UNIVERSITI PUTRA MALAYSIA

SPATIOTEMPORAL EXPRESSION AND MOLECULAR CHARACTERISATION OF miR-344b AND miR-344c IN DEVELOPING MOUSE BRAIN

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By

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Chair : Cheah Pike See, PhD
Faculty : Medicine and Health Sciences

Mammalian brain development requires a meticulous spatiotemporal regulation of gene and protein expression. The developing brain undergoes major construction during the embryonic stage, beginning with the formation of the neural tube that eventually gives rise to a complex nervous system. Studies had shown that microRNAs (miRNAs) played crucial roles in spatiotemporal regulation of the brain development. MiRNAs are small non-coding RNAs of about 22 nucleotides that regulate gene expression through inhibition or repression processes during post-transcriptional or translational stages. A recent study suggested that *miR-344* was neural specific during brain development. In this study, we characterised the expression of *miR-344b* and *miR-344c* during the development of mouse brain. Bioinformatics analysis was employed to identify the potential downstream target genes of *miR344b* and *miR-344c*. Initially, *miR-344b* and *miR-344c* were found to target a total of 1,540 and 863 genes respectively. Genes that are known to be identified by three independent bioinformatics tools and also associated with transcription regulation and nervous system development were selected for further screening. The genes that fulfilled these criteria and targeted by *miR344b* and *miR-344c* were *Olig2* and *Otx2* respectively. Luciferase assay was performed to validate the target genes prediction. Overexpression plasmid was co-transfected with a 3'UTR plasmid and checked for luciferase protein inhibition. However, both *Olig2* and *Otx2* were not suppressed by their respective miRNAs. It may suggest that both *Olig2* and *Otx2* were not the direct targets of *miR-344b* and *miR-344c* or a more complex mechanism is involved. Parallel to bioinformatics study, *in situ* hybridisation analysis study showed that both *miR-344b* and *miR-344c* were strongly expressed in the germinal layer during the early developmental stages of mouse brain. *MiR-344b* was not expressed in the brain from early postnatal until mature adult stage. Interestingly, *miR-344c* remained expressed throughout the P1 brain and its expression was still detectable in the mature adult brain although restricted to the olfactory bulb only. Higher magnification on the expression of *miR-344b* and *miR-344c* revealed that they were expressed in the nucleus. Stemloop RT-qPCR was

employed to further investigate the expression level of these miRNAs in the brain and other multiple organs. The expression of *miR-344b* in the developing brain was peaked at E15.5 and decreased steadily as it progressed to adulthood. On the other hand, *miR-344c* showed high expression at E15.5 and remained steady till the adult stage. Both *miR-344b* and *miR-344c* showed highest expression in adult pancreas when comparing with the adult multiple organs, in line with the previous study reported that *miR-344* was highly expressed in the pancreas. In conclusion, *Olig2* and *Otx2* were not direct targets of *miR-344b* and *miR-344c* respectively as previously predicted. However, this study proves that *miR-344b* and *miR-344c* were expressed in the developing mouse brain, especially localised to the nucleus of the neuronal cells. In addition to whole brain, *miR-344b* and *miR-344c* were highly expressed in pancreas and lowly expressed in muscles.

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EKSPRESI RUANG MASA DAN PENCIRIAN MOLEKUL *miR-344b* DAN *miR-344c* DALAM PERKEMBANGAN OTAK MENCIT

Oleh

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Perkembangan otak mamalia memerlukan pengawalaturan teliti ruang masa ekspresi gen dan protein. Otak yang berkembang sebahagian besarnya terbentuk semasa peringkat embrio, bermula daripada pembentukan tiub saraf yang akhirnya membentuk menjadi sistem saraf yang kompleks. Kajian terkini menunjukkan mikroRNA (miRNA) memainkan peranan penting dalam pengawalaturan ruang masa perkembangan otak. MiRNA merupakan RNA bukan pengekod yang kecil, terdiri daripada 22 nukleotida yang mengawal atur ekspresi melalui proses perencatan atau penahanan semasa peringkat pascatranskripsi atau translasi. Kajian terkini mencadangkan bahawa *miR-344* adalah khusus kepada saraf semasa perkembangan otak. Dalam kajian ini, kami mencirikan ekspresi *miR-344b* dan *miR-344c* sewaktu perkembangan otak mencit. Analisis bioinformatik digunakan bagi mengenal pasti potensial gen sasaran hiliran *miR-344b* dan *miR-344c.* Pada mulanya *miR-344b* dan *miR-344c* masing-masing didapati menyasarkan sejumlah 1,540 gen dan 863 gen. Gen yang diketahui, dikenal pasti dengan tiga alat bioinformatik berasingan, serta yang berhubung kait dengan pengawalaturan transkripsi dan perkembangan sistem saraf dipilih bagi saringan lanjutan. Gen yang memenuhi kriteria ini dan disasarkan oleh *miR-344b* dan *miR-344c* masing-masing ialah *Olig2* dan *Otx2*. Ujian lusiferase dijalankan bagi mengesahkan penjangkaan gen sasaran. Plasmid ekspresi berlebihan ditransfeksi bersama plasmid 3'UTR dan perencatan protein lusiferase diuji. Bagaimanapun, ekspresi *Olig2* mahupun *Otx2* tidak ditahan oleh miRNA masing-masing. Ini menandakan mungkin *Olig2* mahupun *Otx2* bukan sasaran langsung *miR-344b* dan *miR-344c* atau mekanisme yang lebih rumit mungkin terlibat. Analisis penghibridan *in situ* menunjukkan *miR-344b* dan juga *miR-344c* banyak diekspresi di lapisan germa semasa peringkat perkembangan awal otak mencit. *MiR-344b* tidak diekspresi di dalam otak daripada peringkat awal pascalahir sehingga peringkat dewasa matang. Menariknya, *miR-344c* kekal diekspresi di seluruh otak P1 dan ekspresi ini masih dapat dikesan di dalam otak dewasa matang walaupun terbatas di bulba olfaktor sahaja. Magnifikasi lebih tinggi pada ekspresi *miR-344b* dan *miR-344c* menunjukkan ekspresi di dalam nukleus. RT-qPCR *stemloop* digunakan bagi menyiasat lebih lanjut

