UNIVERSITI PUTRA MALAYSIA

MODULATION OF LIPIDOMIC MARKERS IN OXIDATIVE STRESS NEURAL-DERIVED EMBRYONIC STEM CELL CULTURES WITH VITAMIN E SUPPLEMENTATION

AFIFAH BINTI ABD JALIL

FPSK(M) 2017 69

MODULATION OF LIPIDOMIC MARKERS IN OXIDATIVE STRESS NEURAL-DERIVED EMBRYONIC STEM CELL CULTURES WITH VITAMIN E SUPPLEMENTATION

By

AFIFAH BINTI ABD JALIL

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

May 2017

COPYRIGHT

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

MODULATION OF LIPIDOMIC MARKERS IN OXIDATIVE STRESS NEURAL-DERIVED EMBRYONIC STEM CELL CULTURES WITH VITAMIN E SUPPLEMENTATION

By

AFIFAH BINTI ABD JALIL

May 2017

Chairman : Huzwah Binti Khaza'ai, PhD
Faculty : Medicine and Health Sciences

Abnormal lipid metabolism is strongly related to the pathogenesis of Alzheimer's disease (AD). Apolipoprotein E (APO E) is the major apolipoprotein in the CNS that has a role in cholesterol transport. Oxidative stress brain has reduced capacity for neuronal delivery of cholesterol suggesting a defect in cholesterol delivery for neuronal repair mechanism contribute to AD progression. Glutamate is the main excitatory neurotransmitter in the CNS, which can be excitotoxic at high concentration. Vitamin E has been shown to possess potent antioxidant and neuroprotection activities. It has two potent antioxidant isomers which are tocopherol and tocotrienol. In this present study, the effects of Tocotrienol Rich Fraction (TRF) from palm oil and alpha-Tocopherol (α-TCP) in modulating lipidomic markers in oxidative stress neural-derived embryonic stem (ES) cell cultures were elucidated. Transgenic mouse ES cell line (46C) was differentiated into neural lineage by induction with retinoic acid *in vitro.* The cells were then exposed to oxidative stress by a significantly high concentration of glutamate. Reactive oxygen species (ROS) measurement was done upon glutamate excitotoxicity and recovery processes by vitamin E were determined. Gene expression of glutamate receptors (NMDA and Kainate receptors), neuron-specific enolase (NSE), lipidomic markers including APO E, low density related protein (LRP) and 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) were elucidated using real-time PCR. The result reveals downregulation of NMDA, Kainate receptor, NSE and HMGCR upon posttreatment with different concentration of TRF and α-TCP in oxidative stress neural-derived 46C cells, a sign of neurorecovery process. Treatment of vitamin E also reduced the concentration of ROS to 33.05% and 57.2% upon 300ng/mL of TRF and α-TCP treatment respectively, in glutamate-induced oxidative stress neural cells which indicated that vitamin E is one of the potent antioxidants. In conclusion, TRF and α-TCP have protective and antioxidant properties against glutamate toxicity in neural-

derived ES cell and have the possibility to develop into potential treatment agents for AD.

Abstract tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

MODULASI PENANDA LIPIDOMIK DALAM SEL NEURON DARIPADA KULTUR SEL STEM EMBRIONIK DI BAWAH TEKANAN OKSIDATIF DENGAN SUPLEMENTASI VITAMIN E

Oleh

AFIFAH BINTI ABD JALIL

Mei 2017

Pengerusi : Huzwah Binti Khaza'ai, PhD
Faculti : Perubatan dan Sains Kesihatan

Metabolisma lipid yang tidak normal adalah sangat berkaitan dengan patogenesis penyakit Alzheimer (AD). APO E adalah apolipoprotein utama dalam CNS yang berperanan dalam pengangkutan kolesterol. Tekanan oksidatif di dalam otak telah mengurangkan kapasiti penghantaran kolesterol kepada neuron dimana kerosakan dalam mekanisme penghantaran kolesterol untuk pembaikan neuron menyumbang kepada perkembangan AD. Glutamat adalah neurotransmiter yang merupakan perangsang utama dalam CNS dan boleh menjadi toksik pada kepekatan yang tinggi. Vitamin E telah terbukti mempunyai aktiviti antioksidan dan pelindung neuron yang berkesan. Ia mempunyai dua isomer antioksida iaitu tokoferol dan tokotrienol. Dalam kajian ini, kesan fraksi kaya tokotrienol (TRF) di ekstrak daripada kelapa sawit dan α-Tokoferol (α-TCP) dikaji dalam modulasi penanda lipidomik pada tekanan oksidatif neural yang telah diferensiasi daripada sel ES. Tikus transgenik embrionik sel stem (46C) telah diferensiasi kepada sel neural dengan pengaruh asid retinoik *in- vitro* .Sel-sel ini kemudiannya didedahkan kepada tekanan oksidatif dengan kepekatan glutamate yang agak tinggi. Pengukuran spesies reaktif oksigen (ROS) telah dilakukan sebaik proses eksitotoxisiti glutamat dan pemulihan dengan vitamin E telah ditentukan. Ekpresi gen reseptor glutamat (reseptor NMDA and Kainate), "neuron-specific enolase" (NSE) dan penanda lipidomik termasuk APO E, "low density related protein" (LRP) dan "3-hydroxy-3-methylglutaryl-coenzyme A reductase" (HMGCR) telah dijelaskan menggunakan *Real time-PCR.* Hasil ujikaji mendedahkan penurunan ekpresi NMDA, Kainate reseptor, NSE dan HMGCR dengan pasca-rawatan TRF dan α- TCP dengan kepekatan yang berbeza dalam tekanan oksidatif sel neural, yang menandakan proses neuroperlindungan sedang berlaku. Rawatan vitamin E juga mengurangkan kepekatan ROS kepada 33.05% dan 57.2% setiap satu setelah dirawat dengan 300ng/ml TRF dan α-TCP, dalam sel neural dibawah tekanan oksidatif glutamat yang menunjukkan vitamin E adalah salah satu antioksidan yang kuat.

