

# Linagliptin Protects Human SH-SY5Y Neuroblastoma Cells against Amyloid- $\beta$ Cytotoxicity via the Activation of Wnt1 and Suppression of IL-6 Release

Mohsen Sedighi<sup>1\*</sup>, Tourandokht Baluchnejadmojarad<sup>2</sup> and Mehrdad Roghani<sup>3</sup>

<sup>1</sup>Neuroscience Research Center, Iran University of Medical Sciences, Tehran, Iran;

<sup>2</sup>Department of Physiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran;

<sup>3</sup>Neurophysiology Research Center, Shahed University, Tehran, Iran

Received 8 July 2020; accepted 28 September 2020; published online 7 October 2020

## ABSTRACT

**Background:** Alzheimer's disease is one of the neurodegenerative disorders typified by the aggregate of A $\beta$  and phosphorylated tau protein. Oxidative stress and neuroinflammation, because of A $\beta$  peptides, are strongly involved in the pathophysiology of AD. Linagliptin shows neuroprotective properties against AD pathological processes through alleviation of neural inflammation and AMPK activation. **Methods:** We assessed the benefits of linagliptin pretreatment (at 10, 20, and 50 nM concentrations), against A $\beta$ 1-42 toxicity (20  $\mu$ M) in SH-SY5Y cells. The concentrations of secreted cytokines, such as TNF- $\alpha$ , IL-6, and IL-1 $\beta$ , and signaling proteins, including pCREB, Wnt1, and PKC $\epsilon$ , were quantified by ELISA. **Results:** We observed that A $\beta$  led to cellular inflammation, which was assessed by measuring inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ , and IL-6). Moreover, A $\beta$ 1-42 treatment impaired pCREB, PKC $\epsilon$ , and Wnt1 signaling in human SH-SY5Y neuroblastoma cells. Addition of Linagliptin significantly reduced IL-6 levels in the lysates of cells, treated with A $\beta$ 1-42. Furthermore, linagliptin prevented the downregulation of Wnt1 in A $\beta$ 1-42-treated cells exposed. **Conclusion:** The current findings reveal that linagliptin alleviates A $\beta$ 1-42-induced inflammation in SH-SY5Y cells, probably through the suppression of IL-6 release, and some of its benefits are mediated through the activation of the Wnt1 signaling pathway. **DOI: 10.52547/ibj.25.5.343**

**Keywords:** Alzheimer disease, Interleukin-6, Linagliptin, Wnt1 protein

**Corresponding Author:** Mohsen Sedighi

Neuroscience Research Center, Iran University of Medical Sciences, Tehran, Iran; E-mail: Sedighi.mo@iums.ac.ir

## INTRODUCTION

Alzheimer's disease, the most usual neurodegenerative disorder, is typified by the accumulation of extracellular A $\beta$  plaques, intracellular hyperphosphorylated tau protein, and neurofibrillary tangles<sup>[1]</sup>. While the accurate mechanisms of neurodegeneration are not yet fully understood, recent report suggest that the development of neuroinflammation and oxidative stress process strongly contribute to the pathogenesis of neurodegenerative diseases, as well as AD<sup>[2]</sup>. Numerous investigations have shown an upregulation

of inflammatory agents and activated glial cell in the brain of AD patients and AD transgenic animal models<sup>[3]</sup>. IL-6, IL-1 $\beta$ , and TNF- $\alpha$  are strong mediators of neural inflammation, which is responsible for the pathogenesis of AD<sup>[4]</sup>. Release of cytokines due to inflammation recruits circulating monocytes and lymphocytes to promote neural inflammation in the CNS<sup>[5]</sup>.

Recent surveys have indicated that GLP-1 analogues ameliorate neurodegeneration in AD. This incretin hormone can cross the blood-brain barrier and plays a mediatory role in the CNS. Nevertheless, GLP-1 is rapidly inactivated by DPP-4, a serine peptidase in the

### List of Abbreviations:

**AD**, Alzheimer's disease; **AMPK**, AMP-activated protein kinase; **A $\beta$** , amyloid- $\beta$ ; **CNS**, central nervous system; **DPP-4**, dipeptidyl peptidase; **GLP-1**, glucagon-like peptide-1; **pCREB**, phosphorylated cyclic AMP response element-binding protein; **PKC**, protein kinase C; **PKC $\epsilon$** , epsilon isoform of protein kinase C; **MTT**, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **A $\beta$ 1-42**, amyloid-beta1-42

bloodstream, leading to decreased GLP-1 half-life. It has been documented that DPP-4 inhibitors such as linagliptin show neuroprotection by the increase of GLP-1 activity in the circulation. It has been reported that linagliptin improves A $\beta$ -induced cytotoxicity via the induction of AMPK signaling pathway and the Sirt1-elicited antioxidant pathways such as superoxide dismutase in neuronal cells.