tahap ekspresi miRNA ini di dalam otak dan pelbagai organ lain. Ekspresi *miR-344b* di dalam otak yang berkembang memuncak pada E15.5 dan berkurang beransur-ansur apabila semakin dewasa. Sebaliknya, *miR-344c* menunjukkan tahap ekspresi tinggi pada E15.5 dan kekal sehingga peringkat dewasa. *MiR-344b* dan juga *miR-344c* menunjukkan tahap ekspresi paling tinggi di dalam pankreas dewasa apabila dibandingkan dengan pelbagai organ dewasa, sejajar dengan kajian sebelum ini yang melaporkan bahawa *miR-344* banyak diekspresi di pankreas. Kesimpulannya, *Olig2* dan *Otx2* masing-masing bukan sasaran langsung *miR-344b* dan *miR-344c* seperti yang dijangka sebelum ini. Walau bagaimanapun, kajian ini membuktikan bahawa *miR-344b* dan *miR-344c* diekspresi di dalam otak mencit yang berkembang; khususnya ia tertumpu di nukleus sel neuron. Di samping di seluruh otak, *miR-344b* dan *miR-344c* banyak diekspresi di pankreas dan kurang diekspresi di otot.

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I certify that a Thesis Examination Committee has met on 28 December 2015 to conduct the final examination of Angeline Leong Jia Wen on her thesis entitled "Spatiotemporal Expression and Molecular Characterisation of *miR-344b* and *miR-344c* in Developing Mouse Brain" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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The thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Declaration by members of Supervisory Committee

This is to confirm that:

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Supervisory Committee: Syahrilnizam Abdullah, D.Phil

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

˚C	degrees Celsius
±	plus-minus
µg	microgram
µm	micrometre
µM	micromolar
ANOVA	analysis of variance
Aq	aqueduct
CB	cerebellum
CC	cerebral cortex
cDNA	complementary deoxyribonucleic acid
CDS	coding sequence
CP	cortical plate
Cp	crossing point
DEPC	diethylpyrocarbonate
DG	dentate gyrus
DIG	digoxigenin
DMEM	Dulbecco's modified Eagle medium
DNA	deoxyribonucleic acid
E	embryonic day
EDTA	ethylenediaminetetraacetic acid
eGFP	enhanced green fluorescent protein
EGL	external granular layer
ENCODE	Encyclopaedia of DNA elements
FBS	foetal bovine serum

FT	fallopian tube
G	gauge
g	g force (relative centrifugal force)
GCL	granular cell layer
GE	ganglionic eminence
GloCL	glomerular cell layer
gm	gram
HCl	hydrochloric acid
HF	hippocampal formation
Hipp	hippocampus
Hyp or hypothal	hypothalamus
IGL	internal granular layer
IZ	intermediate zone
L I	layer 1 of cerebral cortex
L II/ III	layer 2 or 3 or cerebral cortex
L. intestine	large intestine
LB	Luria-Bertani
LNA	locked nucleic acid
LV	lateral ventricle
M	molar
Mb	midbrain
MCL	mitral cell layer
Med	medulla
Mes	mesencephalon
mg/mL	milligram per millilitre

mg/mL	milligram per millilitre
$MgCl_2$	magnesium chloride
miRNA or miR	microRNA
ML	molecular layer
mL	millilitre
MZ	molecular/marginal zone
n.d.	not detectable
NaCl	sodium chloride
NaOH	sodium hydroxide
NBT/ BCIP	nitro-blue-tetrazolium chloride/ 5-bromo-4-chloro-3-indolyl-phosphate, toluidine salt
ng	nanogram
nM	nanomolar
OB	olfactory bulb
OE	olfactory epithelium
P	postnatal day
PANTHER	Protein analysis through evolutionary relationships
PBS	phosphate buffered saline
PCL	Purkinje cell layer
PCR	polymerase chain reaction
P_{CT}	preferentially conserved targeting
PFA	paraformaldehyde
pmol/µL	picomol per microliter
PP	preplate