Kesimpulannya, TRF dan α-TCP mempunyai sifat pelindung dan antioksidan terhadap ketoksikan glutamat dalam sel-sel neural yang diperolehi daripada ES sel. Ini menunjukan vitamin E berpotensi menjadi ejen rawatan untuk AD.

ACKNOWLEDGEMENTS

In the name of Allah, the Most Gracious, the Most Merciful
All gratification are referred to Allah

All praise to Allah, the Almighty for His consent for giving me the courage and strength in completing my Master study. I am thankful for His blessing in giving me the opportunity, health and strength to learn and gain many priceless experiences in this research field.

First and foremost, I would like to convey my deepest gratitude and how deeply indebted I'm to my supervisor, Dr. Huzwah Khaza'ai, who greatly enriched my knowledge with her guidance and assistance. Devoid of her constant support, the master research would not have accomplished fruitfully. Also, I would like to express my deepest gratitude to my co- supervisor, Dr. Norshariza Nordin for their encouragement, insightful comments and ideas for making this thesis more meaningful. I would like to thank the rest of my beloved lecturer, Dr. Abdah Md. Akim, Assoc. Prof.Dr. Sokhini b.Abd.Mutalib and Dr.Hasiah Hamid for the supports and encouragement.

A sincere gratitude and appreciation for Faculty of Medicine and Health Science, Universiti Putra Malaysia, the place that has granted me the opportunity and amenities to amass the essential practical skills and the keen in fulfilling the research. A special note of thanks goes to all staffs of the Biomedical department, Stem Cell Research, Biochemistry, Cell signaling and Nutrition Laboratory at this faculty for their constructive assistance while grasping the handiness laboratory tasks.

My big thanks to my fellow lab mates: Amirah, Aminah, Henna, Najwa, Aisha, Asma, Afiqah and Nur'izzati for stimulating discussions, the sleepless nights spent working together to meet deadlines and for all tears that for the last 2 years. Also, I thank my best friends: Baizura, Farizatul, Siti Zawanah, Ana Masara, Amielia, Fatin Hannani, Haslina, Mimi, Suhizan for the motivations and courage.

Last but not least, the heartiest thanks to my family: my parents Mr. Abd Jalil bin Mohd and Mrs. Damra Salam for supporting me spiritually throughout my life. Also, heartfelt thanks to my fiancée, Mohd Faizul bin Roslin for the endless support and love.

Thank you and May peace and blessing be upon those who read.

I certify that a Thesis Examination Committee has met on 15 May 2017 to conduct the final examination of Afifah binti Abd Jalil on her thesis entitled "Modulation of Lipidomic Markers in Oxidative Stress Neural-Derived Embryonic Stem Cell Cultures with Vitamin E Supplementation" 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.

Members of the Thesis Examination Committee were as follows:

Abdah binti Md Akim, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Sabrina binti Sukardi, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Mahanem Mat Noor, PhD
Associate Professor
Universiti Kebangsaan Malaysia
Malaysia
(External Examiner)

NOR AINI AB. SHUKOR, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 30 November 2017

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

Huzwah Khaza'ai, PhD
Senior Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Norshariza Bt. Nordin, PhD
Senior Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software

Signature: _____ Date: _____

Name and Matric No.: Afifah Binti Abd Jalil, (GS41511)

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) were adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: Dr. Huzwah Khaza'ai

Signature: _____
Name of Member of Supervisory Committee: Dr. Norshariza Bt. Nordin

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

4-HNE	4-Hydroxynonenal
ABC A1	ATP-binding cassette transporter subtype A1
ACAT	Acetyl coenzyme A-cholesterol
AD	Alzheimer's disease
AGEs	Glycoxidation end products
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANNOVA	Analysis of variance
APO B	Apolipoprotein *B*
APO C1	Apolipoprotein C subtype 1
APO E	Apolipoprotein E. Gene: Apo-E
APO E2	Apolipoprotein E allele 2
APO E3	Apolipoprotein E allele 3
APO E4	Apolipoprotein E allele 4
APO ER2	Apolipoprotein E receptor 2
APO J	Apolipoprotein J
APP	Amyloid precursor protein
ATP	Adenosine triphosphate
ATRA	*All-trans* retinoic acid
Aβ	Beta-amyloid
BAC	Bacterial artificial chromosome
BMP	Bone morphogenetic protein
BSA	Bovine serum albumin
Ca^{2+}	Calcium ion
Caspase	Cysteine-aspartic proteases

cDNA	complementary DNA
CE	Cholesterol ester
CO_2	Carbon dioxide
Cq	Quantitation cycle
CSF	Cerebrospinal fluid
DAPI	4',6-diamidino-2-phenylindole
DCF	2', 7' –dichlorofluorescein
DCFH-DA	2',7' –dichlorofluorescin diacetate
DEPC	Diethyl pyrocarbonate
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
E	Efficiency
EAA	Excitatory amino acid
EAAC1	Excitatory amino acid carrier 1
EAAT	Excitatory amino acid transporter
EAAT1	Excitatory amino acid transporter 1
EAAT2	Excitatory amino acid transporter 2
EAAT3	Excitatory amino acid transporter 3
EAAT5	Excitatory amino acid transporter 5
EB	Embryoid body
EBM	Embryoid body medium
EDTA	Ethylenediaminetetraacetic acid
*e*GFP	Enhanced green fluorescent protein
ELISA	Enzyme-linked immunosorbent assay
ER	Endoplasmic reticulum

