Gene regulatory and pro-tumourigenic mechanisms of the bHLH-PAS Transcription Factor SIM2s

Alexandra Louise Farrall
B.Sc (Jurisprudence) (Hons.)

A thesis submitted in the fulfilment of the requirements for the degree of Doctor of Philosophy

Discipline of Biochemistry
School of Molecular and Biomedical Science
The University of Adelaide, Australia
21st May, 2009

Table of Contents

ABSTRACT

Single-minded 2 (SIM2), a class I basic Helix-Loop-Helix/PAS (bHLH/PAS) transcription factor, is essential for early development, and the short isoform (SIM2s) is selectively up-regulated in pancreatic and prostate tumours. Mechanistic role(s) for SIM2 that are essential for development and in these cancers is unknown, largely due to the fact that few *bona fide* target genes have been described for SIM2. SIM2 must heterodimerise with the obligate class II partner factor ARNT to regulate transcription. Surprisingly, these studies reveal SIM2 plays a role in the regulation of the ARNT homologues, ARNT1 and ARNT2. Two non-exclusive mechanisms were identified; enhanced protein stabilisation, and the specific increased transcription of *ARNT2*. The regulation of ARNT by a class I family member was found to be unique to the SIM homologues. These findings suggest novel insights into how elevated levels of SIM2s in tumours may confer increased transcriptional activities and/or increase the availability of the essential partner factor for other class I family members to promote their respective activities and functions in developmental and/or tumourigeneic processes. Furthermore, microarray studies in prostate DU145 cells identified the pro-cell death gene, *BNIP3* (Bcl-2/adenovirus E1B 19kDa interacting protein 3), as a novel target of SIM2s mediated repression. Further validation showed BNIP3 repression in several prostate and pancreatic carcinoma derived cell lines with ectopic expression of human SIM2s via SIM2s activities at the proximal promoter hypoxia response element (HRE), the site through which bHLH/PAS family member, Hypoxia-Inducible Factor 1α (HIF1α), induces *BNIP3*. SIM2s attenuates *BNIP3* hypoxic induction via the HRE, and increased hypoxic induction of *BNIP3* occurs with siRNA knockdown of endogenous SIM2s in prostate PC3AR+ cells. BNIP3 is implicated in hypoxia-induced cell-death processes. PC3AR+ cells expressing

ectopic SIM2s have enhanced survival upon treatment with hypoxia mimetics, DP and DMOG. LC3-II protein levels fail to induce in PC3AR+/SIM2s DMOG and hypoxia treated cells, suggesting SIM2s may attenuate autophagic cell-death processes, perhaps via BNIP3 repression. These data show, for the first time, SIM2s cross-talk on an endogenous HRE. SIM2s functional interference with HIF1α activities on *BNIP3* may indicate a novel role for SIM2s in promoting tumourigenesis. Moreover, SIM2 expression has previously been implicated in the Hedgehog (Hh) signalling pathway during mouse brain development. The Hh-pathway is known to promote pancreatic and prostate tumour growth, and these studies indicate that SIM2s is indeed implicated in promoting and/or maintaining Hh-signalling in cell lines of these cancer types. Likewise, aberrant Androgen Receptor (AR)-signalling is implicated in prostate tumour development, and androgen-independent AR activity is a hallmark of aggressive prostate cancer. Unexpectedly, SIM2s expression was found to up-regulate endogenous AR protein levels in prostate carcinoma PC3AR+ cells. Furthermore, SIM2s expression is associated with androgen-dependent wtAR-transcriptional responsiveness in these cells, and SIM2s co-immunoprecipitates with endogenous AR in a hormone independent manner. Together these data suggest, for the first time, that SIM2s may function as a co-activator, and concomitant with enhancing AR levels, aid AR-signalling in prostate cancer cells. In summary, these studies sought to identify molecular mechanisms by which aberrant levels of SIM2s expression in solid tumours of the prostate and pancreas may promote tumour development. Several novel mechanisms for SIM2s activities were identified which implicate SIM2s in tumour processes. Namely SIM2s was found to be implicated in:

1) promoting pro-tumourigeneic Hh and AR signalling pathways

2) regulation of the common partner factor ARNT, and

3) attenuation of hypoxically-induced cell-death processes in tumour cells via the direct transcriptional repression of the novel SIM2s target gene, *BNIP3*.

CANDIDATES DECLARATION

To the best of my knowledge, 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, 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.

