Identification and characterisation of novel transcripts involved in the proliferation, differentiation and developmental networks of the mouse cerebral cortex

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ABSTRACT

Cerebral corticogenesis involves specific influence of intrinsic and extrinsic mechanisms, which are triggered spatiotemporally. During mouse embryogenesis, the mouse cerebral cortex develops from a relatively homogenous band of mitotic multipotent progenitor cells into a complex laminated structure between embryonic day 11.5 (E11.5) and 18.5 (E18.5). Identification of molecular targets and regulatory networks involved in cerebral corticogenesis is crucial for the understanding of the development and function of the cortex.

Global transcriptome profiling of the mouse cerebral cortex at various developmental stages using Serial Analysis of Gene Expression (SAGE) technique identified 561 differentially expressed tags/transcripts (DETs). Hierarchical and genomic clustering of DETs showed common functional ontologies and molecular networks that are associated with neurological disorders in human. In addition, 4 genomic loci at *Sox4*, *Sox11*, *Nrgn* and *Camk2n1* were significantly represented by embryonic and adult-specific DETs when compared to other genomic loci. These genomic loci have multiple overlapping sense and natural antisense transcripts (NATs) featuring different polyadenylation signal sites and spatiotemporally regulated expression profiles. The study suggests that these antisense transcripts have an important role in cerebral corticogenesis and neuronal/glial cell differentiation or function.

These NATs were further characterized using Fluorescence *In situ* Hybridization (FISH) probes specific to the sense and antisense transcripts of *Sox4*, *Nrgn* and *Camk2n1* RNA in trypsinized adult brain cells. The analysis showed co-localization of sense and antisense transcripts and confirmed the formation of sense-antisense double stranded RNA (dsRNA) in the cytoplasm. Overexpression of *Sox4* antisense transcripts did not regulate *Sox4* transcription or translation processes. Instead, *Sox4* dsRNAs serve as templates for the generation of a small RNA, namely *Sox4_sir3*, which is an endogenous small interfering RNA (siRNA). Its biogenesis is dependent on Dicer1 activity as well as the formation of dsRNA between *Sox4* sense and antisense transcripts. *Sox4*-sir3 is expressed specifically in the germinative zones and in specialized neurons throughout brain

development. This is the first demonstration in the mammalian system that cytoplasmic sense-NAT dsRNAs serve as templates for the production of novel endogenous siRNAs adding a new dimension to the long-debated controversial role of NATs in the genome.

Small RNAs such as microRNAs (miRNAs) can repress translation of protein-coding mRNA or direct mRNA decay by recruiting RNA-induced silencing complex (RISC) machinery. Massively parallel sequencing of an E15.5 developing mouse brain further identified 4 putative miRNAs and one of them was confirmed as a novel miRNA, M1181. M1181 was spatiotemporally expressed throughout mouse embryo development including mouse embryonic stem cells, E3.5 blastocysts, and embryos aged between E7.5 and E15.5. Between E13.5 and E17.5, M1181 expression was confined to the cortical plate of the cerebral cortex and the ventricular zone of midbrain. In adult mice, M1181 was strongly expressed in the brain, particularly the olfactory bulb, cerebrum, thalamus and midbrain. Taken together, the expression pattern of M1181 implicates its role as a potential novel regulator in early embryonic development involving ES cell pluripotency, neural tube formation and adult central nervous system function.

In a nutshell, novel transcripts involved in the developmental networks of the brain particular the cerebral cortex were identified using a variety of genomic and *in silico* approaches. A new role and related mechanism for novel *Sox4* NATs especially in the biogenesis of small RNA were described and this landmark discovery add to our understanding of the versatility of NAT function in mammalian biology.

STATEMENT

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.

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Date:

* *Published articles*

Methods published in these articles were cited in Chapter 2.

Ling KH, Hewitt CA, Kinkel SA, Smyth GK and Scott HS. High-throughput and complex gene expression validation using the Universal ProbeLibrary and the LightCycler® 480 system. ***Biochemica*** 2008, 2:23-26.

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Materials published in these articles were reused in Chapters 3, 4 and 6.

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* *Submitted manuscript*

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King Hwa Ling

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LIST OF ABBREVIATIONS

5’ RATE	Robust analysis of 5′-transcript ends
A, G, T, C, U	Adenine, Guanini, Thymine, Cytosine, Uracil
AGRF	Australian Genome Research Facility
AMPA	α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
BGEM	Brain Gene Expression Mapping
BLAST	Basic Local Alignment Search Tool
CAGE	Cap Analysis of Gene Expression
cDNA	complementary DNA
CP	cortical plate
cRNA	complementary RNA
DNA	deoxyribonucleic acid
dsDNA	double stranded DNA
dNTP	deoxyribonucleotide triphosphate
dsRNA	double stranded RNA
DV	dorso-ventral
E	embryonic day
ENCODE	ENCyclopedia Of DNA Elements
endo-siRNA	endogenous small interfering RNA
EST	expressed sequence tag
FISH	Fluorescence ISH
gDNA	genomic DNA
GE	ganglionic eminence
GENSAT	The Gene Expression Nervous System Atlas
GEO	Gene Expression Omnibus
IPA	Ingenuity Pathway Analysis
ISH	*In situ* hybridisation
IZ	intermediate zone
LGE	lateral GE
LNA	locked nucleic acid
lncRNA	long noncoding RNA
MGE	medial GE
miRNA	microRNA

MPS	Massively Parallel Sequencing
mRNA	messenger RNA
MZ	marginal zone
NAT	natural antisense transcript
ncRNA	noncoding RNA
NIA	National Institute of Aging of National Institute of Health
NIAID	National Institute of Allergy and Infectious Diseases
NMDA	N-methyl-D-aspartic acid
OMIM	Online Mendelian Inheritance in Man
P	postnatal day
PET	paired-end ditag
PMAGE	polony multiplex analysis of gene expression
RACE	rapid amplification of cDNA ends
RC	rostro-caudal
RNA	ribonucleic acid
SAGE	serial analysis of gene expression
SP	subplate
UCSC	University of California, Santa Cruz
UPL	UniversalProbe Library
UTR	untranslated region