UNIVERSITI PUTRA MALAYSIA

CHARACTERIZATION OF MIR-3099-MEDIATED POSTTRANSCRIPTIONAL OF TARGET GENES REGULATION DURING NEUROGENESIS IN MICE

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By

SHAHIDEE ZAINAL ABIDIN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree Doctor of Philosophy

May 2019

This thesis is dedicated to……..

"For those who do not give up on their dreams"

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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May 2019

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MicroRNAs (miRNAs) are a family of small non-coding RNAs with potent regulatory roles in metabolism, neurodevelopment, neuroplasticity, apoptosis, and other neurobiological processes. MiRNAs function through partial complementary base-pairing with specific target mRNAs, resulting in the repression of translational processes or the promotion of mRNA deadenylation leading to degradation. In 2011, *miR-3099* was found to be expressed as early as in the blastocyst stage, in which the expression was maintained until the developing E11.5 mouse brain. The expression of *miR-3099* was further restricted to the cortical plate of the developing mouse brain between E13.5 and E17.5, coinciding with the time that the majority of the cells are committed to neuronal cell lineage. Moreover, the *miR-3099* was also found to be highly expressed in differentiating P19 cell (2-fold upregulation) when comparing to the proliferating P19 cell. Therefore, this study aims to understand the role of *miR-3099* during neuro-differentiation and corticogenesis in the mouse model. The expression of *miR-3099* was found elevated by 2-3 folds in 46C mouse embryonic stem (mES) cell upon neural induction. Then, predicted target gene of *miR-3099* was further analysed by using four different prediction algorithms (miRDB, miRanda, TargetScan and DIANA-micro-T-CDS) and DAVID bioinformatics analysis with emphasis on target genes related to brain development and function. Based on the prediction, nearly 70% of the predicted target genes were expressed in the nervous system. Of these predicted target genes, *Gfap* was chosen as a candidate for downstream validation because it had been implicated in an important pathway in the brain known as the JAK-STAT signalling pathway, which controls the onset of astrocyte formation. By using the luciferase reporter gene system, *Gfap* was negatively inhibited by *miR-3099*. Furthermore, overexpression of *miR-3099* was performed *in vitro* and *in vivo* for better understanding of the role of m*iR-3099* during neuro-differentiation and brain development. *In vitro,* a transgenic

mES cell that carried *miR-3099* was overexpressed and differentiated for 17 days. The gene expression profile was carried out by using stem-loop RT-qPCR for different marker analysis such as proliferative, neural progenitor, neuron, astrocyte and oligodendrocyte markers. The analysis revealed that the overexpression of *miR-3099* promoted neuronal differentiation and suppressed the astrogliogenesis in the *in vitro* system. In the *in vivo* system, the overexpression of *miR-3099* caused disorganised neuronal migration potentially due to downregulation of *Gfap*. Heretofore, the human homologue of *miR-3099* has not been found or reported. *In silico* analysis via seed sequence similarity search in GEO database found that *mds21* to be novel miRNA that has 100% identical at seed region and 64% closed to *miR-3099* mature sequence. Interestingly, the expression of *mds21* was found to be expressed in various human cell line and tissue, including the brain suggesting that *mds21* might be a potential *miR-3099* homologue in the human genome. Collectively, this study has shown that *miR-3099* plays an essential role in modulating and regulating key markers involved in neuronal differentiation and neural cell function. The degree of functional conservation between *miR-3099* and *mds21* is not clear, and further validations are needed to characterise them further.

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PENCIRIAN SASARAN GEN *miR-3099* DI DALAM PENGAWALAN LEPASAN-TRANSKRIPSI SEMASA NEUROGENESIS PADA MENCIT