ES cell	Embryonic stem cell
ESM	Embryonic stem cell medium
FACS	Fluorescence-activated cell sorting
FBS	Fetal bovine serum
FC	Free cholesterol
FITC	Fluorescein isothiocyanate
g	Gram
GABA	Gamma-aminobutyric acid
GAD	Glutamic acid decarboxylase
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase. Gene: *GAPDH*
gDNA	Genomic deoxyribonucleic acid
GFAP	Glial fibrillary acidic protein
GFP	Green fluorescent protein
GLAST	Glutamate-aspartate transporter
GLT	Glutamate transporter
GLT-1	Glutamate transporter 1
GluK1	Glutamate receptor, kainate 1 (gene)
GluN1	Glutamate receptor,NMDA-1 (gene)
GLUT	Glucose transporter
GLUT 1	Glucose transporter 1
GLUT 2	Glucose transporter 2
GLUT 3	Glucose transporter 3
GLUT 4	Glucose transporter 4
GMEM	Glasgow modified essential medium
gp-130	Glycoprotein-130
GSK-3β	Glycogen synthase kinase 3 beta

H_2O	Water
HDL	High-density lipoprotein
HMGCR	3-hydroxy-3-methyl-glutaryl-coenzyme A reductase. Gene: *HMGCR*
HNF-3α	Hepatocyte nuclear factor 3α
HT-22	Hippocampal neuronal cell line
IC	Inhibitory concentration
ICC	Immunocytochemistry
ICM	Inner cell mass
IGF	Insulin-like growth factors
IgG	Immunoglobulin G
IgG2b	Immunoglobulin G subtype 2b
iGluR	Ionotropic glutamate receptor
JAK	Janus kinase
K^+	Potassium ion
kD	Kilodalton
LDL	Low-density lipoprotein
LIF	Leukemic inhibitory factor
LPL	Lipoprotein lipase
LRP	Low-density lipoprotein receptor-related protein
LRP-1	Low-density lipoprotein receptor-related protein 1. Gene: *Lrp-1*
MEF	Mouse embryonic fibroblasts
MEM	Minimum Essential Medium
mg	Milligram
mGluR	Metabotropic glutamate receptor
mL	Millilitre

mM	Millimolar
MTP	Mitochondrial permeability transition pore
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
mV	miliVolt
Na^+	Sodium ion
NADPH	Nicotinamide adenine dinucleotide (reduced form)
NaOH	Sodium hydroxide
NFT	Neurofibrillary tangles
ng	Nanogram
NLC	Neural –like cell
nm	nanometer
nM	Nanomolar
NMDA	N-methyl-D-aspartate
NO	Nitric oxide
NPC	Neural precursor cell
NSE	Neuron-specific enolase. Gene: *NSE*
NTC	Non-template control
ORF	Open reading frame
PA 6	Stromal cell lines
pA/pF	picoamperes per picofarad
Pac	Puromycin *N*-acetyl-transferase
pax 3	Phosphatidylinositol 3-Kinase
PBS	Phosphate buffered saline
PC 12	Pheochromocytoma cell line
PD	Parkinson's diseases
PDL	Poly-D-Lysine

PET	Positron emission tomography
pH	Potential of hydrogen
PL	Phospholipids
PS-1	Presenilin-1
PS-2	Presenilin-2
PUFA	Polyunsaturated fatty acid
qPCR	Quantitative polymerase chain reaction
R^2	Correlation coefficient
RA	Retinoic acid
RAR	Retinoic acid receptor
RFU	Relative fluorescence unit
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
Rpm	Revolutions per minute
rRNA	Ribosomal ribonucleic acid
RT	Room temperature
RT-PCR	Real-time PCR
RxR	Retinoid X receptor
SDIA	Stromal-derived inducing activity
SEM	Standard error mean
SGG	Salt glucose glycine
SK-N-S-H	Neuroblastoma
SOD-1	superoxide dismutase 1
STAT	Signal transducer and activator of transcription
TAE	Tris-acetate-*EDTA*

TBARS	Thiobarbituric acid reactive substances
TBE	Tris-borate-EDTA
TBI	Traumatic brain injury
TRF	Tocotrienol-rich fraction
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
UK	United kingdom
USA	Unites states of America
UV	Ultraviolet
α-TCP	Alpha-tocopherol

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Vitamin E is a fat-soluble compound with antioxidant properties that exist in eight forms in nature (alpha-, beta-, gamma- and delta-tocopherol and alpha-, beta-, gamma- and delta- tocotrienol) each with its own biological properties (Sen *et al*., 2006). The difference between tocopherol and tocotrienol is that they have different number and position of methyl groups attached to the aromatic ring (Osakada *et al*., 2004). In short, tocopherols are saturated forms of vitamin E, whereas tocotrienol is unsaturated and possess an isoprenoid side chain. This variant of vitamin E only occurs at very low levels in nature, with the highest concentration found in palm oil. Currently, there is an increase in interest on Tocotrienol Rich Fraction (TRF) from palm oil. TRF consist of 25% of alpha-tocopherol (α-TCP) and 75% of tocotrienol. TRF of palm oil has been shown to possess potent antioxidant, anticancer, and cholesterol-lowering activities (Khor *et al*., 2016; Osakada *et al*., 2004;).

At normal concentrations, glutamate plays a role as a major neurotransmitter in the brain, important for cognition, memory, and learning. However, elevated levels of glutamate can cause overstimulation of glutamate receptor including NMDA, AMPA and kainate receptors that cause an influx of calcium ions in the postsynaptic membrane. High energy in the form of ATP is needed to rectify back to the normal concentrations influx of intracellular calcium ion. The high requirement of energy will cause the mitochondrion to generate more reactive oxygen species (ROS) as a natural byproduct. ROS is a chemically reactive species containing oxygen including peroxides, superoxide, hydroxyl radical and singlet oxygen (Dayem *et al*., 2010). Generally, it is a byproduct of DNA, amino acid, and lipid oxidation which can cause significant damage to cells. Oxidative stress is term for a condition where the production of ROS is greater than the capacity of the body to reduce oxidation.