Wnt signaling cascade is involved in the regulation of synaptic transmission and plasticity in the brain<sup>[6]</sup>. Research reports have indicated that Wnt signaling impairment plays a pivotal role in AD pathogenesis<sup>[7]</sup>. Interestingly, Wnt signaling actively contributes to the A $\beta$  formation and impairment of Wnt signaling pitches in the appearance of tau phosphorylation<sup>[8,9]</sup>. In addition, Wnt1 exhibits neuroprotective effects against amyloid plaque formation and phosphorylation of tau protein. Therefore, impairment of the Wnt/ $\beta$ -catenin pathway could act in AD development<sup>[10,11]</sup>.

PKC is a family member of isoenzymes of serine/threonine protein kinases acting distinctly in the regulation of cellular signal transduction<sup>[12]</sup>. It has been reported that PKC $\epsilon$  significantly involves in the regulation of APP metabolism and regulation of diverse functions in neuronal cells, including the modulation of gene expression<sup>[13]</sup>. Moreover, CREB is crucial for neuronal survival and function<sup>[14]</sup>. It is well documented that CREB-mediated gene expression is damaged in the AD brain, and the level of phosphorylated CREB declines in the hippocampal neurons of PS1/APP double mutant transgenic mice<sup>[15]</sup>. The human SH-SY5Y cell line has been widely used in neuroscience research, particularly in the generation of the cellular model of neurodegenerative diseases<sup>[16-18]</sup>. The current investigation was aimed to explore the protective effects of linagliptin as a DPP-4 inhibitor on the A $\beta$ -induced cytotoxicity in SH-SY5Y cells through the evaluation of Wnt1, PKC $\epsilon$ , and CREB signaling, as well as inflammatory cytokines.

## MATERIALS AND METHODS

### Materials

Human neuroblastoma SH-SY5Y cells (Pasteur Institute of Iran, Teran) and MTT assay (BIO-IDEA, Iran) were used in this study. Chemicals, such as A $\beta$ 1-42 and pure linagliptin were acquired from R&D Systems (USA) and Cayman Chemical (USA), respectively. Antibodies purchased for ELISA were as follow: IL-6, IL-1 $\beta$ , Wnt1, PKC $\epsilon$  (MyBioSource, USA), TNF- $\alpha$  (Sigma-Aldrich, USA), and pCREB (R&D Systems).

### Preparation of A $\beta$ and linagliptin

Recombinant A $\beta$ 1-42 was prepared according to a previously described method<sup>[19]</sup>. Briefly, A $\beta$ 1-42 peptide was solubilized in 10% DMSO to acquire a 2- $\mu$ M solution, which was consisted of fibrillar and monomer forms of A $\beta$ 1-42. For the preparation of linagliptin, pure linagliptin powder was dissolved in DMSO for 24 h to obtain 10, 20, and 50  $\mu$ M solutions. The range of concentrations for linagliptin was opted based on an earlier investigation on the protective effect of linagliptin against A $\beta$ -induced cytotoxicity and insulin signaling impairment in SK-N-MC human neuronal cells<sup>[20]</sup>. All preparations were incubated at 4 °C for 24 h and stored at -20 °C.

