Defining the role of p75 neurotrophin receptor ($p75^{NTR}$) in the development of Alzheimer's disease

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THESIS SUMMARY

The dysregulation of neurotrophins and their receptors plays a crucial role in the pathological process of sporadic Alzheimer's disease (AD). Here, we investigated the potential functions of $p75^{NTR}$ in the development of AD. We have found that $p75^{NTR}$ interacts with APP and Aβ, as a $p75^{NTR}$ ligand, promotes the interaction. To address the significance of this $p75^{NTR}$/APP interaction in AD, we discovered that $p75^{NTR}$ transfection increased amyloidogenic processing of APP in CHO^{APP695}. Aβ enhances APP amyloidogenic processing in mouse cortical neurons of AD/$p75^{+/+}$, but not in AD/$p75^{-/-}$ neurons via upregulation of APP and BACE1 expression. $A\beta_{42}$ increases the internalization of APP and the internalization of BACE1 through $p75^{NTR}$. In addition, Aβ and proNGF increased the APP/BACE1 interaction. The $A\beta_{42}$/$p75^{NTR}$ association regulates the phosphorylation of APP-Thr668 and phosphorylation of Tau in mouse cortical neurons.

It was shown that Sortilin interacts with BACE1, mediates retrograde trafficking of BACE1 and promotes Aβ generation. We have elucidated that BACE1, the rate-limiting enzyme processing APP, interacts with $p75^{NTR}$, as a co-receptor for Sortilin, and regulates its proteolytic processing. Our results present that BACE1 interacts with p75ECD. Aβ and proNGF significantly enhanced the BACE1/$p75^{NTR}$ interaction. The ratio of p75ECD/p75FL in $BACE^{+/+}$ mouse brain was significantly higher than in $BACE^{-/-}$ mouse brain. p75ECD is increased in cell lysates, but reduced in culture medium, of HEK-293T cells co-transfected with BACE1/$p75^{NTR}$ plasmids. To address the physiological function of p75ECD in AD, we found that p75ECD significantly rescued Aβ and proNTs-induced impairment of neurite outgrowth in cortical neurons.

The neurotrophin receptor $p75^{NTR}$ mediates both neurotrophic and neurodegenerative signals and its ectodomain shedding from the cell surface are physiologically regulated. We have conducted an *in vivo* study to investigate the effects of p75ECD-Fc recombinant protein on cognitive function and neuropathology features of AD in an AD mouse model. Our data showed that i.p delivery of p75ECD-Fc was not effective on cognitive function in APPswe/PS1DE9 (AD) mouse. p75ECD-Fc improved the process of learning, but not memory impairment in tau pathology-related tyrosine phosphorylation (PR5) mouse model. p75ECD-Fc significantly decreased the size and number of Aβ plaques in AD mouse brain through inhibition of BACE1 expression. p75ECD-Fc significantly reduced GFAP levels in AD mouse. Moreover, p75ECD-Fc was not effective in restoring the level of synaptic proteins, including the vesicle-associated membrane protein (VAMP2) and synaptosomal-associated protein 25 (SNAP-25) in AD mouse brain. p75ECD-Fc did not change ChAT levels, but it significantly reduced Tau phosphorylation and inhibited BACE1 expression in PR5 mouse brain.

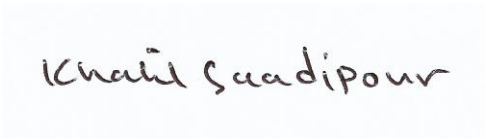
We further investigated the expression and regulation of Sortilin, as a $p75^{NTR}$ co-receptor, in AD. Our data showed that Sortilin expression is significantly increased in human AD brains and in brains of 6-month old APPswe/PS1dE9 transgenic mice in comparison with relevant control groups. $A\beta_{42}$ enhanced the protein and mRNA expression level of Sortilin in SH-SY5Y cells. In addition, proBDNF also significantly increased the mRNA and protein expression of Sortilin. We found the inhibition of $p75^{NTR}$ and ROCK, but not JNK, suppressed constitutive and $A\beta_{42}$-induced expression of Sortilin.

Taken together, the full length of $p75^{NTR}$ mediates APP processing and contributes to AD pathogenesis via Aβ-induced upregulation of BACE1, APP and Sortilin, whereas

the p75ECD fragment is a novel neurotrophic molecule and protects the brain from toxicity induced by Aβ and proNTs.