Apolipoprotein E (APO E) is the major apolipoprotein in the CNS that has a role in cholesterol transport. Cholesterol is needed by neurons to build up their cellular membranes such as cell membrane of the axons, dendrites, and synapses (Poirier *et al*.,1993). This current study postulates that in an oxidative stressed brain, cholesterol recycling is unable to be performed accurately due to the deterioration of cholesterol delivery mechanism in injured neuronal cells. The understanding of the cholesterol metabolism and it's delivery in the brain and its role in neurodegenerative diseases therefore warrant further investigation. Thus, factors such as APO E, LRP receptor, HMGCR are the lipidomic markers involved in cholesterol homeostasis which became the main interest in this study.

This study aims to elucidate the protective role of vitamin E against glutamate toxicity and to understand how vitamin E is involved in modulating lipidomic markers, antioxidant activity and neurons-specific enolase expression in accomplishing the neurorecovery. It is expected that both forms of vitamin E (TRF and α-TCP) would have a neuroprotective effect against oxidative stress in the brain.

1.2 Problem statement

Neurodegenerative diseases are considered one of the major problems in our aging society as it can be serious and life-threatening. Prevalence of these diseases is increasing yearly; however, there is a lack of effective therapies or specific drug to treat this disease. Current medication only alleviates symptoms, relieves pain and helps to improve patients' quality of life. A high concentration of glutamate that contributes to oxidative stress in CNS is believed to reduce the capacity of cholesterol delivery to neuronal cells. Failure of the repair mechanism may be one of the factors contributing to the progression of neurodegenerative diseases such as AD. Thus, free radical scavenger compounds such as vitamin E in the form of TRF and α-TCP are the great interest knowing its protective properties is well documented against oxidative stress. Therefore, this study was designed to evaluate the potency of both isomers of vitamin E in protecting neural-derived embryonic stem (ES) cells from glutamate induced oxidative stress and modulating lipidomic markers for repair mechanism of the cells.

1.3 Research objective

1.3.1 General objective

To elucidate modulation of lipidomic markers in oxidative stress neural- derived ES cell upon supplementation of vitamin E.

1.3.2 Specific objective

- To differentiate transgenic mouse embryonic stem (ES) cell line (46C) into neural commitment and confirmation with immunocytochemistry.
- To determine the dose response and time course of glutamate toxicity in neural-derived 46C cells by using MTT assay.
- To develop *in-vitro* oxidative stress model by using glutamate in neural- derived 46C cells.
- To determine the ROS activity as a neural oxidative stress marker upon glutamate toxicity and the role of TRF and α-TCP as an antioxidant.
- To assess the gene expression of glutamate receptors (NMDA and kainate receptor), NSE and lipidomic markers (APO E, HMGCR, and LRP-1) upon glutamate toxicity and the recovery process after supplementation with TRF and α-TCP.

1.4 Hypothesis

TRF and α-TCP are able to overcome oxidative stress hence, modulating lipidomic markers in *in-vitro* oxidative stress model.

REFERENCES

Angelov, D. N., Arnhold, S., Andressen, C., Grabsch, H., Puschmann, M., Hescheler, J., & Addicks, K. (1998). Temporospatial relationships between macroglia and microglia during in vitro differentiation of murine stem cells. *Developmental Neuroscience*, *20*(1), 42–51.

Arabadjiev, B., Petkova, R., Momchilova, A., Chakarov, S., & Pankov, R. (2016). Of mice and men – differential mechanisms of maintaining the undifferentiated state in mESC and hESC. *BioDiscovery, 3* (1), 1–18.

Arriza, J. L., Eliasof, S., Kavanaugh, M. P., & Amara, S. G. (1997). Excitatory amino acid transporter 5, a retinal glutamate transporter coupled to a chloride conductance. *Proceedings of the National Academy of Sciences of the United States of America, 94*(8), 4155–60.

Aubert, J., Stavridis, M. P., Tweedie, S., Reilly, M. O., Vierlinger, K., Li, M.,Smith, A. (2003). Screening for mammalian neural genes via fluorescence-activated cell sorter purification of neural precursors from Sox1 – gfp knock-in mice. *Proceedings of the National Academy of Sciences of the USA.100*

Bain, G., Kitchens, D., Yao, M., Huettner, J. E., & Gottlieb, D. I. (1995). Embryonic stem cells express neuronal properties in vitro. *Developmental Biology*, *168*(2), 342–357.

Balmer, J. E., & Blomhoff, R. (2002). Gene expression regulation by retinoic acid. *Journal of Lipid Research. 43*.

Behl, C., Davis, J. B., Lesley, R., & Schubert, D. (1994). Hydrogen peroxide mediates amyloid beta protein toxicity.*Cell*, *77*(6), 817–27.

Bekman, E., Castro, C. V. De, Fior, R., & Henrique, D. (2006). Neuronal production in vitro from embryonic stem cells. *Actas Bioq, 7*,61-59.

Bibel, M., Richter, J., Schrenk, K., Tucker, K. L., Staiger, V., Korte, M.Barde, Y. (2004). Differentiation of mouse embryonic stem cells into a defined neuronal lineage. *Nature, 7*(9), 1003–1009.

Blennow, K., Wallin, A., & Ekman, R. (1994). Neuron specific enolase in cerebrospinal fluid: a biochemical marker for neuronal degeneration in dementia disorders? *Journal of Neural Transmission*, *8*, 183–191.

Brewer, G. J., Torricelli, J. R., Evege, E. K., & Price, P. J. (1993). Optimized Survival of Hippocampal Neurons in B27-Supplemented Neurobasalm , a New Serum-free Medium Combination. *Journal of Neuroscience Research, 35*,567-576

Bullock, R., Ph, D., Zauner, A., Woodward, J. J., Ph, D., Myseros, J., … Young, H. F. (1998). Factors affecting excitatory amino acid release following severe human head injury.*Neurosurgical found.*

Butterfield, D. A., Reed, T., Newman, S. F., & Sultana, R. (2007). Roles of amyloid beta- peptide-associated oxidative stress and brain protein modifications in the pathogenesis of Alzheimer's disease and mild cognitive impairment. *Free Radical Biology and Medicine*, *43*(5), 658–677.