### Cell culture and viability assay

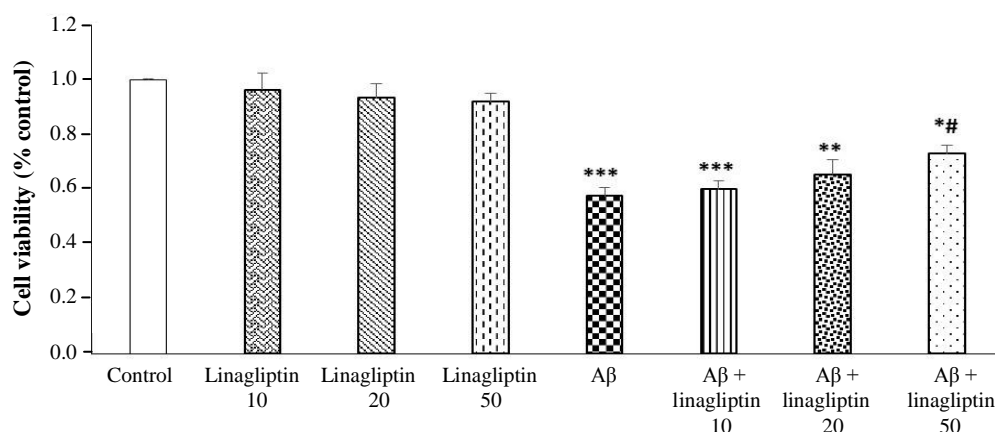
DMEM/F12 supplemented with penicillin 100 U/ml, 10% FBS, and streptomycin 100  $\mu$ g/ml was used at conditions of 37 °C, 5% CO<sub>2</sub> and 95% air to culture SH-SY5Y cells. The cells were seeded onto 96-welled plates to reach a density of 5000 cell/well and incubated for 24 h. Pretreatment of cells was carried out with various doses of linagliptin for 24 h and followed by the exposure of cells to A $\beta$ 1-42 overnight. Experimental groups included SH-SY5Y cells without treatment (control group), linagliptin groups (SH-SY5Y cells pretreated with 10, 20, and 50  $\mu$ M of linagliptin), A $\beta$  group (SH-SY5Y cells treated with 20  $\mu$ M of A $\beta$ ), A $\beta$  + linagliptin groups (SH-SY5Y cells treated with 20  $\mu$ M of A $\beta$  and 10, 20, and 50  $\mu$ M linagliptin). To conduct the viability assay, we added MTT solution to the medium following the linagliptin pretreatment and A $\beta$  challenge. After 4 h, the reaction product was solubilized by DMSO. Culture plates containing SH-SY5Y cells were placed overnight (37 °C), and a microplate reader was applied to measure absorbance at 570 nm. The results were compared with the untreated cells, as the control group.

### ELISA technique

Treated SH-SY5Y cells were incubated with hypotonic lysis buffer after harvesting. Cells were centrifuged at 10,000  $\times$ g in a centrifuge at 4 °C to obtain the cell lysate. The levels of secreted cytokines (IL-6, IL-1 $\beta$ , and TNF- $\alpha$ ) and signaling proteins (pCREB, Wnt1, and PKC $\epsilon$ ) in the cell lysate were quantified using ELISA.

### Statistical analysis

SPSS package (version 22, Chicago, IL, USA) was applied for data analysis, and one-way ANOVA followed by Turkey's post hoc test was utilized for the comparison of experimental groups. Outcomes were presented as mean  $\pm$  SEM, and a *p* value <0.05 was considered statistically significant in all experiments.



**Fig. 1.** The effect of A $\beta$  1-42 (20  $\mu$ M) and linagliptin (10, 20, and 50 nM) on the viability of human SH-SY5Y neuroblastoma cells. Cells were incubated with linagliptin for 24 h, and then A $\beta$  was added for an additional 24 h. \*\*\*  $p < 0.001$ , \*\*  $p < 0.01$ , \*  $p < 0.05$  (as compared to control); #  $p < 0.05$  (as compared to A $\beta$  1-42).

## RESULTS

### Effects of A $\beta$ 1-42 and linagliptin on the viability of SH-SY5Y cells

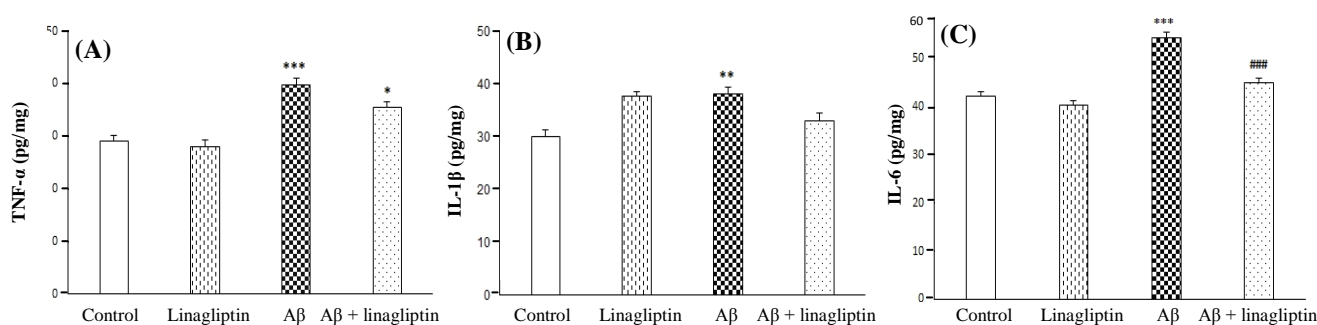
Pretreatment of SH-SY5Y cells with linagliptin (10, 20, and 50  $\mu$ M) was accomplished for 24 h, and the cell viability was assessed by the MTT test in order to explore whether linagliptin induces cytotoxicity in these cells. As represented in Figure 1, cell viability in the treated cells with linagliptin (10, 20, and 50  $\mu$ M) did not show any significant change. Therefore, we selected 50  $\mu$ M of linagliptin in later experiments. Moreover, the toxicity of A $\beta$ 1-42 on cell viability was assessed via incubating SH-SY5Y cell with A $\beta$ 1-42 (20  $\mu$ M). Exposure of cells to A $\beta$ 1-42 significantly reduced the cell viability of cultured SH-SY5Y cells ( $p < 0.001$ ). Furthermore, pretreatment of cells with 50  $\mu$ M of linagliptin significantly prevented the decrease of viability in cells treated with A $\beta$ 1-42.