Oleh

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RNA mikro (miRNAs) adalah keluarga kecil RNA bukan pengekod yang mempunyai peranan yang penting dalam mengawal metabolisme, perkembangan saraf, neuroplastisitas, apoptosis dan pelbagai proses neurobiologikal. MiRNA berfungsi melalui padanan bes komplimentari separa dengan sasaran spesifik mRNAs yang menyebabkan penindasan proses translasi atau membawa kepada proses penyahadenilatan mRNA lalu mengakibatkan degradasi. Pada tahun 2011, *miR-3099* didapati telah diekspreskan seawal peringkat blastosis, yang mana ekspresi ini kekal sehingga perkembangan E11.5 dalam otak mencit. Ekspresi *miR-3099* juga tertumpu pada plet kortikal dalam otak mencit yang sedang berkembang di antara E13.5 and E17, serentak dengan waktu di mana majoriti sel komited kearah nasabah neuron. Tambahan pula, ekpresi *miR-3099* didapati tinggi di dalam sel P19 yang sedang mengalami proses pembezaan, (sebanyak 2 kali ganda) berbanding dengan sel P19 yang sedang mengalami proses proliferasi. Oleh itu, kajian ini dijalankan bagi memahami peranan *miR-3099* semasa pembezaan sel neuron dan perkembangan korteks di dalam model mencit. Ekspresi *miR-3099* didapati meningkat sebanyak 2-3 kali ganda dalam sel 46C sel stem embrionik mencit (mES) sewaktu aruhan neural. Kemudian, gen sasaran *miR-3099* diramal dengan menggunakan empat algoritma ramalan yang berbeza (miRDB, miRanda, TargetScan and DIANA-micro-T-CDS) serta analisis bioinformatik DAVID telah menekankan gen sasaran yang berkait dengan perkembangan otak dan fungsinya. Berdasarkan ramalan tersebut, hampir 70% daripada gen sasaran mempunyai ekspresi di dalam sistem saraf. Di antara gen-gen ramalan tersebut, *Gfap* telah dipilih untuk pengesahan kerana gen ini terlibat dalam laluan penting dalam otak yang dikenali sebagai laluan pengisyaratan JAK-STAT, yang mengawal permulaan pembentukan astrosit. Dengan menggunakan sistem gen pelapor *luciferase*, ekspresi *Gfap* telah dihalang secara negatif oleh *miR-3099*. Tambahan pula, ekspresi-lampau *miR-3099* telah dilakukan secara *in vitro* dan *in vivo* bagi memahami dengan

lebih baik peranan *miR-3099* sewaktu pembezaan neuron sel dan perkembangan otak. Dalam sistem *in vitro*, mES transgenik yang membawa *miR-3099* telah diekspresi-lampau serta dibezakan selama 17 hari. Profil ekspresi gen telah dijalankan melalui kaedah RT-qPCR *stemloop* bagi menganalisa penanda yang berbeza seperti penanda proliferatif, penanda leluhur neural, penanda neuron, penanda astrosit dan penanda oligodendrosit. Analisis menunjukkan ekspresi-lampau *miR-3099* menggalakkan pembezaan neuron dan merencat pembentukan sel astroglia secara *in vitro*. Di dalam system *in vivo*, ekspresi-lampau *miR-3099* telah menyebabkan ketidakaturan penghijrahan neuron yang mungkin disebabkan oleh kemerosotan *Gfap*. Sehingga kini, homolog *miR-3099* bagi manusia belum dijumpai atau dilaporkan. Berdasarkan analisis *in silico* melalui persamaan jujukan benih dalam pangkalan data GEO mendapati *mds21* sebagai miRNA terbaharu yang mempunyai persamaan 100% pada kawasan benih dan 64% kesamaan dengan jujukan-matang *miR-3099*. Menariknya, ekspresi *mds21* telah didapati dipelbagai titisan sel manusia dan tisu termasuk otak. Penemuan gen *mds21* melalui analisa bioinformatik menunjukkan bahawa *mds21* adalah homolog *miR-3099* dalam genom manusia. Secara kolektifnya, *miR-3099* memainkan peranan yang penting dalam modulasi dan kawalatur penanda utama yang terlibat dalam pembezaan neuron. Walaubagaimanapun, darjah pemeliharan fungsi *miR-3099* dan *mds21* dalam manusia masih kurang jelas dan memerlukan pengesahan serta pencirian yang selanjutnya.