Cañete, A., Cano, E., & Carmona, R. (2017). Role of Vitamin A / Retinoic Acid in Regulation of Embryonic and Adult Hematopoiesis, *1*(2), 1–18.

Chen, Y., & Swanson, R. a. (2003). Astrocytes and brain injury. *Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, *23*(2), 137–149.

Chung, S., Sonntag, K.-C., Andersson, T., Bjorklund, L. M., Park, J.-J., Kim, D.-W., Kim, K.-S. (2002). Genetic engineering of mouse embryonic stem cells by Nurr1 enhances differentiation and maturation into dopaminergic neurons. *The European Journal of Neuroscience*, *16*(10), 1829–38.

Clementi, M. E., Pezzotti, M., Orsini, F., Sampaolese, B., Mezzogori, D., Grassi, C., … Misiti, F. (2006). Alzheimer's amyloid beta-peptide (1-42) induces cell death in human neuroblastoma via bax/bcl-2 ratio increase: an intriguing role for methionine 35. *Biochemical and Biophysical Research Communications*, *342*(1), 206–13.

Conti, F., DeBiasi, S., Minelli, A., Rothstein, J. D., & Melone, M. (1998). EAAC1, a high-affinity glutamate tranporter, is localized to astrocytes and gabaergic neurons besides pyramidal cells in the rat cerebral cortex. *Cerebral Cortex)*, *8*(2), 108–16.

Coucouvanis, E., & Martin, G. R. (1995). Signals for death and survival: a two-step mechanism for cavitation in the vertebrate embryo. *Cell*, *83*(2), 279–87.

Danbolt, N. C. (2001). Glutamate uptake. *Progress in Neurobiology*, *65*(1), 1–105.

Dauer, W., & Przedborski, S. (2003). Parkinson's Disease. *Neuron*, *39*(6), 889–909.

Dayem, A. A., Choi, H.-Y., Kim, J.-H., & Cho, S.-G. (2010). Role of Oxidative Stress in Stem, Cancer, and Cancer Stem Cells. *Cancers*, *2*(2), 859–884.

Dekroon, R. M., & Armati, P. J. (2001). Synthesis and processing of apolipoprotein E in human brain cultures. *Glia*, *33*(4), 298–305.

Dingledine, R., Borges, K., Bowie, D., & Traynelis, S. F. (1999). The glutamate receptor ion channels. *Pharmacological Reviews*, *51*(1), 7–61.

Drake, J., Link, C. D., & Butterfield, D. A. (2003). Oxidative stress precedes fibrillar deposition of Alzheimer's disease amyloid beta-peptide (1-42) in a transgenic Caenorhabditis elegans model. *Neurobiology of Aging*, *24*(3), 415–420.

El-maraghi, S. (2013). The prognostic value of neuron specific enolase in head injury. *The Egyptian Journal of Critical Care Medicine*, *1*(1), 25–32.

Enhances, H., Glutamate, E., Subjected, R., & Ischemia, F. (2000). Hyperglycemia Enhances Extracellular Glutamate Accumulation in Rats Subjected to Forebrain Ischemia, *31*(1), 183–192.

Ercal, N., Gurer-orhan, H., & Aykin-burns, N. (2001). Toxic Metals and Oxidative Stress Part I : Mechanisms Involved in Metal induced Oxidative Damage, *1*(573), 529–539.

Evans, M. (2011). Discovering pluripotency: 30 years of mouse embryonic stem cells. *Nature Reviews Molecular Cell Biology*, *12*(10), 680–686.

Fairman, W. A., Vandenberg, R. J., Arriza, J. L., Kavanaugh, M. P., & Amara, S. G. (1995). An excitatory amino-acid transporter with properties of a ligand-gated chloride channel. *Nature*, *375*(6532), 599–603.

Fathi, F., Altiraihi, T., Mowla, S. J., & Movahedin, M. (2009). Formation of embryoid bodies from mouse embryonic stem cells cultured on silicon-coated surfaces. *Cytotechnology*, *59*(1), 11–16.

Fraichard, A., Chassande, O., Bilbaut, G., Dehay, C., Savatier, P., & Samarut, J. (1995). In vitro differentiation of embryonic stem cells into glial cells and functional neurons. *Journal of Cell Science*, *108 (*1), 3181–3188.

Gao, L., Syahril, A., & Rozita, R. (2014). Neural Commitment of Embryonic Stem Cells through the Formation of Embryoid Bodies (EBs), *21*(5), 8–16.

Gottlieb, D. I., & Huettner, J. E. (1999). An in vitro pathway from embryonic stem cells to neurons and glia. *Cells, Tissues, Organs*, *165*(3–4), 165–172.

Guan, K., Chang, H., Rolletschek, A., & Wobus, A. M. (2001). Embryonic stem cell-derived neurogenesis: Retinoic acid induction and lineage selection of neuronal cells. *Cell and Tissue Research*, *305*(2), 171–176.

Guan, K., Rohwedel, J., & Wobus, A. M. (1999). Embryonic stem cell differentiation models : cardiogenesis , myogenesis , neurogenesis , epithelial and vascular smooth muscle cell differentiation in vitro. *Cytotechnology*, *30*, 211–226

Guillaume, D., Bertrand, P., Dea, D., Davignon, J., & Poirier, J. (1996). Apolipoprotein E and low-density lipoprotein binding and internalization in primary cultures of rat astrocytes: isoform-specific alterations. *Journal of Neurochemistry*, *66*(6), 2410–8.

Gupta, K., Hardingham, G. E., & Chandran, S. (2013). NMDA receptor-dependent glutamate excitotoxicity in human embryonic stem cell-derived neurons. *Neuroscience Letters*, *543*, 95–100.

Ha, J. S., & Park, S. S. (2006). Glutamate-induced oxidative stress , but not cell death , is largely dependent upon extracellular calcium in mouse neuronal HT22 cells. *Neuroscience Letters*,*393*, 165–169.