Pre-miRNA	precursor miRNA
Pri-miRNA	primary miRNA
PS	pial surface
qPCR	quantitative polymerase chain reaction
RISC	RNA-induced silencing complex
RLU	relative light unit
RMS	rostral migratory stream
RNA	ribonucleic acid
rpm	revolution per minute
RT-qPCR	reverse transcription qPCR
S. intestine	small intestine
SEM	standard error of mean
SGZ	subgranular zone
SOC	super optimal broth with catabolite repression
SP	subplate
SSC	saline sodium citrate
SSCF	saline sodium citrate – formamide
SVZ	subventricular zone
TE	tris-EDTA
Thal	thalamus
UTR	untranslated region
V	ventricle
v/v	volume to volume
VZ	ventricular zone
W	IUPAC nucleotide code for A or T

w/v	weight to volume
WB	whole brain

CHAPTER 1

INTRODUCTION

Mammalian brain development requires a meticulous spatiotemporal regulation of gene and protein expression. The developing brain undergoes major construction during foetal life, beginning with the formation of the neural tube that eventually gives rise to the nervous system. This process involves neurogenesis, a stage where neurone develops from neural stem/progenitor cells, followed by neuronal migration, differentiation and speciation (Taupin and Gage, 2002). The brain is divided into three key regions; the forebrain (cerebral cortex), midbrain and hindbrain (cerebellum). The cerebral cortex has a diverse functional roles, spanning from a simple motor control to a more complex cognitive processes while the midbrain is one of the three-part structure of a brain stem that relays information between the forebrain and hindbrain. The third region, hindbrain, coordinates motor activity. Therefore, spatiotemporal development of the brain is vital to determine its functionality and maturity.

MicroRNAs (miRNAs) are short regulatory, non-coding RNAs with an average length of 20-25 nucleotides (Bartel, 2004). *Lin-4* was the first miRNA discovered in nematodes *Caenorhabditis elegans* (*C. elegans*). It was first postulated that *lin-4* played a role in *C. elegans* developmental stages (Lee et al., 1993). To date, various studies have subsequently shown that miRNA plays a significant role in modulating gene expression at a post-transcriptional level. MiRNA degrades or represses the expression of targeted mRNAs by binding to the 3' UTR of the gene (Lee et al., 1993). Recent study had shown that miRNAs also target 5' UTR of the mRNA. Additionally, most miRNAs, like *miR-34a*, that target 5' UTR have simultaneous interaction in the 3' UTR target site of the mRNA, resulting in large-scale protein changes (Lee et al., 2009). Thus, miRNA plays a crucial role in regulating cellular functions, which include cell growth, differentiation and apoptosis. Various miRNAs have been implicated in the development and progression of various neurological disorders such as Alzheimer's disease (Maes et al., 2009), Parkinson's disease (Harraz et al., 2011) as well as in individuals with intellectual disabilities caused by genetic factors (Siew et al., 2013).

Various studies have shown that miRNAs played a vital role in brain development. For instance, a miRNA-array study demonstrated that *miR-9* and *miR-131* played crucial roles in spatiotemporal regulation of brain development in rat and mouse (Krichevsky *et al.*, 2003). *MiR-9* is a neural-specific miRNA and not expressed in other tissues. It is primarily expressed in neural precursor cells, whilst a lower expression was also observed in mature post-mitotic neurones (Motti et al., 2012). Moreover, *miR-134* is localised at the synapto-dendritic area of the rat hippocampal neurones and regulates synaptic development, maturation and plasticity (Schratt et al., 2006). *MiR-124* is perhaps the most abundant and well characterised brain-specific miRNA

(Lagos-Quintana et al., 2002). *MiR-124* was found expressed in mature neurones but was upregulated in differentiating neurones in an adult mouse brain (Åkerblom et al., 2012). Brain-enriched *miR-124* had been known to promote neurogenesis. It is a vital regulator in adult neurogenesis in mice by repressing *Sox9*, which leads to neurone formation (Cheng et al., 2009). In addition, *miR-124* promotes neurite outgrowth by engaging in cytoskeletal regulation during neuronal differentiation (Yu et al., 2008).

Mir-344 is a novel miRNA that was first described in 2004 (Kim et al., 2004). It is located in chromosome 7 of the mouse (MI0014095). According to miRBase database, it is expressed in rats and mice while no human homologues were found to date. The *miR-344* family contains 19 mature sequences (Liu et al., 2014). It was one of the 29 miRNAs identified to inhibit adipogenesis *via* Wnt signalling pathway activation (Qin et al., 2010). Subsequent study showed *miR-344* inhibited cell differentiation by targeting the Wnt/β-catenin signalling pathway (Chen et al., 2014). Recently, *miR-344* is an emerging miRNA involved in the developing mouse brain (Ling et al., 2011). A study showed that *miR-344-3p* was expressed in neural-specific region during mouse embryonic development (Liu et al., 2014). Moreover, *miR-344* was shown to be downregulated in Huntington disease in mouse model (Lee et al., 2011), thus implicating it with a potential role in motor neurone disorders.
MiR-344 family had nine known isoforms, *miR-344a* to *miR-344i*. However, limited studies were carried out on a few of these isoforms and implicated with roles in various pathological disorders. *MiR-344a* was found to be upregulated in the myocardium of lipopolysaccharide-treated rats. It was postulated that *miR-344a* was involved in endotoxin-induced myocardial injury (Ding et al., 2015). *MiR-344h* was one of the miRNA identified in a study that observed miRNA expressional alteration of a mouse hippocampus after a traumatic brain injury (Bao et al., 2014). Another study also had showed that *miR-344b*, *miR-344d* and *miR-344h* were downregulated in a neurotoxin-induced apoptosis in mouse MN9D cell line (Li et al., 2013).