DECLARATION

‘I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the test.’



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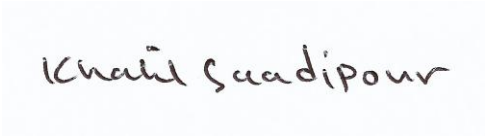
At the end, I proudly dedicate my thesis to my lovely family, neuroscientists and all patients who are suffering from Alzheimer's disease.



PUBLICATIONS AND SEMINARS ARISING FROM THIS THESIS

Publications:

1. Wang YJ, Zeng F, **Saadipour K**, Lu JJ, Zhou XF. $p75^{NTR}$- A molecule with multiple functions in amyloid-beta metabolism and neurotoxicity (2014); J. Neurotoxicity Research (*Handbook*).

2. **Saadipour K**, Yang M, Lim Y, Georgiou K, Sun Y, Keating DJ, Liu J, Wang YR, Gai WP, Zhong JH, Wang YJ, Zhou XF. Amyloid beta $_{1\text{-}42}$ ($A\beta_{42}$) up-regulates the expression of Sortilin via the $p75^{NTR}$/RhoA signalling pathway (2013); J. Neurochemistry 127(2):152-62. doi: 10.1111/jnc.12383.
   This article is Highlighted in an editorial piece: doi: 10.1111/jnc.12389. Epub 2013 Aug 28.

3. Yang M, Virassamy B, Lekha Vijayaraj S, Lim Y, **Saadipour K**, Wang YJ, Han YC, Zhong JH, Carlos R. Morales CR, Zhou XF. The Intracellular Domain of Sortilin Interacts with Amyloid Precursor Protein and Regulates Its Lysosomal and Lipid Raft Trafficking (2013); PLoS One **8**(5): e63049. doi: 10.1371/journal.pone.0063049.

4. Yao X[¥], Jia S[¥], **Saadipour K**[¥], Wang S, Zeng F, Wang Q, Wang Y. Zhong J, Zhou H, Zhou XF and Wang YJ. $p75^{NTR}$ ectodomain is a physiological neuroprotective molecule against amyloid-beta toxicity in the brain of Alzheimer's disease (Under review). ***¥ First equal co-authors***

5. **Saadipour K**, Lim Y, Zhou XF. A simplified method for the brain meninges removal of neonatal mouse for cortical neuron culture (Under review).

6. **Saadipour K**, Lim Y, Keating DJ, Liu J, Wang YR, Zhong JH, Wang YJ, Zhou XF. A complex of $p75^{NTR}$/APP/Aβ interaction mediates a positive-forward loop promoting APP processing and Aβ generation in Alzheimer's disease (Manuscript).

7. **Saadipour K**, Lim Y, Keating DJ, Zhong JH, Wang YJ, Zhou XF. BACE1 regulates the proteolytic processing of $p75^{NTR}$ and mitigates neurodegenerative signals in the brain (Manuscript).

8. **Saadipour K**, Lim Y, Keating DJ, Zhong JH, Wang YJ, Zhou XF. Effects of extracellular domain of $p75^{NTR}$ (p75ECD-Fc) on behavioural deficits and neuropathology features in Alzheimer's disease mouse models (Manuscript).

Conference abstracts:

1. **Khalil Saadipour**, Yoon Lim, Jia Liu, Damien J. Keating, YeRan Wang, Jin-hua Zhong, Yan-Jiang Wang and Xin-Fu Zhou. Aβ induces BACE1 upregulation and enhances APP processing through cross-talk with $p75^{NTR}$. Alzheimer's association International Conference (AAIC), 12^{th}-17^{th} of July 2014, Copenhagen, Denmark. Poster presentation.

2. **Khalil Saadipour**, Yoon Lim, Jia Liu, YeRan Wang, Damien J. Keating, Yan-Jiang Wang and Xin-Fu Zhou. BACE1 regulates the proteolytic processing of $p75^{NTR}$ via interacting with its extracellular domain. Australasian Neuroscience Society 34^{th} Annual Meeting, Jan 2014, Adelaide, Australia. Oral presentation.