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Shahidee Zainal Abidin

Declaration by Members of Supervisory Committee

This is to confirm that:

- The research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____
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Signature: _____
Name of Member of Supervisory Committee: Norshariza Nordin

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5-HT	Serotonin
ADAR	Adenosine deaminases acting on ribonucleic acid
AJ	Adherens Junction
Ago	Argonaute
AP	Apical progenitor
Aq	Aqueduct
aRGC	Apical radial glial cell
bFGF	Basic fibroblast growth factor
BP	Basal progenitor
bp	Base pair
bRGC	Basal radial glial cell
BSA	Bovine serum albumin
CB	Cerebellum
CC	Cerebral cortex
C.elegans	*Caenorhabditis elegans*
cDNA	Complementary DNA
CIP	Calf intestinal alkaline phosphatase
CNS	Central nervous system
CP	Cortical plate
Cp	Crossing point
Cp	Caudo-putamen
DGCR8	DiGeorge Syndrome Critical Region Gene 8
DEPC	Diethyl pyrocarbonate
dien	Diencephalon
DIV	Day *in vitro*
DMEM	Dulbecco's modified eagle media
DMEM/F12	Dulbecco's modified essential medium: Nutrient mixture F-12
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
Dox	Doxycycline
dsDNA	Double-stranded deoxyribonucleic acid
dsRBD	Double-stranded RNA-binding domains
dsRNA	Double-stranded ribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
eGFP	Enhanced green fluorescent protein
EST	Expressed sequence tag
FACS	Fluorescent activated cells sorting
FBS	Foetal bovine serum
FGF	Fibroblast growth factor
GABA	γ-aminobutyric acid
GE	Ganglionic eminence
GEO	Gene expression omnibus
GFP	Green fluorescent protein
GMEM	Glasgow's minimum essential media

GO	Gene ontology
HPLC	High performance liquid chromatography
Hpf	Hippocampal formation
IC	Inferior colliculus
IPC	Intermediate progenitor cell
IRES2	Internal ribosomal entry site 2
IUE	*In utero* electroporation
IZ	Intermediate zone
KEGG	Kyoto Encyclopedia of Genes and Genome
Let-7	Lethal 7
LIF1010	Leukaemia inhibitory factor human
LNA	Locked nucleic acid
LSD1	Lysine-specific demethylase 1
LTP	Long-term potentiation
LV	Lateral ventricle
mES	Mouse embryonic stem
mes	Mesencephalon
met	Metencephalon
MFE	Minimum free energy
Mib1	Mind bomb 1
miRNA	MicroRNA
mo	Molecular layer
MOI	Multiplicity of infection
MPall	Medial pallium (hippocampal allocortex)
mRNA	Messenger ribonucleic acid
MZ	Marginal zone
NCBI	National Center for Biotechnology Information
NE	Neuroepithelium
NF1	Nuclear factor 1
nt	Nucleotide
OCT	Optimal cutting temperature
OPC	Oligodendrocytes precursor cell
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PCW	Post-conception week
PES	Polyethersulfone
PFA	Paraformaldehyde
PIR	Piriform cortex
piRNA	Piwi-interacting RNA
PP	Preplate
pre-miRNA	Precursor microRNA
pri-miRNA	Primary microRNA
PS	Pial surface
pSTAT3	Phosphorylation of STAT3
PTBP1	Polypyrimidine tract binding protein 1
RE	Restriction endonucleases
RHA	Ribonucleic acid helicase A

RISC	Ribonucleic acid-induced silencing complex
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
SAP	Sub-apical progenitor
SC	Superior colliculus
SCPI	Small C-terminal domain phosphatase 1
SEM	Standard error mean
siRNA	Small interfering ribonucleic acid
SRA	Sequence read archive
ssRNA	Single-stranded ribonucleic acid
snoRNA	Small nucleolar ribonucleic acid
SNP	Short neural precursor
SP	Subplate
Stem-loop qPCR	Pulsed stem-loop-quantitative polymerase chain reaction
Str	Striatum
SVZ	Subventricular zone
TAE	Tris-Acetate-EDTA
Tel	Telencephalon
TF	Transcription factor
TRBP	*Trans*-activator ribonucleic acid-binding protein
TRE	Tetracycline response element
tRNA	Transfer ribonucleic acid
Tudor-SN	Tudor *staphylococcus* nuclease
UCSC	University of California Santa Cruz
UPL	Universal probe library
UTR	Untranslated region
XPO5	Exportin 5
VZ	Ventricular zone
V	Ventricle

LIST OF SYMBOLS

C19MC	Primate-specific microRNA gene cluster
CO_2	Carbon dioxide
H_2O	Water
HCl	Hydrochloric acid
NaCl	Sodium chloride
NaOH	Sodium hydroxide

CHAPTER 1

INTRODUCTION

1.1. Background of study

MicroRNA (miRNA) is a small non-coding RNA that is approximately 22 nucleotide (nt) in length. The miRNA is involved in gene silencing, and it was first described in *Caenorhabditis elegans* (*C.elegans*) (Lee, Feinbaum, & Ambros, 1993; Wightman, Ha, & Ruvkun, 1993). In the genome, miRNA is transcribed either at the coding region or within the introns of coding genes (Baskerville & Bartel, 2005). Some of the miRNAs shared similar promoters and are transcribed as a single primary transcript, also known as polycistronic primary transcripts (Baskerville & Bartel, 2005). Majority of miRNAs are transcribed by RNA polymerase II, subsequently, give rise to primary miRNAs (pri-miRNAs) that contain poly-A tails.