Given the significance of *miR-344* family in brain development, the novel *miR-344b* and *miR-344c* may play a crucial role in a normal physiological as well as pathological processes in brain. These miRNAs are not fully characterised to date. Hence, this provide an exciting opportunity to study and explore the potential functions, roles and mechanism of their miRNA on their target genes during brain development. This may yield valuable insights and knowledge to enhance the ever-growing literature on the importance of miRNA in brain development.

This study hypothesised that two miRNAs, *miR-344b* and *miR-344c* may play a role in regulating brain development and function and both are expressed spatiotemporally in the developing mouse brain. Therefore, the main objective of this study is to explore the potential roles of both *miR-344b* and *miR-344c* and to characterise their expression profile during the development of the mouse brain.

Three specific objectives in this study are, to:

1. predict downstream target genes of *miR-344b* and *miR-344c* via *in silico* analysis,
2. validate the selected downstream targets of the miRNAs via Luciferase assay,
3. investigate spatiotemporal expression of *miR-344b* and *miR-344c* at different stages of the developing mouse brain.

REFERENCES

Acampora, D., Barone, P., Simeone, A., 1999. Otx genes in corticogenesis and brain development. Cereb. Cortex 9, 533–542. doi:10.1093/cercor/9.6.533

Adam, Y., Mizrahi, A., 2010. Circuit formation and maintenance--perspectives from the mammalian olfactory bulb. Curr. Opin. Neurobiol. 20, 134–40. doi:10.1016/j.conb.2009.11.001

Åkerblom, M., Sachdeva, R., Barde, I., Verp, S., Gentner, B., Trono, D., Jakobsson, J., 2012. MicroRNA-124 is a subventricular zone neuronal fate determinant. J. Neurosci. 32, 8879–89. doi:10.1523/JNEUROSCI.0558-12.2012

Alder, J., Cho, N.K., Hatten, M.E., 1996. Embryonic precursor cells from the rhombic lip are specified to a cerebellar granule neuron identity. Neuron 17, 389–99.

Alvarez-Garcia, I., Miska, E.A., 2005. MicroRNA functions in animal development and human disease. Development 132, 4653–62. doi:10.1242/dev.02073

Ambros, V., Bartel, B., Bartel, D.P., Burge, C.B., Carrington, J.C., Chen, X., Dreyfuss, G., Eddy, S.R., Griffiths-Jones, S., Marshall, M., Matzke, M., Ruvkun, G., Tuschl, T., 2003. A uniform system for microRNA annotation. RNA 9, 277–279. doi:10.1261/rna.2183803

Ang, S.L., Jin, O., Rhinn, M., Daigle, N., Stevenson, L., Rossant, J., 1996. A targeted mouse Otx2 mutation leads to severe defects in gastrulation and formation of axial mesoderm and to deletion of rostral brain. Development 122, 243–252.

Bao, T.H., Miao, W., Han, J.H., Yin, M., Yan, Y., Wang, W.W., Zhu, Y.H., 2014. Spontaneous running wheel improves cognitive functions of mouse associated with miRNA expressional alteration in hippocampus following traumatic brain injury. J. Mol. Neurosci. 622–629. doi:10.1007/s12031-014-0344-1

Barca-Mayo, O., De Pietri Tonelli, D., 2014. Convergent microRNA actions coordinate neocortical development. Cell. Mol. Life Sci. 71, 2975–2995. doi:10.1007/s00018-014-1576-5

Bartel, D.P., 2004. MicroRNAs: Genomics, Biogenesis, Mechanism, and Function. Cell. doi:10.1016/S0092-8674(04)00045-5

Berry, M., Rogers, a W., 1965. The migration of neuroblasts in the developing cerebral cortex. J. Anat. 99, 691–709.

Betel, D., Koppal, A., Agius, P., Sander, C., Leslie, C., 2010. Comprehensive modeling of microRNA targets predicts functional non-conserved and non-canonical sites. Genome Biol. 11, R90. doi:10.1186/gb-2010-11-8-r90

Betel, D., Wilson, M., Gabow, A., Marks, D.S., Sander, C., 2008. The microRNA.org resource: targets and expression. Nucleic Acids Res. 36, D149–53. doi:10.1093/nar/gkm995

Birney, E., Stamatoyannopoulos, J. a, Dutta, A., Guigó, R., Gingeras, T.R., Margulies, E.H., Weng, Z., Snyder, M., et. al., 2007. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. Nature 447, 799–816. doi:10.1038/nature05874

Bohnsack, M.T., Czaplinski, K., Gorlich, D., 2004. Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. RNA 10, 185–91. doi:10.1261/rna.5167604

Castanotto, D., Lingeman, R., Riggs, A.D., Rossi, J.J., 2009. CRM1 mediates nuclear-cytoplasmic shuttling of mature microRNAs. Proc. Natl. Acad. Sci. 106, 21655–21659. doi:10.1073/pnas.0912384106

Chandran, S., Harmey, J., Toomey, S., 2012. Inhibition of the IGF signalling pathway in MDA MB 231 triple negative breast cancer cells. R. Coll. Surg. Irel. Student Med. J. 5, 39–45.

