Expression and functional analysis of SOX3 in murine neurogenesis

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Discipline of Biochemistry

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Thesis Amendments

Introduction:

Page 12- “Originally” not “Original”

Page 20- “severe” not “server”

Page 22- “affected” not “effected”

Introduction figure 10 (legend)- “Antigen peptide sequence used to generate” not “Binding site of”

Page 27-“ C57BL/6” not “ black6”

Page 28- “effector” not “effectors”

Page 29- “A recent” not “A Recent”

Materials and Methods:

Pages 37- “C57BL/6” not “c57Bl/6”

Page 41- “Wild type Male on the Sox3 null background” not “Male Sox3 null +/Y”

Acknowledgments:

“Patience” not “patients”

Contents

Abstract

The Sox (SRY-related HMG box) family of proteins are transcription factors. There are, in total, 30 different genes in the Sox family. Each Sox protein contains a HMG box (high-mobility-group) which functions as a DNA binding domain. The HMG box is highly conserved (>50% identity) throughout the entire Sox family. *Sox3* belongs to the SoxB1 subgroup.

SOX3 has been associated with human CNS related disorders. Duplication and mutations of *SOX3* have been identified in patients with X-linked hypopituitarism (XH). Afflicted XH patients suffer from varying levels of mental retardation and pituitary hormone deficiencies which can lead to short stature.

Previous studies have shown that *Sox3* is expressed in nascent neuroprogenitor cells and is functionally required in mammals for development of the dorsal telencephalon and hypothalamus. Using a SOX3-specific antibody, data within my thesis shows that murine SOX3 expression is maintained throughout telencephalic neurogenesis and is restricted to progenitor cells with neuroepithelial and radial glial morphologies. In addition, characterisation of SOX3 expression within the adult neurogenic regions indicates that it is a lifelong marker of neuroprogenitor cells.

In contrast to the telencephalon, *Sox3* expression within the developing hypothalamus is up-regulated in developing neurons and is maintained in a subset of differentiated hypothalamic cells through to adulthood.

In addition, using genome wide expression analysis examining a *Sox3* null neural progenitor population, I identified a number of putative *Sox3* targets. The data identified *Dbx1* as a robust Sox3 target with Dbx1 down-regulation, at both the

mRNA and Protein level, within Sox3 null mice at early stages of CNS development. I also independently confirm a number of SOX3 binding sites surrounding *Dbx1*, with one site showing clear enrichment *in vivo*. In addition, correlation between these putative targets and that of a previously published SOX3-ChIP data set show a clear enrichment for SOXB1 binding sites near the mis-regulated genes suggesting they are direct targets of *Sox3*.

Taken together, data presented within my thesis identifies new regions of *Sox3* expression and putative Sox3 targets. This data helps advance our knowledge of *Sox3* regulation and function within CNS development.

Original publications

This thesis is based on the following publications:

Results Chapters 1 (Rogers et al, 2013):

Rogers, N., Cheah, P. S., Szarek, E., Banerjee, K., Schwartz, J. and Thomas, P. (2013). Expression of the murine transcription factor SOX3 during embryonic and adult neurogenesis. *Gene Expr Patterns* **13**, 240-8

Results Chapter 2 (Hughes et al, 2013):

Hughes, J., Piltz, S., **Rogers, N.,** McAninch, D., Rowley, L. and Thomas, P. (2013). Mechanistic insight into the pathology of polyalanine expansion disorders revealed by a mouse model for X linked hypopituitarism. *PLoS Genet* **9**, e1003290.

Results Chapter 2 (Rogers et al, Manuscript):

Rogers,N.,McAninch,D. And Thomas P. Dbx1 is a direct target of Sox3 in the spinal cord. (Manuscript)

Declaration

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Abbreviations:

3V	Third ventricle
ANOVA	Analysis of variance
ARC	Arcuate nucleus
cDNA	complementary DNA
ChIP	Chromatin immunoprecipitation
CNS	Central nervous system
DNA	Deoxyribonucleic acid
dpc	Days post coitum
ES	Embryonic stem
HMG	High mobility group
H&E	Haematoxylin and eosin staining
IHC	Immunohistochemsitry
Kb	Kilobase(s)
LOF	Loss of function
LV	Lateral ventrical
ME	Median eminence
MPOA	Medial preoptic area
Ne	Neurohypophysis
NP	Neural progenitors
P	Postnatal
PCR	Polymerase Chain Reaction
PL	Pial Layer
qRT-PCR	quantitative Real Time PCR
RIN	RNA integrity number
RNA	Ribonucleic acid
SGZ	Sub granular Zone
SOX	Sry-related HMG Box

SRY	Sex determining region Y
SVZ	Sub ventricular zone
TV	Telencephalic vesicles
VZ	Ventricular Zone
VD	Ventral Diencephalon
XH	X-linked hypopituitarism