In the canonical pathway, the pri-miRNA is further processed in the nucleus by Drosha and DiGeorge Syndrome Critical Region Gene 8 (DGCR8) protein to generate ~70 nt precursor miRNA (pre-miRNA). The pre-miRNA is transported into the cytoplasm by exportin-5 (XPO5)/RanGTP complex. In the cytoplasm, the pre-miRNA is further processed by RNase III endonuclease DICER and form ~20 bp miRNA duplex. The miRNA duplex is unwound and one of the strands is incorporated into the RNA-induced silencing complex (RISC). Generally, the other strand known as passenger strand will be released and degraded, however, in rare instances, both strands can be loaded into different RISC. The activated RISC containing a miRNA is recruited to bind complementarily at the 3'untranslated region (UTR) of messenger RNAs (mRNAs). As a result, the complex suppresses the translation process and lead to degradation of the targeted transcript (Krol, Loedige, & Filipowicz, 2010).

Mammalian brain development requires meticulous spatiotemporal regulation of gene/protein expression, from the transcription of DNA within the nucleus to translation of mRNA in the cytoplasm (Gunnersen et al., 2002; Ling et al., 2009). Throughout development, the brain undergoes rapid cellular and anatomical changes involving neuronal migration, the proliferation of neural progenitor cell, cell fate determination, gliogenesis, axonogenesis and rostro-lateral to caudo-medial structure patterning (Gupta, Tsai, Wynshaw-Boris, & Medical, 2002; Smart, 1984). The process requires millions of different cell to generate and organise into specific tissues or organs. This mechanism requires cell fate determination throughout the development. In neurogenesis, the diversity of the neuronal population is achieved by asymmetrical cell division of neural stem cell, subsequently produced two daughter cells.

One of the daughter cells will differentiate into the neurone, and the other will remain as the stem cell. The miRNAs play a regulatory role in determining the fate of the neurons and glia.Both cells are derived from the neural progenitor cells, and the neuronal cell differentiation normally occurs in the early stage of embryonic development prior to glia differentiation in the early postnatal nervous system. Interestingly, the previous study found that a different set of miRNAs is specifically involved in neuronal differentiation as well as glial differentiation (Jovičić et al., 2013). In this study, the gene expression profile that extracted from rat cortical differentiated stem cell for 14 days followed by microarray analysis indicated that the expression of miRNAs is remarkable distinct across four cell types, neurone, astrocytes, oligodendrocytes and microglia.

The analysis revealed that multiple miRNAs are enriched and diminished for neurone are relatively fewer found in astrocytes, microglia and oligodendrocyte, *vice versa*. For example, *miR-124*, *miR-127*, *miR-129*, *miR-129**, *miR-136*, *miR-136**, *miR-137*, *miR-154*, *miR-300-3p* and *miR-551b* are found restricted in neurone while *miR-143* and *miR-449a* are specifically expressed in astrocytes. Another miRNA such as *miR-219-2-3p* is expressed particularly in oligodendrocytes. Moreover, the miRNAs are expressed restricted in microglia are *miR-126*, *miR-126**, *miR-141*, *miR-142-3p*, *miR-142-5p*, *miR-146a*, *miR-150*, *miR-200c* and *miR-223* (Jovičić et al., 2013).

Among brain-enriched miRNAs, *miR-124* is well-characterised, and it is highly expressed in the brain during development. The *miR-124* has been proposed to promote neuronal cell differentiation through the suppression of small C-terminal domain phosphatase 1 (SCP1), polypyrimidine tract binding protein 1 (PTBP1) and *Sox9*, which is involved in anti-neuronal differentiation pathway (Cheng, Pastrana, Tavazoie, & Doetsch, 2009a; Makeyev, Zhang, Carrasco, & Maniatis, 2007; Visvanathan, Lee, Lee, Lee, & Lee, 2007). The *Let-7* family is known to express throughout the cortical development. The *Let-7* encoded for *Lethal-7* (*Let-7*) which plays a significant role in the proliferation of neural stem cell as well as neural progenitor cell (Roush & Slack, 2008). Both *Let-7* and *miR-9* are involved in cell proliferation and commitment by converging on a common target, TLX protein.