Chen, H., Wang, S., Chen, L., Chen, Y., Wu, M., Zhang, Y., Yu, K., Huang, Z., Qin, L., Mo, D., 2014. MicroRNA-344 inhibits 3T3-L1 cell differentiation via targeting GSK3β of Wnt/β-catenin signaling pathway. FEBS Lett. 588, 429–435. doi:10.1016/j.febslet.2013.12.002

Chen, L., Song, J., Cui, J., Hou, J., Zheng, X., Li, C., Liu, L., 2013. microRNAs regulate adipocyte differentiation. Cell Biol. Int. 9999, 1–14. doi:10.1002/cbin.10063

Cheng, L.-C., Pastrana, E., Tavazoie, M., Doetsch, F., 2009. miR-124 regulates adult neurogenesis in the subventricular zone stem cell niche. Nat. Neurosci. 12, 399–408. doi:10.1038/nn.2294

Cullen, B.R., 2004. Transcription and processing of human microRNA precursors. Mol. Cell 16, 861–865. doi:10.1016/j.molcel.2004.12.002

Ding, T., Li, Y., Tang, R., Zhang, X., Yun, Y., Li, J., Wang, D., 2015. [Differential expression of miRNAs in myocardial tissues of rats with

lipopolysaccharide- induced endotoxemia]. Nan Fang Yi Ke Da Xue Xue Bao 35, 213–217. doi:10.3969/j.issn.1673-4254.2015.02.11

Fox, A.H., Lam, Y.W., Leung, A.K.L., Lyon, C.E., Andersen, J., Mann, M., Lamond, A.I., 2002. Paraspeckles. Curr. Biol. 12, 13–25. doi:10.1016/S0960-9822(01)00632-7

Friedman, R.C., Farh, K.K.-H., Burge, C.B., Bartel, D.P., 2009. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res. 19, 92–105. doi:10.1101/gr.082701.108

Gammill, L.S., Bronner-Fraser, M., 2003. Neural crest specification: migrating into genomics. Nat Rev Neurosci 4, 795–805.

Ghashghaei, H.T., Lai, C., Anton, E.S., 2007. Neuronal migration in the adult brain: are we there yet? Nat. Rev. Neurosci. 8, 141–51. doi:10.1038/nrn2074

Glickstein, M., Strata, P., Voogd, J., 2009. Cerebellum: history. Neuroscience 162, 549–59. doi:10.1016/j.neuroscience.2009.02.054

Gregory, R.I., Yan, K.-P., Amuthan, G., Chendrimada, T., Doratotaj, B., Cooch, N., Shiekhattar, R., 2004. The Microprocessor complex mediates the genesis of microRNAs. Nature 432, 235–40. doi:10.1038/nature03120

Gupta, A., Tsai, L.-H., Wynshaw-Boris, A., 2002. Life is a journey: a genetic look at neocortical development. Nat. Rev. Genet. 3, 342–55. doi:10.1038/nrg799

Harraz, M., Dawson, T., Dawson, V., 2011. MicroRNAs in Parkinson's disease. J. Chem. Neuroanat. 42, 127–130. doi:10.1016/j.jchemneu.2011.01.005.MicroRNAs

Hatten, M.E., 1999. Central nervous system neuronal migration. Annu. Rev. Neurosci. 22, 511–39. doi:10.1146/annurev.neuro.22.1.511

Hatten, M.E., Heintz, N., 1995. Mechanisms of neural patterning and specification in the developing cerebellum. Annu. Rev. Neurosci. 18, 385–408. doi:10.1146/annurev.ne.18.030195.002125

Hobert, O., 2008. Gene regulation by transcription factors and microRNAs. Science 319, 1785–6. doi:10.1126/science.1151651

Höck, J., Meister, G., 2008. The Argonaute protein family. Genome Biol. 9, 210. doi:10.1186/gb-2008-9-2-210

Huang, C., Xiao, X., Chintagari, N.R., Breshears, M., Wang, Y., Liu, L., 2014. MicroRNA and mRNA expression profiling in rat acute respiratory distress syndrome. BMC Med. Genomics 7, 46. doi:10.1186/1755-8794-7-46

Hutvágner, G., Zamore, P.D., 2002. A microRNA in a multiple-turnover RNAi enzyme complex. Science 297, 2056–60. doi:10.1126/science.1073827

Jaskiewicz, L., Filipowicz, W., 2008. Role of Dicer in posttranscriptional RNA silencing. Curr. Top. Microbiol. Immunol. 320, 77–97.

