Submitted for partial fulfillment of Doctor of Philosophy (PhD)

ANALYSING THE BIOLOGICAL FUNCTION OF PS2V: AN ABERRANT SPLICING PHENOMENON OR EVOLUTIONARILY CONSERVED MECHANISM IN ALZHEIMER'S DISEASE

Seyyed Hani Moussavi Nik

Supervised by Dr. Michael Lardelli and Dr. Joan Kelly

Discipline of Genetics
School of Molecular and Biomedical Sciences
The University of Adelaide
Australia

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LIST OF PUBLICATIONS CONTRIBUTED TO DURING PH.D. CANDIDATURE

The Guinea pig as a model for sporadic Alzheimer's disease (AD): the impact of cholesterol intake on expression of AD-related genes.
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BMC Research Notes, 2014

Alzheimer's disease-related peptide PS2V plays ancient, conserved roles in stimulation of γ-secretase activity and suppression of the unfolded protein response under hypoxia.
Seyyed Hani Moussavi-Nik, Morgan Newman, Lachlan Wilson, Esmaeil Ebrahimie, Simon Wells, Mark Van Der Hoek, Giuseppe Verdile, Ralph N. Martins and Michael Lardelli.
Human Molecular Genetics, 2014 (Under review)

A zebrafish homologue of Alzheimer's disease-associated PRESENILIN isoform PS2V regulates inflammatory and other responses to hypoxic stress.
Esmaeil Ebrahimie, Seyyed Hani Moussavi-Nik, Morgan Newman, Mark Van Der Hoek, and Michael Lardelli**.**
Human Molecular Genetics, 2014 (Under review)

ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disorder with pathologies such as neuron loss, glial cell proliferation, extracellular deposition of senile plaques from the accumulation of amyloid beta (Aβ) peptides and deposition of intracellular neurofibrillary tangles. Aβ is generated from the cleavage of the Amyloid Precursor Protein (AβPP) by two different types of aspartyl proteases, β- and γ-secretase. The majorities of AD cases are sporadic and have a late onset. Mutations in genes encoding APP, PRESENILIN1 and *2* (*PSEN1* and *PSEN2*) cause an autosomal dominant inherited form of the disease with an early onset known as familial AD. In some sporadic cases an aberrant splice variant of *PSEN2* named PS2V is formed that can be found in inclusion bodies in the brain. PS2V results from the binding of the High Mobility Group A1a (HMGA1a) protein close to the splice donor site of exon 5 of *PSEN2*. HMGA1a is widely expressed during embryo development but not in adults. Its expression can be induced in adult neurons by hypoxia/oxidative stress and it is commonly reactivated in many types of cancer.

Zebrafish embryos have a unique combination of characteristics that allows genetic manipulation and analysis of molecular pathways implicated in neurodegenerative diseases. The embryos are numerous, macroscopic, external to the mother and transparent making them easy to inject and observe. Changes in different aspects of their rapid development can be used as bioassays to assess gene activity. In **chapter II** of this thesis, we present evidence from a number of different assays that acute exposure to hypoxia or chemical mimicry of hypoxia increases oxidative stress in zebrafish brain tissue. We demonstrated that intracellular ROS levels are significantly increased in zebrafish brains exposed to actual hypoxia or chemical mimicry of hypoxia using NaN_3 hypoxia. In **chapter III** of this thesis we examine the evolutionary conservation of PS2V and investigate its effect on gene expression profiles, γ- secretase activity. In this chapter we show evidence for an important role of PRESENILIN genes in cellular responses to low oxygen (hypoxia). The PS2V splicing isoform of human *PSEN2* transcripts is generated under hypoxic conditions through induction of HMGA1a that binds to exon 5 sequence in transcripts. We show that an orthologue of the PS2V isoform, PS1IV, exists in the zebrafish. The novel splice product of zebrafish *psen1*, "PS1IV" codes for a much smaller peptide then PS2V but, nevertheless is capable of boosting γ-secretase activity. In **chapter IV** we utilised microarray to analysis the function of PS1IV in modulation of a wide variety of gene products. We show that that production of this PS1IV is accompanied with activation of stress response genes such as interleukin 1 Beta (*IL1B),* tyrosine hydroxylase *(TH)*, and myelin expression factor (*MYEF)* which leads to triggering apoptosis and autophagy. We also demonstrate that PS1IV is an important contributor in signaling pathways associated with AD.

In **chapter V** we investigated the guinea pig, *Cavia porcellus*, as a model for Alzheimer's disease (AD), both in terms of the conservation of genes involved in AD and the regulatory responses of these to a known AD risk factor - high cholesterol intake. We demonstrate that PS2V formation is up-regulated by hypoxia and a high-cholesterol diet while, consistent with observations in humans, Aβ concentrations are raised in some brain regions but not others. We have previously identified two paralogues (co-orthologues) of *MAPT* in zebrafish, denoted *mapta* and *maptb* and have shown that both genes are expressed in the developing central nervous system. In **chapter VI** we extend our examination of expression of the zebrafish tau co-orthologues to study their response to actual hypoxia and chemical mimicry of hypoxia in explanted adult fish brains. We observed increases in the overall levels of both *mapta* and *maptb* transcripts due to specific increases in the levels of *mapta* 6R and *maptb* 4R transcript isoforms. This is consistent with dramatically decreased levels of transcripts of the zebrafish orthologue of the human *TRA2B* gene that codes for a splicing factor proposed to regulate alternative splicing of *MAPT* transcripts.