3. **Khalil Saadipour**, Miao Yang, Kristen Georgiou, Yoon Lim, Shen Liu, Ying Sun, Wei-Ping Gai, Damien Keating and Xin-Fu Zhou. Amyloid $beta_{1-42}$ up-regulates expression of Sortilin mRNA and protein in SH-SY5Y human neuroblastoma cells. Australian Neuroscience Society 33^{rd} Annual Meeting, Feb 2013, Melbourne, Australia. Poster presentation.

4. **Khalil Saadipour**, Miao Yang, Yoon Lim, Kevin Smith, Shen Liu, Ying Sun, Yan-Jiang Wang and Xin-Fu Zhou. Amyloid beta mediates APP processing through $p75^{NTR}$ in Alzheimer's disease. Australian Society for Medical Research (ASMR), 6^{th} June 2012, Adelaide, Australia. Poster presentation.

AWARDS

- Endeavour International Postgraduate Research Scholarship (EIPRS) for PhD study in Neuroscience by Flinders University, 2010.

- Best student publication award (AU$250) for "Amyloid beta$_{1-42}$ (Aβ_{42}) up-regulates the expression of Sortilin via the p75^{NTR}/RhoA signalling pathway. J Neurochemistry 2013" article by Australian Society for Biochemistry and Molecular Biology (ASBMB), 2013.

- International conference travel grant (AU$2000) by Flinders University, 2014.

ABBREVIATIONS

AA	Amino acid
Aβ	Amyloid beta/ Beta amyloid
AD	Alzheimer's disease
ADAM	A disintegrin and metalloproteinase
AICD	APP intracellular domain
ANOVA	Analysis of variance
AP	Anteroposterior (axis)
APLP	Amyloid precursor-like protein
ApoE	Apolipoprotein E
APP	Amyloid precursor protein
BACE1	Beta-site amyloid precursor protein cleaving enzyme 1
BBB	Blood–brain barrier
BCA	Bicinchoninic acid (kit)
BDNF	Brain-derived neurotrophic factor
BF	Basal forebrain
BFCN	Basal forebrain cholinergic neurons
bp	base pairs
BSA	Bovine serum albumin
cAMP	Cyclic adenosine monophosphate
cdk5	Cyclin-dependent protein kinase 5
CFP	Cyan fluorescent protein
CGNs	Sensory and cerebellar granule neurons
ChAT	Choline acetyltransferase
CHO^{APP695}	Chinese hamster ovary cells expressing APP695 protein
CNS	Central nervous system

CO2	Carbon dioxide
Co-IP	Co-immunoprecipitation
CREB	cAMP responsive element binding (signalling)
CSF	Cerebrospinal fluid
CTF	C-terminal fragment
Cy3	Cyanine-3 fluorescence dye
DAB	3,3'-Diaminobenzidine
DAPI	4' 6-Diamidino-2-phenylindole
DMEM	Dulbecco's Modified Eagle's Medium
DNA	Deoxyribonucleic acid
DNaseI	Deoxyribonuclease I
DRG	Dorsal root ganglion
DS	Down syndrome
DV	Dorsoventral (axis)
Dyrk1A	Dual specificity tyrosine-phosphorylation-regulated kinase 1A
ECD	Extracellular domain
EDTA	Ethylene diamine tetraacetic acid
EEA1	Early endosome antigen 1
EGF	Epidermal growth factor
EGTA	Ethylene glycol tetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
ER	Endoplasmic reticulum
ERK	Extracellular signal-regulated kinase
FAD	Familial Alzheimer’s disease
FBS	Fetal bovine serum
Fc	related to IgG “Fc” chain
FRET	Förster resonance energy transfer

FRET AB	FRET Acceptor bleaching
Gab1	GRB2-associated-binding protein 1
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GFAP	Glial fibrillary acidic protein
GFP	Green fluorescent protein
GM130	cis-Golgi matrix protein
GO	Glucose oxidase
GRP78	Glucose-regulated protein
GSK-3β	Glycogen synthase kinase 3β
GTP	Guanosine triphosphate
HA-tag	Hemagglutinin-tag
HAB	Head activator binding protein
HEK-293T	Human embryonic kidney-293T cells
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HFIP	1,1,1,3,3,3-hexafluoro-2-propanol
HRP	Horseradish peroxidase
ICD	Intracellular domain
ICC	Immunocytochemistry
IGF-1R	Insulin-like growth factor 1 receptor
IHC	Immunohistochemistry
IPTG	Isopropyl β-D-1-thiogalactopyranoside
IS	Interstitial (fluid/space)
JNK	c-Jun N-terminal kinases
kb	kilobase
kDa	kilodalton
KPI	Kunitz protease inhibitor
LAMP1	Lysosomal-associated membrane protein 1

