinically distinct, although etiologically similar α-synucleinopathies, namely MSA and PD. Through investigation of a link between overexpression of hemoglobins and function of oligodendrocytes, which are the main target for α-synuclein aggregation, we established foundation of our understanding of molecular networks which are specific to MSA.