Study of miRNA and circular RNA Role and Mechanism in Synaptic Plasticity and the Pathogenesis of Schizophrenia

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DECLARATION

Statement of Originality

I hereby certify that the work embodied in the thesis is my own work, conducted under normal supervision. The thesis contains no material which has been accepted, or is being examined, 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. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository, subject to the provisions of the Copyright Act 1968 and any approved embargo.

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28/05/2019

Ebrahim Mahmoudi **Date**

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LIST OF ABBREVIATIONS

ADAR1	RNA-specific adenosine deaminase 1
AD	Alzheimer's disease
AGO	Argonaute
ALB	albumin
Alu	Arthrobacter luteus
ANRIL	antisense noncoding RNA in the INK4 locus
ARC	apoptosis repressor with CARD domain
CDK2	cyclin-dependent kinase 2
CDR1as	cerebellar degeneration-related protein 1 antisense
ceRNA	competitive endogenous RNA
circRNAs	circular RNA
CSPP1	centrosome spindle pole-associated protein 1
E2F1	E2F Transcription Factor 1
ECM	extracellular matrix
EIciRNA	exon-intron circular RNA
EMT	epithelial–mesenchymal transition
ESCC	esophageal squamous cell carcinoma
EXOC6B	Exocyst Complex Component 6B
FAK	focal adhesion kinase
FBXW7	F-box and WD repeat domain containing 7
FOXO3	forkhead box O3
HEK 293T	human embryonic kidney 293 T antigene
HCC	hepatocellular carcinoma
HDAC2	histone deacetylase 2
HIF-1	hypoxia-inducible factor-1
Homer1b/c	homer scaffold protein 1

HRCR	heart-related circRNA
ICAM1	intercellular adhesion molecule 1
ID-1	inhibitor of DNA binding 1
IGF2BP	insulin-like growth factor 2 binding protein
LncRNA	long noncoding RNA
MBL	muscleblind
miRNA	microRNA
MMP-13	matrix metalloproteinase-13
mRNA	messenger RNA
MYRIP	myosin viia and rab interacting protein
OGD/R	oxygen-glucose deprivation/reoxygenation
PABP	Polyadenylate-binding protein
PAK1	p21-activated kinase 1
PAX6	paired box 6
PD	Parkinson's disease
PrPSc	particles composed of scrapie prion protein
PWMD	periventricular white matter damage
QKI	quaking
RBPs	RNA binding proteins
RIMS2	regulating synaptic membrane exocytosis 2
rRNA	ribosomal RNA
TNF	tumor necrosis factor
VEGF	vascular endothelial growth factor
YAP	yes-associated protein 1

ABSTRACT

Schizophrenia is a severe psychiatric disorder attributed to neurodevelopmental changes in connectivity and neurotransmission. While the acute psychotic symptoms usually respond to antipsychotic treatment, the chronic negative and cognitive symptoms are less responsive and represent a major unmet need in psychiatry. With a greater understanding of the molecular basis of the disorder, especially the debilitating cognitive symptoms it should be possible to refine the treatment options and improve the outcome for millions of people. With heritability around 80%, genetics has the potential to achieve important new insights into the biology of the disorder. One of the most interesting candidates to emerge from genome wide association studies is *MIR137*, a gene encoding the microRNA miR-137 whose expression seems essential for neural processes and brain development. As this gene encodes a small non-coding RNA, most of the functionally significant variation is likely to modify transcription and this is supported by postmortem analysis with reduced expression from the risk allele. One of these in close proximity to the miR-137 encoding segment is a 15-bp Variable Number Tandem Repeat (VNTR) (rs58335419). To investigate possible regulatory role of this variant in disease associated changes in cognitive and neuroanatomical features, DNA sequencing was performed on a cohort of schizophrenia and non-psychiatric controls with respect to their neurocognitive and neuroimaging phenotypes established by a battery of cognitive testing and magnetic resonance imaging. The results revealed VNTR was associated with cognitive performance, with the 4-repeat variant enriched in the cognitive deficit subtype of schizophrenia. Surface-based morphometry of imaging data also revealed that the VNTR carriers have significantly thinner grey matter in the left inferior temporal gyrus, deeper right mid-cingulate, and deeper right postcentral sulci relative to non-carrier individuals. These findings suggest that *MIR137* VNTR has biological function in the brain development and etiology of schizophrenia, particularly in relation to cognitive symptoms.

There is recent evidence to suggest that miRNA expression, more broadly, is important for brain function and synaptic plasticity, and is implicated in schizophrenia. The expression of these molecules is dynamically regulated by environmental exposures, including those associated with psychiatric disorders. Their function can also be modulated by another class of noncoding RNAs, known as circular RNA (circRNA). These transcripts, which are highly enriched in the brain, contain binding sites for miRNA, enabling them to act as endogenous competitors. To establish a more comprehensive model of gene regulatory networks in the neuronal biology, we profiled the expression of circRNA and analyzed their differential expression in neuronal development and aging, neuronal excitation, and in the pathophysiology of schizophrenia using RNA sequencing. Interestingly, the brain showed the highest level of enrichment and expression change during aging with an increased trend detected throughout the life span of the rats. Bioinformatic analysis of the circRNA-miRNA interaction indicated that the age-associated circRNAs might be involved in ageing processes by regulating mRNAs expression through sponging miRNAs.

The analysis of circRNA regulation in neuronal depolarization revealed a significant alteration in circRNA abundance which coincided with a change in miRNA and mRNA abundance, suggesting a circRNA-mediated gene regulation mechanism in the cellular response to neural activity. This was supported by both *in silico* and functional analysis suggesting that circular transcripts have the capacity to impact mRNA expression through interaction with common miRNAs. Finally, exploration of circRNA in neuropsychiatric disorder of schizophrenia revealed a substantial depletion of these transcripts in the disorder. A significant enrichment of neural functions and neurological disorders was observed for the differentially expressed circRNAs host genes in gene set analysis. Many of the depleted circRNAs have the potential to sequester miRNAs that were previously implicated in the neuropathology of schizophrenia, potentially exacerbating the functional impact of their dysregulation via posttranscriptional gene silencing.

In summary, the data presented in this thesis provide evidence of miRNA and circRNA association with neuronal development and neuronal activity, and their alteration in the pathogenesis of schizophrenia.