Han, S. H., Hulette, C., Saunders, A. M., Einstein, G., Pericak-Vance, M., Strittmatter, W. J.,Schmechel, D. E. (1994). Apolipoprotein E is present in hippocampal neurons without neurofibrillary tangles in Alzheimer's disease and in age-matched controls. *Experimental Neurology*, *128*(1), 13–26.

Herz, J., & Bock, H. H. (2002). Lipoprotein receptors in the nervous system. *Annual Review of Biochemistry*, *71*(1), 405–434.

Hoe, H. S., Harris, D. C., & Rebeck, G. W. (2005). Multiple pathways of apolipoprotein E signaling in primary neurons. *Journal of Neurochemistry*, *93*(1), 145–155.

Hosomi, A., Arita, M., Sato, Y., Kiyose, C., Ueda, T., Igarashi, O., Inoue, K. (1997). Affinity for alpha-tocopherol transfer protein as a determinant of the biological activities of vitamin E analogs. *FEBS Letters*, *409*(1), 105–8.

Hu, W.-H., Walters, W. M., Xia, X.-M., Karmally, S. A., & Bethea, J. R. (2003). Neuronal glutamate transporter EAAT4 is expressed in astrocytes. *Glia*, *44*(1), 13–25.

Kawasaki, H., Mizuseki, K., Nishikawa, S., Kaneko, S., Kuwana, Y., Nakanishi, S.,Sasai, Y. (2000). Induction of midbrain dopaminergic neurons from ES cells by stromal cell-derived inducing activity. *Neuron*, *28*(1), 31–40.

Kidder, B. L., Yang, J., & Palmer, S. (2008). Stat3 and c-Myc Genome-Wide Promoter Occupancy in Embryonic Stem Cells. *Plus One*, *3*(12), 1–14.

Kim, J., Castellano, J. M., Jiang, H., Basak, J. M., Parsadanian, M., Pham, V., Holtzman, D. M. (2009). Overexpression of low-density lipoprotein receptor in the brain markedly inhibits amyloid deposition and increases extracellular Aβ clearance. *Neuron*, *64*(5), 632–644.

Kim, M., Habiba, A., Doherty, J. M., Mills, J. C., Mercer, R. W., & Huettner, J. E. (2009a). Regulation of mouse embryonic stem cell neural differentiation by retinoic acid. *Developmental Biology*, *328*(2), 456–471.

Kim, M., Habiba, A., Doherty, J. M., Mills, J. C., Mercer, R. W., & Huettner, J. E. (2009b). Regulation of mouse embryonic stem cell neural differentiation by retinoic acid. *Developmental Biology*, *328*(2), 456–71.

Kinoshita, A., Whelan, C. M., Irizarry, M. C., Arelin, K., Rebeck, G. W., Strickland, D. K., & Hyman, B. T. (2002). LRP and senile plaques in Alzheimer ' s disease : colocalization with apolipoprotein E and with activated astrocytes. *Molecular Brain Research,104* 38–46.

Khor, S. C., Razak, A. M., Wan Ngah, W. Z., Mohd Yusof, Y. A., Abdul Karim, N., & Makpol, S. (2016). The Tocotrienol-Rich Fraction Is Superior to Tocopherol in Promoting Myogenic Differentiation in the Prevention of Replicative Senescence of Myoblasts. *PloS One*, *11*(2).

Konno, T., Akita, K., Kurita, K., & Ito, Y. (2005). Formation of embryoid bodies by mouse embryonic stem cells on plastic surfaces. *Journal of Bioscience and Bioengineering*, *100*(1), 88–93.

Kunishima, N., Shimada, Y., Tsuji, Y., Sato, T., Yamamoto, M., Kumasaka, T.,Morikawa, K. (2000). Structural basis of glutamate recognition by a dimeric metabotropic glutamate receptor. *Nature*, *407*(6807), 971–7.

Kurosawa, H., Imamura, T., Koike, M., Sasaki, K., & Amano, Y. (2003). A simple method for forming embryoid body from mouse embryonic stem cells. *Journal of Bioscience and Bioengineering*, *96*(4), 409–411.

Leduc, V., Jasmin-Bélanger, S., & Poirier, J. (2010). APOE and cholesterol homeostasis in Alzheimer's disease. *Trends in Molecular Medicine*, *16*(10), 469–477.

Lewerenz, J., Hewett, S. J., Huang, Y., Lambros, M., Gout, P. W., Kalivas, P. W.,Smith, S. B. (2013). The Cystine / Glutamate Antiporter System x c - in Health and Disease. *Molecular Mechanisms*, *18*(5), 522–555.

Li. (2002). Lineage Selection for Generation and Amplification of Neural Precursor Cells, *185*, 205–215.

Liu, C., Hu, J., Tsai, C., Yue, M., Melrose, H. L., Kanekiyo, T., & Bu, G. (2015). Neuronal LRP1 Regulates Glucose Metabolism and Insulin Signaling in the Brain. *Journal of Neuroscience*, *35*(14), 5851–5859.

Liu, C.-C., Liu, C.-C., Kanekiyo, T., Xu, H., & Bu, G. (2013). Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. *Nature Reviews. Neurology*, *9*(2), 106–18.

Loebel, D. A. F., Watson, C. M., Young, R. A. De, & Tam, P. P. L. (2003). Lineage choice and differentiation in mouse embryos and embryonic stem cells. *Developmental biology. 264*(1), 1–14.

Lovell, M. A., Robertson, J. D., Teesdale, W. J., Campbell, J. L., & Markesbery, W. R. (1998). Copper , iron and zinc in Alzheimer ' s disease senile plaques, *158*, 47–52.

Malhotra, H., Sharma, P., Malhotra, B., Bhargava, S., Jasuja, S., & Kumar, M. (2016). Molecular response to imatinib & its correlation with mRNA expression levels of imatinib influx & efflux transporters in patients with chronic myeloid leukaemia in chronic phase. *Indian Journal of Medical Research*, *142*(2), 175–182.