The author acknowledges that copyright of published works contained within this thesis (as listed below) resides with the copyright holder(s) of those works. 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, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Alexandra Farrall

21st of May 2009

1. Woods, S., Farrall, A., Procko, C., & Whitelaw, M. The bHLH/Per-Arnt-Sim transcription factor SIM2 regulates muscle transcript myomesin2 via a novel, non-canonical E-box sequence. *Nucleic Acids Res.* 2008 Jun;36(11):3716-27

PUBLISHED & PRESENTED WORK ARISING FROM THIS THESIS

Publications:

1. Woods, S., Farrall, A., Procko, C., & Whitelaw, M. The bHLH/Per-Arnt-Sim transcription factor SIM2 regulates muscle transcript myomesin2 via a novel, non-canonical E-box sequence. *Nucleic Acids Res.* 2008 Jun;36(11):3716-27

2. Farrall, A., and Whitelaw, M. The HIF-1α inducible pro-cell death gene BNIP3 is a novel target of SIM2s repression via cross talk on the Hypoxia Response Element. *(Manuscript under peer review)*

Conferences and Symposia Presentations:

Published abstract:

Farrall, A., and Whitelaw, M. SIM2s functional interference with HIF1α-inducible expression of the pro-cell death factor BNIP3 - A novel target of SIM2s repression via the hypoxia response element. 2009 (*in press*) *Annals of the NYAS*.

Invited oral presentations:

1. Flinders University Medical School Seminar Series, SA, Australia, July 23rd, 2008
Presentation Title: "Is anything SIMple in tumourigenesis?: Putative role for the bHLH-PAS transcription factor, SIM2, in tumour progression through regulation of pro-apoptotic BNIP3" A. Farrall & M. Whitelaw

2. Network in Genes and Environment in Development [NGED] Forum:
Cross Disciplinary Workshop Attendance Award, 2007, June 13-15th 2007 - Palm Cove, QLD, Australia

Presentation Title: “NGED Epigenetics Workshop, QIMR, 2006: Investigating a putative role for the transcription factor SIM2 in tumourigenesis” A. Farrall & M. Whitelaw

Poster presentations:

1. Howard Hughes Medical Institute [HHMI]: Modern Technologies in Gene Expression Detection and Data Integration Course, July 2006

The University of Debrecen, Debrecen, HUNGARY

Poster: “Investigating a role for the transcription factor SIM2s in tumourigenesis” A. Farrall & M. Whitelaw

2. New York Academy of Sciences: “Hypoxia and Consequences: from Molecule to Malady” Conference, March 2009 – New York, NY, USA

Poster: “SIM2s functional interference with hif1α-inducible expression of the pro-cell death factor bnip3 - a novel target of SIM2s repression via the hypoxia response element” A. Farrall, & M. Whitelaw

3. 28th Lorne Genome Conference 2007 – Lorne, VIC, Australia

Millennium Science Student Poster Prize –

Poster presentation entitled “Investigating a role for the transcription factor SIM2 in tumourigenesis”. A. Farrall & M. Whitelaw

4. 20th Lorne Cancer Conference 2008 – Lorne, VIC, Australia

Poster: “Interplay of bHLH/PAS transcription factors in tumourigenesis: Single Minded 2 Competes with the Hypoxia Inducible Factor for Regulation of the Pro-apoptotic gene BNIP3” A. Farrall & M. Whitelaw

ACKNOWLEDGEMENTS

Firstly, to my supervisor and mentor Murray Whitelaw; thank you for all the insightful conversations, inspiring excitement and passion for science, and allowing me the opportunity to study and work in a supportive, stimulating and fun environment. I am particulary grateful for all the opportunities to extend my professional, and personal, development via study and work both at home and abroad. I am also grateful to Daniel Peet for inspired advice and many laughs, to Anne Chapman-Smith, for her patience and insight, and to Keryn Williams, for her encouragement and sincere interest in my professional development. I am particulary most thankful for the friendship and mentorship of Susi Woods, a brilliant mind with a big heart. I was honoured to be your 'partner in (SIM2) crime'! Fiona Whelan, my 'bay buddy' and friend; thanks for your quick wit and mind, all the daily laughs, science-talk, life-talk and encouragement along the way. It was wonderful to share the 'PhD journey' with you! I sincerely thank Margo van Bekkum and Colleen Bindloss for their friendship and brilliant technical assistance, and to all past and present Whitelaw and Peet Lab members, and many in the School of Molecular & Biomedical Science who have all been supportive friends and collegues and contributed to making a dynamic and fun work environment. Big thanks to Jo, Mike, Sebastian, Anne R, Dave, Andrew and Adrienne – the next carrier of the SIM2 torch, with your mind behind it, it will surley be bright! I am privileged to have had the opportunity to work with our collaborators Lorenz Poellinger and Katarina Gradin, a most patient teacher and friend, at the Karolinska Institutet in Stockholm, Sweden, also in collaboration with Helena Edlund and Elisabet Palsson at the Univeristy of Umeå, Sweden. I would also like to thank the staff of the Adelaide Microarray Centre. Importantly, my deepest thanks go my supportive parents, wonderful brothers and sisters, and friends for all the fun, love, friendship and understanding. Lastly, and most importantly, my heartfelt gratitude and appreciation goes to my husband Pelham; ever constant, ever understanding and supportive, and a source of inspiration and continued encouragement.