### Effect of A $\beta$ 1-42 and linagliptin on inflammatory cytokines on SH-SY5Y cells

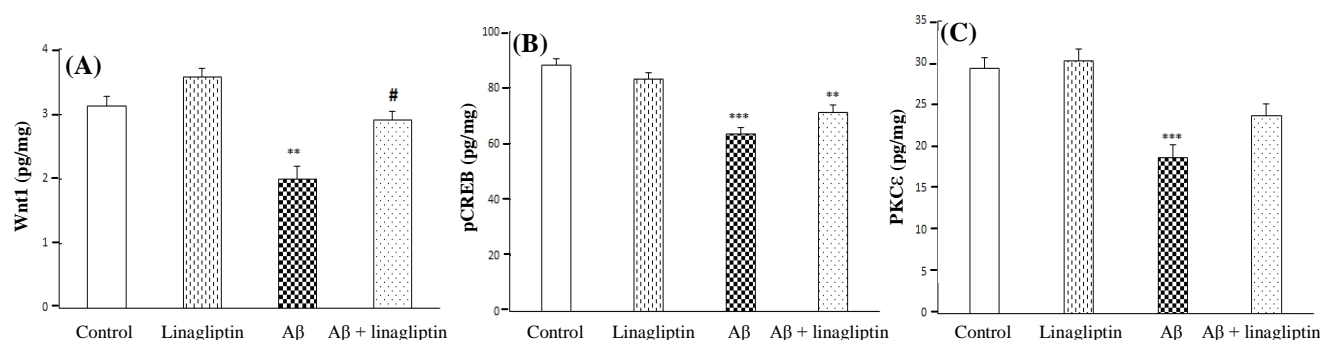
To investigate the effects of A $\beta$ 1-42 and linagliptin on inflammatory biomarkers, we measured the concentrations of TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 in cell lysate. The concentrations of TNF- $\alpha$  ( $p < 0.001$ ), IL-1 $\beta$  ( $p < 0.01$ ), and IL-6 ( $p < 0.001$ ) in treated cells were risen significantly after exposure to A $\beta$ 1-42. Furthermore, the pretreatment of cells with linagliptin could significantly decrease the levels of IL-6 in A $\beta$ 1-42 exposed cells ( $p < 0.001$ ; Fig 2).

### Effects of A $\beta$ 1-42 and linagliptin on pCREB, PKC $\epsilon$ , and Wnt1 levels on SH-SY5Y cells

The lysate levels of Wnt1 ( $p < 0.001$ ), pCREB ( $p < 0.001$ ), and PKC $\epsilon$  ( $p < 0.001$ ) noticeably declined in cells treated with A $\beta$  1-42, as shown in Figure 3. Furthermore, the pretreatment of cells with linagliptin prevented the down-regulation of the lysate level of Wnt1, in cells treated with A $\beta$  1-42.



**Fig. 2.** The effect of A $\beta$  1-42 (20  $\mu$ M) and linagliptin (50 nM) on TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 levels in human SH-SY5Y neuroblastoma cells. Cells were incubated with linagliptin for 24 h, and then A $\beta$  was added for an additional 24 h. \*\*\*  $p < 0.001$ , \*\*  $p < 0.01$  (as compared to control); ###  $p < 0.001$  (as compared to A $\beta$  1-42).