Mariani, E., Polidori, M. C., Cherubini, A., & Mecocci, P. (2005). Oxidative stress in brain aging , neurodegenerative and vascular diseases : An overview, *827*(4), 65–75.

Markesbery, W. R. (1997). Oxidative stress hypothesis in Alzheimer's disease. *Free Radical Biology & Medicine*, *23*(1), 134–47.

Martin, F., Mergl, R., Stach, B., Jahn, I., Gertz, H., & Schönknecht, P. (2014). Elevated levels of cerebrospinal fluid neuron-specific enolase (NSE) in Alzheimer ' s disease. *Neuroscience Letters*, *570*, 81–85.

Masliah, E., Alford, M., Mallory, M., Rockenstein, E., Moechars, D., & Van Leuven, F. (2000). Abnormal glutamate transport function in mutant amyloid precursor protein transgenic mice. *Experimental Neurology*, *163*(2), 381–7.

Matthews, R. T., & Beal, M. F. (1996). Increased 3-nitrotyrosine in brains of Apo E-deficient mice. *Brain Research*, *718*(1–2), 181–4.

May, P. C., Lampert-Etchells, M., Johnson, S. A., Poirier, J., Masters, J. N., & Finch, C. E. (1990). Dynamics of gene expression for a hippocampal glycoprotein elevated in Alzheimer's disease and in response to experimental lesions in rat. *Neuron*, *5*(6), 831–839.

Min, Y. H., Khaza, H., Sokhini, M., Mutalib, A., & Musa, I. (2013). The comparative effects between tocotrieonol-rich fraction (TRF) and α- tocopherol on glutamate toxicity in neuron-astrocyte mono- and co- culture systems. *International Journal of Biomedical and Advance Research Research, 4*(6), 403-409.

Monte, S. M. De. (2012). Brain Insulin Resistance and Deficiency as Therapeutic Targets in Alzheimer ' s Disease, *Current Alzheimer Research, 9*, 35–66.

Moskovitz, J., Bar-Noy, S., Williams, W. M., Requena, J., Berlett, B. S., & Stadtman, E. R. (2001). Methionine sulfoxide reductase (MsrA) is a regulator of antioxidant defense and lifespan in mammals. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(23), 12920–5.

Murray, I. V. J., Sindoni, M. E., & Axelsen, P. H. (2005). Promotion of oxidative lipid membrane damage by amyloid beta proteins. *Biochemistry*, *44*(37), 12606–13.

Näslund, J., Schierhorn, A., Hellman, U., Lannfelt, L., Roses, A. D., Tjernberg, L. O., Greengard, P. (1994). Relative abundance of Alzheimer A beta amyloid peptide variants in Alzheimer disease and normal aging. *Proceedings of the National Academy of Sciences of the United States of America*, *91*(18), 8378–82.

Nordin, N., Li, M., & Mason, J. O. (2008). Expression profiles of Wnt genes during neural differentiation of mouse embryonic stem cells. *Cloning and stem cells*, *10*(1), 37-47.

Ohtsuka, M., Ishii, K., Kikuti, Y. Y., Warita, T., Suzuki, D., Sato, M., & Kimura, M. (2006). Construction of Mouse 129 / Ola BAC library for targeting experiments using e14 embryonic stem cells. *Genes and genetic system*, *81*, 143–146.

Okabe, S., Forsberg-nilsson, K., Spiro, A. C., Segal, M., & Mckay, R. D. G. (1996). Development of neuronal precursor cells and functional postmitotic neurons from embryonic stem cells in vitro. *Mechanisms of Development ,59*, 89–102.

Osakada, F., Hashino, A., Kume, T., Katsuki, H., Kaneko, S., & Akaike, A. (2004). α-Tocotrienol provides the most potent neuroprotection among vitamin E analogs on cultured striatal neurons. *Neuropharmacology*, *47*(6), 904–915.

Pan, G. J., Zeng, Y. C., Er, h., & Pei, D. (2002). Stem cell pluripotency and transcription factor Oct4. *Cell research*, *12*, 321–329.

Pin, J. P., & Duvoisin, R. (1995). The metabotropic glutamate receptors: structure and functions. *Neuropharmacology*, *34*(1), 1–26.

Platt, S. R. (2007). The role of glutamate in central nervous system health and disease - A review. *Veterinary Journal*, *173*(2), 278–286.

Podratz, J. L., Rodriguez, E. H., & Windebank, A. J. (2004). Antioxidants are necessary for myelination of dorsal root ganglion neurons , in vitro. *GLIA*, *58*, 54–58.

Poirier, J. (2003). Apolipoprotein E and cholesterol metabolism in the pathogenesis and treatment of Alzheimer's disease. *Trends in Molecular Medicine*, *9*(3), 94–100.

Poirier, J. (2008). Apolipoprotein E represents a potent gene-based therapeutic target for the treatment of sporadic Alzheimer's disease. *Alzheimer's and Dementia*, *4*(1), 91–97.

Qian, A., & Johnson, J. W. (2002). Channel gating of NMDA receptors. *Physiology & Behavior*, *77*(4–5), 577–82.

Reynolds, I. J., & Hastings, G. (1995). Glutamate Induces Cultured Forebrain the Production of Reactive Oxygen Species in Neurons Following NMDA Receptor Activation. *The Journal of Neuroscience*,*15*(5), 3318–3327.

Rothstein, J. D., Dykes-hoberg, M., Pardo, C. A., Bristol, L. A., Jin, L., Kuncl, R. W., … Welty, D. F. (1996). Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. *Neuron, 16,* 675–686.

Schubert, D., & Piasecki, D. (2001). Oxidative glutamate toxicity can be a component of the excitotoxicity cascade. *The Journal of Neuroscience, 21*(19), 7455–7462.