The convergent action of *Let-7* and *miR-9* reduces the levels TLX, thereby inhibits self-renewal of neural stem cell/neural progenitor cell thus accelerating neuronal differentiation (Sun, Yu, Evans, & Shi, 2007; Zhao et al., 2010a; Zhao, Sun, Li, & Shi, 2009). Another miRNA that plays an essential role in promoting neurogenesis is *miR-137*. The *miR-137* targets ubiquitin ligase mind bomb 1 (*Mib1*), which is known to activate the Notch signalling pathway by promoting ubiquitination and internalisation of Notch ligand Delta (Smrt et al., 2010).

In addition, *miR-137* regulates neuronal maturation via the inhibition of dendrite morphogenesis by binding to the 3'UTR of *Mib1* (Smrt et al., 2010). Knockdown *miR-124a* and *miR-9* elevated the expression of STAT3, thus leading to astrogliogenesis (Li & Jin, 2010; Wen, Li, & Liu, 2009). Besides, the expression of *miR-23* is involved in astrocyte specification and promote glial cell commitment by suppressing HES1 gene (Hiroaki Kawasaki & Taira, 2003; Smirnova et al., 2005). In different studies, gain- and loss- of function studying in *in vitro* and *in vivo* have demonstrated that *miR-219* and *miR-338* controlled the oligodendrocytes differentiation by suppressing *Hes5* and *Sox6* (Galloway & Moore, 2016; Zhao et al., 2010b).

Furthermore, one of the miRNAs that potentially plays an important role during brain development is *miR-3099*. The *miR-3099* was discovered through deep sequencing of small RNAs extracted from E15.5 developing mouse brain (Ling et al., 2011). The expression profile of *miR-3099* showed that it is highly expressed in the blastocyst stage and throughout the developing mouse embryos (Ling et al., 2011). Later at E13.5 until E18.5, the expression of *miR-3099* is restricted at the central nervous system (CNS) (Ling et al., 2011). Interestingly, the expression level of *miR-3099* is higher in differentiating P19 mouse embryo teratocarcinoma cell when compared to proliferative P19 cell (Ling et al., 2011) suggesting a prominent role in CNS development particularly neuronal differentiation. *MiR-3099* was shown to negatively regulate *Vcan* and *Nap1l1* genes that are involved in cell growth, division and proliferation (Vesely et al., 2014).

Based on the pathway analysis of *miR-3099* predicted target genes by using KEGG (Kyoto Encyclopedia of Genes and Genome) database, *miR-3099* was proposed to regulate glutamatergic synapse, axon guidance and Notch signalling pathway (Vesely et al., 2014). Again, these findings suggest that *miR-3099* has a significant role in regulating brain development, particularly in neuronal differentiation and function. The functional role of *miR-3099* during neuro-differentiation and development is understudied. Therefore, in this study, molecular characterisation of *miR-3099* was performed using *in vivo* and *in vitro* systems via gain-of-function approach to provide a better understanding of its role during neuronal cell development and function.

The *in vitro* characterisation was carried out based on a mouse embryonic stem (mES) cell culture system with a stable and regulatable expression for *miR-3099*. To understand the role of *miR-3099* during neurogenesis, the *miR-3099* was overexpressed at different time-point upon neural induction. The expression of neural lineage markers was further evaluated. While in *in vivo* study, the precursor *miR-3099* was introduced and electroporated into the lateral ventricle of the E15.5 mouse embryo brain.

Later, the brain was harvested on E18.5 and further analysed by using immunohistochemistry. The immunolabelling between immature neurone (Tuj1)/astrocyte (Gfap) markers with *miR-3099* were quantified. The *in utero* electroporation is a technique that used electrical pulse to create a small pore and allow nucleic acid to pass through the cell membrane. This condition allows to deliver gene of interest into developing mouse brain to access the role of transgene. Besides that, the technique also temporary creation a transgenic model and provide rapid investigation as well as direct examination of the function of delivered gene.