**Fig. 3.** The effect of A $\beta$  1-42 (20  $\mu$ M) and linagliptin (50 nM) on Wnt1, pCREB, and PKC $\epsilon$  in human SH-SY5Y neuroblastoma cells. Cells were incubated with linagliptin for 24 h, and then A $\beta$  was added for an additional 24 h. \*\*\*  $p < 0.001$ , \*\*  $p < 0.01$  (as compared to control); #  $p < 0.05$  (as compared to A $\beta$  1-42).

## DISCUSSION

AD is a prevalent form of neurodegenerative diseases characterized by progressive memory loss and a slow decrease in cognitive function. Normally, The accumulation of extracellular senile plaques containing A $\beta$ , neurofibrillary tangles, and abnormal phosphorylated tau protein are involved in the AD pathogenesis, especially in the hippocampus and cortex<sup>[20,21]</sup>.

The present study was designed to test the potential effect of linagliptin on A $\beta$ -induced cytotoxicity. Our results revealed that the exposure of SH-SY5Y cells to A $\beta$ 1-42 exerts cellular cytotoxicity, which leads to an inflammatory response and induced release of cytokines such as TNF- $\alpha$ , IL-6, and IL-1 $\beta$ . Our findings implicate the role of the inflammatory process in AD pathophysiology<sup>[3,22,23]</sup>. It has been well defined that cytokines released from activated astrocytes and microglia are the main effectors of neural inflammation signals that can affect cognitive function and memory in AD<sup>[4,24,25]</sup>. Moreover, the cytotoxic effect of A $\beta$ 1-42 is mediated through the inhibition of Wnt1, pCREB, and PKC $\epsilon$  signaling pathways<sup>[26-28]</sup>.

Recent investigation has reported that incretins may be a feasible choice for AD treatment<sup>[29]</sup>. In addition to the increase of GLP-1 levels, one study has shown that linagliptin, as a DPP-4 inhibitor, exhibits a neuroprotective effect on A $\beta$ -induced neurotoxicity associated with AD<sup>[27]</sup>. Our prior investigation has discovered that the treatment of peripheral mononuclear blood cells with linagliptin decreases the cellular levels of IL-1 $\beta$  in healthy individuals and TNF- $\alpha$  in AD patients<sup>[11]</sup>. In the current research, findings indicated that linagliptin pretreatment attenuates increased IL-6 in cells cultured with A $\beta$ 1-42. Typically, IL-6 is the most inflammatory marker released by activated microglia and astrocytes in different regions of the brain. It is also capable of

stimulating microglia and astrocytes to produce a cascade of proinflammatory cytokines<sup>[30]</sup>. Former studies have established that IL-6 involves in the APP processing and production in primary rat cortical neurons<sup>[31,32]</sup>. Nakamura *et al.*<sup>[33]</sup> have reported that linagliptin remarkably reduces p65 expression, p38 MAPK phosphorylation, and IL-6 production in the endothelial cells of the umbilical vein, which were treated with lipopolysaccharides. Furthermore, research findings have provided evidence for this point that linagliptin ameliorates A $\beta$ -induced cytotoxicity via the stimulation of AMPK and the Sirt1-elicited antioxidant pathways<sup>[20]</sup>.

The Wnt signaling cascade regulates plasticity and synaptic transmission in the nervous system and the link between the Wnt signaling pathway and AD pathogenesis has been well documented<sup>[34,35]</sup>. Accordingly, research findings have proposed that Wnt signaling deficiency is an important contributing factor in the formation of A $\beta$  and the etiology of AD<sup>[36]</sup>. Tapia-Rojas and Nibaldo<sup>[6]</sup> have reported that the suppression of the Wnt signaling leads to a rise in A $\beta$  42 levels and the A $\beta$ 42/A $\beta$ 40 ratio, which is in favor of A $\beta$  oligomerization *in vitro*. In this study, exposure of neuroblastoma cells to A $\beta$ 1-42 significantly decreased Wnt1 level, which is consistent with an investigation<sup>[26]</sup>. The activation of canonical Wnt signaling resulted in the protective effect of Trolox and vitamin C against A $\beta$ -induced cytotoxicity in hippocampal cultured neurons. A recent discovery has shown that the treatment of isolated hippocampal neurons with some antioxidants protects neurons against A $\beta$  neurotoxicity by a mechanism involving the activation of Wnt signaling and control of oxidative stress<sup>[37]</sup>. Interestingly, some antidiabetic drugs, including DPP-4 inhibitors, have demonstrated a beneficial effect in the CNS of experimental models of AD<sup>[20,38-40]</sup>. We found the increased expression of Wnt1 level in the lysate of SH-SY5Y cells that were

pretreated with linagliptin before A $\beta$  challenge. Our data support this hypothesis that the modulation of Wnt1 signaling might play an important role in the protection against A $\beta$ -induced cytotoxicity<sup>[37,41]</sup>. Moreover, we add this evidence that DPP-4 inhibitor linagliptin has ability to protect SH-SY5Y cells against A $\beta$  challenge, possibly via the activation of Wnt1 signaling and control of oxidative stress.