Kim, J., Krichevsky, A., Grad, Y., Hayes, G.D., Kosik, K.S., Church, G.M., Ruvkun, G., 2004. Identification of many microRNAs that copurify with polyribosomes in mammalian neurons. Proc. Natl. Acad. Sci. U. S. A. 101, 360–365. doi:10.1073/pnas.2333854100

Kim, V.N., 2005. MicroRNA biogenesis: coordinated cropping and dicing. Nat. Rev. Mol. Cell Biol. 6, 376–85. doi:10.1038/nrm1644

Kim, V.N., Han, J., Siomi, M.C., 2009. Biogenesis of small RNAs in animals. Nat. Rev. Mol. Cell Biol. 10, 126–39. doi:10.1038/nrm2632

Knecht, A.K., Bronner-Fraser, M., 2002. Induction of the neural crest: a multigene process. Nat. Rev. Genet. 3, 453–61. doi:10.1038/nrg819

Krichevsky, A.M., King, K.S., Donahue, C.P., Khrapko, K., Kosik, K.S., 2003. A microRNA array reveals extensive regulation of microRNAs during brain development. RNA 9, 1274–1281. doi:10.1261/rna.5980303

Lagos-Quintana, M., Rauhut, R., Yalcin, A., Meyer, J., Lendeckel, W., Tuschl, T., 2002. Identification of tissue-specific MicroRNAs from mouse. Curr. Biol. 12, 735–739. doi:10.1016/S0960-9822(02)00809-6

Landgraf, P., Rusu, M., Sheridan, R., Sewer, A., 2007. A mammalian microRNA expression atlas based on small RNA library sequencing. Cell 129, 1401–1414. doi:10.1016/j.cell.2007.04.040.A

Lee, I., Ajay, S.S., Yook, J.I., Kim, H.S., Hong, S.H., Kim, N.H., Dhanasekaran, S.M., Chinnaiyan, A.M., Athey, B.D., 2009. New class of microRNA targets containing simultaneous 5'-UTR and 3'-UTR interaction sites. Genome Res. 19, 1175–83. doi:10.1101/gr.089367.108

Lee, R.C., Feinbaum, R.L., Ambros, V., 1993. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75, 843–854. doi:10.1016/0092-8674(93)90529-Y

Lee, S.-T., Chu, K., Im, W.-S., Yoon, H.-J., Im, J.-Y., Park, J.-E., Park, K.-H., Jung, K.-H., Lee, S.K., Kim, M., Roh, J.-K., 2011. Altered microRNA

regulation in Huntington's disease models. Exp. Neurol. 227, 172–9. doi:10.1016/j.expneurol.2010.10.012

Lee, Y., Ahn, C., Han, J., Choi, H., Kim, J., Yim, J., Lee, J., Provost, P., Rådmark, O., Kim, S., Kim, V.N., 2003. The nuclear RNase III Drosha initiates microRNA processing. Nature 425, 415–9. doi:10.1038/nature01957

Lein, E.S., Hawrylycz, M.J., Ao, N., Ayres, M., Bensinger, A., Bernard, A., et. al., 2007. Genome-wide atlas of gene expression in the adult mouse brain. Nature 445, 168–176. doi:10.1038/nature05453

Lewis, B.P., Burge, C.B., Bartel, D.P., 2005. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 120, 15–20. doi:10.1016/j.cell.2004.12.035

Lewis, B.P., Shih, I.H., Jones-Rhoades, M.W., Bartel, D.P., Burge, C.B., 2003. Prediction of Mammalian MicroRNA Targets. Cell 115, 787–798. doi:10.1016/S0092-8674(03)01018-3

Li, H., de Faria, J.P., Andrew, P., Nitarska, J., Richardson, W.D., 2011. Phosphorylation regulates OLIG2 cofactor choice and the motor neuron-oligodendrocyte fate switch. Neuron 69, 918–29. doi:10.1016/j.neuron.2011.01.030

Li, L., Chen, H.-Z., Chen, F.-F., Li, F., Wang, M., Wang, L., Li, Y.-Q., Gao, D.-S., 2013. Global microRNA expression profiling reveals differential expression of target genes in 6-hydroxydopamine-injured MN9D cells. Neuromolecular Med. 15, 593–604. doi:10.1007/s12017-013-8244-z

Ling, K.-H., Brautigan, P.J., Hahn, C.N., Daish, T., Rayner, J.R., Cheah, P.-S., Raison, J.M., Piltz, S., Mann, J.R., Mattiske, D.M., Thomas, P.Q., Adelson, D.L., Scott, H.S., 2011. Deep sequencing analysis of the developing mouse brain reveals a novel microRNA. BMC Genomics 12, 176. doi:10.1186/1471-2164-12-176

Liu, Q., He, H., Zeng, T., Huang, Z., Fan, T., Wu, Q., 2014. Neural-specific expression of miR-344-3p during mouse embryonic development. J. Mol. Histol. 45, 363–72. doi:10.1007/s10735-013-9555-y

Lois, C., Alvarez-Buylla, A., 1994. Long-distance neuronal migration in the adult mammalian brain. Science 264, 1145–8.