Moreover, this study also adopted an *in silico* approach based on *miR-3099* seed sequence similarity search and evolutionary analysis of across the human genome. The *miR-3099* sequence is only found conserved in the rat genomes (Ling et al., 2011). To date, the human homologue of *miR-3099* has not been reported. The structural conservation of miRNA gene can be very different from the conservation of regular protein-coding gene among vertebrate genomes. Since the affinity of a miRNA is determined mainly by the seed region of the miRNA (the 2^{nd} – 7^{th} bases of the 5'-phosphate terminal), searching for *miR-3099* homologues in other organisms should not be based on the conventional criteria adapted for gene conservation analysis.

Screening of *miR-3099* homologues in the human genome may suggest the evolutionarily conserved role of *miR-3099* in the development of the human nervous system. Thus, the present study provides a fundamental understanding of *miR-3099* role during neurogenesis, neuronal differentiation and function. It also establishes the miRNA as a possible regulator of an associated molecular pathway involved in neuronal development, particularly in the mammalian brain. Moreover, identification of a homologue of *miR-3099* in the human will give a new insight into the novel miRNA in the development of the human brain.

1.2. Hypothesis

miR-3099 plays a crucial role during neural differentiation and neuronal cell function.

1.3. Objectives

This study consists of one general objective and four specific objectives.

- **General objective**

To elucidate the functional role of *miR-3099* during neuro-differentiation *in vivo* and *in vitro*.

- **Specific objectives**

i. To determine the expression of *miR-3099* during cellular differentiation of 46C mouse embryonic stem cells upon neural induction.

ii. To predict the target genes of *miR-3099* via *in silico* analysis using four independent prediction algorithms (miRDB, miRanda, TargetScan and DIANA-micro-T-CDS) with emphasis on target genes related to brain development and function.

iii. To construct and validate an expression vector of *miR-3099* for future gain-of-function and loss-of-function studies.

iv. To elucidate the functional role of *miR-3099* at the cellular level during neuro-differentiation by using 'gain-of-function` approach and identification of *miR-3099* homologue in the human genome.

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BIODATA OF STUDENT

During childhood, Shahidee loved to watch science programmes on television like National Geography, Discovery's channel, and so forth. Those kinds of programmes had cultivated him an eagerness to explore something new that could contribute to society. So, with the ambition of wanting to be a scientist led him to pursue his study in the science stream. The journey has begun in 2014 when he enrolled in Diploma Microbiology at Universiti Teknologi MARA (UiTM). Three years later, he pursued bachelor degree in Biomolecular Science at the same university. Upon graduation, he continued his study in master degree by research at Faculty of Medicine, UiTM under supervision of Assoc. Prof. Dr. Nuraliza Abdul Satar. His master dissertation research aims to explore the potential of tocotrienol in preventing the adverse effect of corticosterone-induced DNA damage in mouse embryonic development. Once he accomplished his master degree, he furthers his study in a doctoral programme in the field of neuroscience supervised by Dr. Ling King Hwa from the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (UPM).

Throughout his PhD training, he managed to publish five first-authored peer-reviewed papers and four co-authored peer-reviewed papers. He also has presented in seven scientific conferences; local as well as internationals. Moreover, he has received various and prestigious awards from international and national levels. Among his accomplishments, Shahidee was the sole recipient of UPM fellowship to attend the prestigious Nobel Laureate meeting at Lindau, Germany from 24 till 29 June 2018. He also received three international travel grants such as the International Brain Research Organization (IBRO) Asia-Pacific Region Council (APRC) Grant in 2013 to attend 2-week courses in Banaras Hindu University in India. In 2017, he received an award from Society of Neuroscience (SFN)–IBRO to attend 47th Annual Meeting Neuroscience 2017 in Washington D.C., US and Postgraduate Travel Grant to attend the 14th meeting of Asian Pacific Society for Neurochemistry in 2016.

Shahidee had an opportunity of doing one year attachment at Harvard Medical School, at Professor Bruce Yankner's Laboratory. During the internship, he worked on cutting edge techniques such as CRISPR-dCas9 genome editing and human induced pluripotent cells derived from Alzheimer's disease patients. With his impressive academic and track record, he passed his PhD viva with distinction.

LIST OF PUBLICATIONS

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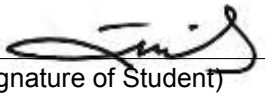

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