Selkoe, D. J. (2001). Alzheimer's disease: genes, proteins, and therapy. *Physiological Reviews, 81*(2), 741–66.

Selvaraju, R., Khaza, H., Vidyadaran, S., Sokhini, M., & Mutalib, A. (2014). The neuroprotective eff ects of tocotrienol rich fraction and alpha tocopherol against glutamate injury in astrocytes. *Journal of Basic Medical Science,* 14(4):195-204

Sen, C. K., Khanna, S., & Roy, S. (2006). Tocotrienols: Vitamin E beyond tocopherols. *Life Sciences, 78*(18), 2088–2098.

Seri, B., Garcı, J. M., Mcewen, B. S., & Alvarez-buylla, A. (2001). Astrocytes Give Rise to New Neurons in the Adult Mammalian Hippocampus, *21*(18), 7153–7160.

Sen, C. K., Khanna, S., Roy, S., & Packer,L. (2000). Molecular basis of vitamin e action tocotrienol potently inhibits glutamate-induced pp60$^{c\text{-}src}$ kinase activation and death of ht4 neuronal cells. *The journal of Biology Chemistry, 275* (17),13049-13055

Sepkuty, J. P., Cohen, A. S., Eccles, C., Rafiq, A., Behar, K., Ganel, R., … Rothstein, J. D. (2002). A neuronal glutamate transporter contributes to neurotransmitter GABA synthesis and epilepsy. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 22*(15), 6372–9.

Sheldon, A. L., & Robinson, M. B. (2007). The role of glutamate transporters in neurodegenerative diseases and potential opportunities for intervention. *Neurochemistry International, 51*(6–7), 333–355.

Smith,A,G., Heath,J,K., Donaldson,D,D.,Wong,G,G., Moreau,J.,Stahl,M., & Rogers,D (1988). Inhibition of pluripotential embryonic stem cell differentiation by purified polypeptides. *Nature, 336,* 688-690

Song, H., Stevens, C. F., & Gage, F. H. (2002). Neural stem cells from adult hippocampus develop essential properties of functional CNS neurons, *5*(5).

Soulie, C., Sergeant, N., Wrieze, N. W., & Delacourte, A. (1997). Apoe synthesis in human neuroblastoma cells. *Neurobiology of Disease, 4,* 356–364.

Strubing, C., Ahnert-Hilger, G., Shan, J., Wiedenmann, B., Hescheler, J., & Wobus, a. M. (1995). Differentiation of pluripotent embryonic stem cells into the neuronal lineage in vitro gives rise to mature inhibitory and excitatory neurons. *Mechanisms of Development*, *53*(2), 275–287.

Sun, Y., Wu, S., Bu, G., Onifade, M. K., Patel, S. N., LaDu, M. J., … Holtzman, D. M. (1998). Glial fibrillary acidic protein-apolipoprotein E (apoE) transgenic mice: astrocyte-specific expression and differing biological effects of astrocyte-secreted apoE3 and apoE4 lipoproteins. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *18*(9), 3261–3272.

Tanaka, K. (2000). Functions of glutamate transporters in the brain. *Neuroscience Research*, *37*(1), 15–19.

Uttara, B., Singh, A. V, Zamboni, P., & Mahajan, R. T. (2009). Oxidative stress and neurodegenerative diseases : a review of upstream and downstream antioxidant therapeutic options. *Current Neuropharmacology*,*7*, 65–74.

Wands, J. R. (2008). Alzheimer ' s Disease Is Type 3 Diabetes — Evidence Reviewed. *Journal of Diabetes Science and Technology* , *2*(6), 1101–1113.

Winblad, B., Amouyel, P., Andrieu, S., Ballard, C., Brayne, C., Brodaty, H., Curie-paris, P. M. (2015). Defeating Alzheimer ' s disease and other dementias : a priority for European science and society. *The Lancet Neurology Commission*,*15*, 455-532.

Xu, P. T., Gilbert, J. R., Qiu, H. L., Ervin, J., Rothrock-Christian, T. R., Hulette, C., & Schmechel, D. E. (1999). Specific regional transcription of apolipoprotein E in human brain neurons. *The American Journal of Pathology*, *154*(2), 601–611.

Ying, Q., Nichols, J., Chambers, I., & Smith, A. (2003). BMP Induction of Id Proteins Suppresses Differentiation and Sustains Embryonic Stem Cell Self-Renewal in Collaboration with STAT. *Cell*, *115*(3), 281–292.

Ying, Q., Stavridis, M., Griffiths, D., Li, M., & Smith, A. (2003). Conversion of embryonic stem cells into neuroectodermal precursors in adherent monoculture. *Nature*, *21*(2), 183–186.

Yokel, R. (2000). The Toxicology of Aluminum in the Brain : A Review, *21*(5), 813–828.

Zeineddine, D., Hammoud, A. A., Mortada, M., & Boeuf, H. (2014). The Oct4 protein : more than a magic stemness marker, *3*(2), 74–82.

Zhang, J., Dawson, V. L., Dawson, T. M., & Snyder, S. H. (1994). Nitric oxide activation of poly(ADP-ribose) synthetase in neurotoxicity. *Science*, *263*(5147), 687–9.

Zhang, S., & Cui, W. (2014). Sox2 , a key factor in the regulation of pluripotency and neural differentiation. *World Journal of Stem cells*, *6*(3), 305–311.

Zoia, C., Cogliati, T., Tagliabue, E., Cavaletti, G., Sala, G., Galimberti, G., Ferrarese, C. (2004). Glutamate transporters in platelets: EAAT1 decrease in aging and in Alzheimer's disease. *Neurobiology of Aging*, *25*(2), 149–57.

Zoia, C. P., Tagliabue, E., Isella, V., Begni, B., Fumagalli, L., Brighina, L., Ferrarese, C. (2005). Fibroblast glutamate transport in aging and in AD: correlations with disease severity. *Neurobiology of Aging*, *26*(6), 825–32.