Luu-The, V., Paquet, N., Calvo, E., Cumps, J., 2005. Improved real-time RT-PCR method for high-throughput measurements using second derivative calculation and double correction. Biotechniques 38, 287–93.

Maes, O.C., Chertkow, H.M., Wang, E., Schipper, H.M., 2009. MicroRNA: Implications for Alzheimer disease and other human CNS disorders. Curr. Genomics 10, 154–168. doi:10.2174/138920209788185252

Matsuo, I., Kuratani, S., Kimura, C., Takeda, N., Aizawa, S., 1995. Mouse Otx2 functions in the formation and patterning of rostral head. Genes Dev. 9, 2646–2658. doi:10.1101/gad.9.21.2646

Megías, M., Emri, Z., Freund, T.F., Gulyás, A.I., 2001. Total number and distribution of inhibitory and excitatory synapses on hippocampal CA1 pyramidal cells. Neuroscience 102, 527–40.

Mi, H., Muruganujan, A., Thomas, P.D., 2013. PANTHER in 2013: Modeling the evolution of gene function, and other gene attributes, in the context of phylogenetic trees. Nucleic Acids Res. 41, 377–386. doi:10.1093/nar/gks1118

Ming, G.-L., Song, H., 2011. Adult neurogenesis in the mammalian brain: significant answers and significant questions. Neuron 70, 687–702. doi:10.1016/j.neuron.2011.05.001

Mori, K., Nagao, H., Yoshihara, Y., 1999. The olfactory bulb: coding and processing of odor molecule information. Science 286, 711–5.

Motti, D., Bixby, J.L., Lemmon, V.P., 2012. MicroRNAs and neuronal development. Semin. Fetal Neonatal Med. 17, 347–52. doi:10.1016/j.siny.2012.07.008

Paraskevopoulou, M.D., Georgakilas, G., Kostoulas, N., Vlachos, I.S., Vergoulis, T., Reczko, M., Filippidis, C., Dalamagas, T., Hatzigeorgiou, a G., 2013. DIANA-microT web server v5.0: service integration into miRNA functional analysis workflows. Nucleic Acids Res. 41, W169–73. doi:10.1093/nar/gkt393

Purves, D., Augustine, G.J., Fitzpatrick, D., Katz, L.C., LaMantia, A.-S., McNamara, J.O., Williams, S.M., 2001. Circuits within the Cerebellum.

Qin, L., Chen, Y., Niu, Y., Chen, W., Wang, Q., Xiao, S., Li, A., Xie, Y., Li, J., Zhao, X., He, Z., Mo, D., 2010. A deep investigation into the adipogenesis mechanism: profile of microRNAs regulating adipogenesis by modulating the canonical Wnt/beta-catenin signaling pathway. BMC Genomics 11, 320. doi:10.1186/1471-2164-11-320

Reczko, M., Maragkakis, M., Alexiou, P., Grosse, I., Hatzigeorgiou, A.G., 2012. Functional microRNA targets in protein coding sequences. Bioinformatics 28, 771–6. doi:10.1093/bioinformatics/bts043

Roberts, T.C., 2014. The MicroRNA Biology of the Mammalian Nucleus. Mol. Ther. Nucleic Acids 3, e188. doi:10.1038/mtna.2014.40

Saba, R., Schratt, G.M., 2010. MicroRNAs in neuronal development, function and dysfunction. Brain Res. 1338, 3–13. doi:10.1016/j.brainres.2010.03.107

Schratt, G.M., Tuebing, F., Nigh, E.A., Kane, C.G., Sabatini, M.E., Kiebler, M., Greenberg, M.E., 2006. A brain-specific microRNA regulates dendritic spine development. Nature 439, 283–9. doi:10.1038/nature04367

Schultz, W., 2010. Multiple functions of dopamine neurons. F1000 Biol. Rep. 2. doi:10.3410/B2-2

Serbedzija, G.N., Bronner-Fraser, M., Fraser, S.E., 1994. Developmental potential of trunk neural crest cells in the mouse. Development 120, 1709–18.

Seto, Y., Ishiwata, S., Hoshino, M., 2014. Characterization of Olig2 expression during cerebellar development. Gene Expr. Patterns 15, 1–7. doi:10.1016/j.gep.2014.02.001

Siew, W.-H., Tan, K.-L., Babaei, M.A., Cheah, P.-S., Ling, K.-H., 2013. MicroRNAs and intellectual disability (ID) in Down syndrome, X-linked ID, and Fragile X syndrome. Front. Cell. Neurosci. 7, 41. doi:10.3389/fncel.2013.00041

Simeone, A., 2000. Positioning the isthmic organizer where Otx2 and Gbx2 meet. Trends Genet. 16, 237–40.