LM	Lateromedial
LRP	Lipoprotein receptor-related protein
LTD	Long-term depression
LTP	Long term potentiation
MAG	Myelin-associated glycoprotein
MAPK	Mitogen-activated protein kinase
MAP-2	Microtubule-associated protein-2 (antibody)
MAPs	Microtubule-associated proteins
MARK	Microtubule-affinity-regulating kinase
MBGIs	Myelin-based growth inhibitors
mRNA	Messenger RNA
MTT	Methyl Thiazoly Blue Tetrazolium Bromide (assay)
MW	Molecular weight
MWM	Morris water maze
N	Normal
NaCl	Sodium chloride
NADPH	Nicotinamide adenine dinucleotide phosphate
NBM	Nucleus Basalis of Meynert
NC	Negative control
NEB	New England Biolabs
NEP	Neprilysin
NFTs	Neurofibrillary tangles
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NGF	Nerve growth factor
NgR	Nogo receptor
NT-3	Neurotrophin-3
NT-4	Neurotrophin-4

NTs	Neurotrophins
OB	Olfactory bulb
OD	Optical density
OmGP	Oligodendrocyte myelin glycoprotein
OS	Oxidative stress
p3	Peptide 3
p53	Tumour protein p53
p75ECD-Fc	Extracellular domain of $p75^{NTR}$
p75KO	p75knockout or $p75^{-/-}$ (mouse)
$p75^{NTR}$	p75 neurotrophin receptor
p75WT	p75wild type or $p75^{+/+}$ or 129sv (mouse)
PBS	Phosphate-buffered saline
PBS-CM	Phosphate-buffered saline with calcium chloride and magnesium chloride
PBST	Phosphate-buffered saline with Tween-20
PC	Positive control
PC12 cells	Rat adrenal pheochromocytoma cells
PCR	Polymerase chain reaction
PDL	Poly-D-Lysine
PF (4%)	Paraformaldehyde solution
PHFs	Paired helical filaments
PI3K	Phosphoinositide 3-kinase
PKA	Protein kinase A
PKC	Protein kinase C
PLC-γ1	Phospholipase C-γ1
PMSF	Phenyl methane sulfonyl fluoride
PNS	Peripheral nervous system

PR5	Tau pathology-related tyrosine phosphorylation (mouse)
proBDNF	Precursor form of brain-derived neurotrophic factor
proNGF	Precursor form of Nerve Growth Factor
proNTs	Precursor form of neurotrophins
PS1 or 2	Presenilin-1 or 2 (enzyme)
RAGE	Receptor for advanced glycation end products
RhoA	Ras homolog gene family, member A
RIPA	Radioimmunoprecipitation assay (buffer)
ROCK	Rho-associated protein kinase
ROI	Region of interests
RPM	Revolutions per minute
RT-PCR	Real-time quantitative PCR
SAPK1b	Stress activated protein kinase 1b
sAPPα	non-Amyloidogenic soluble form of APP
sAPPβ	Amyloidogenic soluble form of APP
Scr.	Scramble
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEM	Standard error of mean
SNAP-25	Synaptosomal-associated protein 25
SORCS	Sortilin-related Vps10p domain containing receptor 1
SorLA	Sorting protein-related receptor with A-type repeats
SPSS	Statistical Package for the Social Sciences
SVZ	Sub-ventricular zone
TACE	Tumour necrosis factor-alpha converting enzyme
TBS	Tris-Buffered Saline
TBST	Tris-Buffered Saline with Tween 20

2x Tg	Double transgenic (mouse)
TGN	Trans-Golgi network
Tm	Melting temperature
TMD	Transmembrane domain
TNF-alpha	Tumour necrosis factor-alpha
Trk	Tyrosine protein kinase/ Tropomyosin-related kinase (receptor)
V	Voltage
VAMP2	Vesicle-associated membrane protein 2
Vps10p	Vacuolar protein sorting 10 protein
vs	versus
WT	Wild type
YFP	Yellow fluorescent protein