Molecular responses to low oxygen levels/oxidative stress in Zebrafish

Seyyed Hani Moussavi Nik

Supervised by Michael Lardelli and Joan Kelly

Discipline of Genetics

School of Molecular and Biomedical Sciences

The University of Adelaide

Australia

March 2011

Contents

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 created from the cleavage of the Amyloid Precursor Protein (APP) by two different types of aspartyl proteases, β- and γ-secretase.The majority of AD cases are sporadic and have a late onset.Mutations in the genes encoding APP,PRESENILIN1 and *2* (*PSEN1* and *PSEN2*) genes 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*.The High Mobility Group A1 protein,HMGA1, 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.

Hypoxia can be a direct consequence of hypoperfusion, a common vascular component among Alzheimer's disease risk factors and may play an important role in AD pathogenesis. BETA-SITE AMYLOID BETA A4 PRECURSOR PROTEIN-CLEAVING ENZYME 1, BACE1 is responsible, with γ -secretase, for cleavage of AMYLOID PRECURSOR PROTEIN, APP to produce Aβ peptide. A recent study observed that oxidative stress upregulates BACE1 expression via a regulatory pathway that is dependent on γ -secretase cleavage of APP and that results in increased Aβ peptide production.

In this thesis, we define strategies for exposure of zebrafish to hypoxia and “chemical hypoxia”. We identifiyendogenous zebrafish *hmga1* in an attempt to investigate PS2V formation in fish. We also demonstrate that responses to low oxygen/oxidative stress by genes involved in Alzheimer’s disease are evolutionarily conserved in fish. Paper1 (thesis chapter in the form of a manuscript) describes the identification of the *hmga1* gene in zebrafish which is an orthologue of human *HMGA1*. It also examines the regulation of this gene under hypoxia/oxidative stress conditions and demonstrates that*hmga1* expression is induced under these conditions. However no PS2V-like splice variant of zebrafish *psen2* is observed. Paper 2 (thesis chapter in the form of a manuscript) describes the identification of the zebrafish *bace1* gene which is orthologous to human *BACE1*. It also examines the regulation of AD-related genes under hypoxia/oxidative stress. We show that the response of the *BACE1-PSEN-APP*regulatory axisto hypoxia/oxidative stress is evolutionarily conserved between fish and mammals. Therefore, we also demonstrate that zebrafish are a valid model system for analysis of the effects of hypoxia/oxidative stress on genes associated with Alzheimer’s disease.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Seyyed Hani Moussavi Nik and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Sign

Date

List of Publications

The response of *HMGA1* to changes in oxygen availability is evolutionarily conserved

Seyyed Hani Moussavi Nik[a,*], Morgan Newman[a] and Michael Lardelli[a]

Journal of Experimental Cell Research, manuscript submitted 18 December 2010

The *BACE1-PSEN-APP* regulatory axis has an ancient role in response to low oxygen/oxidative stress

Seyyed Hani Moussavi Nik[a,*],Lachlan Wilson[a], Morgan Newman[a] and Michael Lardelli[a]

Journal of Alzheimer's disease, manuscript submitted for publication on 6April 2011

Acknowledgements

I am heartily thankful to:

My supervisor, Michael Lardelli, for his guidance and encouragement and support during the last 1.5 years.

Dr. Morgan Newman for her advice, assistance and support. Members of our lab, Simon Wells, Lachlan Wilson, Yuya Sugano and Anne Lim and the Discipline of Genetics for their advice and support in any respect during the compilation of the work.

Finally, my dear family and friends for all their love and support.

Without these people (science related or not), none of this would be possible.