Slaughterbeck, C.R., Datta, S., Smith, S., Ritchie, D., Wohnoutka, P., 2007. High-Throughput Automated Microscopy Platform for the Allen Brain Atlas. JALA - J. Assoc. Lab. Autom. 12, 377–383. doi:10.1016/j.jala.2007.07.003

Spruston, N., 2008. Pyramidal neurons: dendritic structure and synaptic integration. Nat. Rev. Neurosci. 9, 206–21. doi:10.1038/nrn2286

Stefan, M., Simmons, R. a, Bertera, S., Trucco, M., Esni, F., Drain, P., Nicholls, R.D., 2011. Global deficits in development, function, and gene expression in the endocrine pancreas in a deletion mouse model of Prader-Willi syndrome. Am. J. Physiol. Endocrinol. Metab. 300, E909–22. doi:10.1152/ajpendo.00185.2010

Sun, Y., Meijer, D.H., Alberta, J. a, Mehta, S., Kane, M.F., Tien, A.-C., Fu, H., Petryniak, M. a, Potter, G.B., Liu, Z., Powers, J.F., Runquist, I.S., Rowitch, D.H., Stiles, C.D., 2011. Phosphorylation state of Olig2

regulates proliferation of neural progenitors. Neuron 69, 906–17. doi:10.1016/j.neuron.2011.02.005

Takebayashi, H., Nabeshima, Y., Yoshida, S., Chisaka, O., Ikenaka, K., Nabeshima, Y.I., 2002. The basic helix-loop-helix factor Olig2 is essential for the development of motoneuron and oligodendrocyte lineages. Curr. Biol. 12, 1157–1163. doi:10.1016/S0960-9822(02)00926-0

Tang, R., Li, L., Zhu, D., Hou, D., Cao, T., Gu, H., Zhang, J., Chen, J., Zhang, C.-Y., Zen, K., 2012. Mouse miRNA-709 directly regulates miRNA-15a/16-1 biogenesis at the posttranscriptional level in the nucleus: evidence for a microRNA hierarchy system. Cell Res. 22, 504–15. doi:10.1038/cr.2011.137

Tang, X., Gal, J., Zhuang, X., Wang, W., Zhu, H., Tang, G., 2007. A simple array platform for microRNA analysis and its application in mouse tissues. RNA 13, 1803–22. doi:10.1261/rna.498607

Tarantino, C., Paolella, G., Cozzuto, L., Minopoli, G., Pastore, L., Parisi, S., Russo, T., 2010. miRNA 34a, 100, and 137 modulate differentiation of mouse embryonic stem cells. FASEB J. 24, 3255–63. doi:10.1096/fj.09-152207

Taupin, P., Gage, F.H., 2002. Adult neurogenesis and neural stem cells of the central nervous system in mammals. J. Neurosci. Res. 69, 745–9. doi:10.1002/jnr.10378

Thomas, P., Smart, T.G., 2005. HEK293 cell line: A vehicle for the expression of recombinant proteins. J. Pharmacol. Toxicol. Methods 51, 187–200. doi:10.1016/j.vascn.2004.08.014

Wang, V.Y., Zoghbi, H.Y., 2001. Genetic regulation of cerebellar development. Nat. Rev. Neurosci. 2, 484–491. doi:10.1038/35081558

Wang, X., 2008. miRDB : A microRNA target prediction and functional annotation database with a wiki interface. RNA 14, 1012–1017. doi:10.1261/rna.965408.was

Wei, Y., Li, L., Wang, D., Zhang, C.-Y., Zen, K., 2014. Importin 8 regulates the transport of mature microRNAs into the cell nucleus. J. Biol. Chem. 289, 10270–5. doi:10.1074/jbc.C113.541417

Wightman, B., Ha, I., Ruvkun, G., 1993. Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans. Cell 75, 855–62.

Wingate, R.J., 2001. The rhombic lip and early cerebellar development. Curr. Opin. Neurobiol. 11, 82–8.

Yao, M.-J., Chen, G., Zhao, P.-P., Lu, M.-H., Jian, J., Liu, M.-F., Yuan, X.-B., 2012. Transcriptome analysis of microRNAs in developing cerebral cortex of rat. BMC Genomics 13, 232. doi:10.1186/1471-2164-13-232

Yates, D., 2011. Development: directing development through phosphorylation. Nat. Rev. Neurosci. 12, 248–9. doi:10.1038/nrn3030

Yu, J.-Y., Chung, K.-H., Deo, M., Thompson, R.C., Turner, D.L., 2008. MicroRNA miR-124 regulates neurite outgrowth during neuronal differentiation. Exp. Cell Res. 314, 2618–33. doi:10.1016/j.yexcr.2008.06.002

Yue, D., Liu, H., Huang, Y., 2009. Survey of Computational Algorithms for MicroRNA Target Prediction. Curr. Genomics 10, 478–492. doi:10.2174/138920209789208219

Zhao, C., Sun, G., Li, S., Shi, Y., 2009. A feedback regulatory loop involving microRNA-9 and nuclear receptor TLX in neural stem cell fate determination. Nat. Struct. Mol. Biol. 16, 365–371. doi:10.1038/nsmb.1576

Zisoulis, D.G., Kai, Z.S., Chang, R.K., Pasquinelli, A.E., 2012. Autoregulation of microRNA biogenesis by let-7 and Argonaute. Nature 486, 541–4. doi:10.1038/nature11134