Our findings in this study exhibit that linagliptin ameliorates A $\beta$ -induced cytotoxicity in human neuroblastoma cells, and a part of this protective effect is mediated through the activation of Wnt1 signaling and control of cellular inflammation. Hence, linagliptin may be a promising therapeutic candidate to treat AD, but more investigations are needed to support this hypothesis.

## ACKNOWLEDGEMENTS

This research study was approved and financially supported (grant # 95-01-87-27994) by Iran University of Medical Sciences (Tehran, Iran).

**CONFLICT OF INTEREST.** None declared.

## REFERENCES

- Kumar A, Singh A. A review on Alzheimer's disease pathophysiology and its management. *Pharmacological reports* 2015; **67**(2): 195-203.
- Dursun E, Gezen-Ak D, Hanağası H, Bilgiç B, Lohmann E, Ertan S, Atasoy İL, Alaylıoğlu M, Selin Araz Ö, Önal B, Gündüz A, Apaydın H, Kızıltan G, Ulutin T, Gürvit H, Yılmaz S. The interleukin1 alpha, interleukin 1 beta, interleukin 6 and alpha-2-macroglobulin serum levels in patients with early or late onset Alzheimer's disease, mild cognitive impairment or Parkinson's disease. *Journal of neuroimmunology* 2015; **283**: 50-57.
- Liu L, Chan C. The role of inflammasome in Alzheimer's disease. *Ageing research reviews* 2014; **15**: 6-15.
- Sedighi M, Baluchnejadmojarad T, Fallah S, Moradi N, Afshin-Majd S, Roghani M. The association between circulating klotho and dipeptidyl peptidase-4 activity and inflammatory cytokines in elderly patients with Alzheimer disease. *Basic and clinical neuroscience* 2020; **11**(3): 349-358.
- Das S, Basu A. Inflammation: a new candidate in modulating adult neurogenesis. *Journal of neuroscience research* 2008; **86**(6): 1199-1208.
- Tapia-Rojas C, Inestrosa NC. Wnt signaling loss accelerates the appearance of neuropathological hallmarks of Alzheimer's disease in J20 APP transgenic and wild type mice. *Journal of neurochemistry* 2018; **144**(4): 443-465.
- Ferrari DV, Avila ME, Medina MA, Pérez-Palma E, Bustos B, Alarcon M. Wnt/ $\beta$ -catenin signaling in Alzheimer's disease. *CNS and neurological disorders drug targets* 2014; **13**(5): 745-754.
- Forlenza OV, de Paula VJ, Machado-Vieira R, Diniz BS, Gattaz WF. Does lithium prevent Alzheimer's disease? *Drugs and aging* 2012; **29**(5): 335-342.
- Parr C, Mirzaei N, Christian M, Sastre M. Activation of the Wnt/ $\beta$ -catenin pathway represses the transcription of the  $\beta$ -amyloid precursor protein cleaving enzyme (BACE1) via binding of T-cell factor-4 to BACE1 promoter. *The FASEB journal* 2015; **29**(2): 623-635.
- Yao Y, Chen X, Bao Y, Wu Y. Puerarin inhibits  $\beta$ -amyloid peptide 1-42-induced tau hyperphosphorylation via the Wnt/ $\beta$ -catenin signaling pathway. *Molecular medicine reports* 2017; **16**(6): 9081-9085.
- Sedighi M, Baluchnejadmojarad T, Fallah S, Moradi N, Afshin-Majd S, Roghani M. Klotho ameliorates cellular inflammation via suppression of cytokine release and upregulation of mir-29a in the pbmcs of diagnosed Alzheimer's disease patients. *Journal of molecular neuroscience* 2019; **69**(1): 157-165.
- Akita Y. Protein kinase C- $\epsilon$  (PKC- $\epsilon$ ): its unique structure and function. *The journal of biochemistry* 2002; **132**(6): 847-852.
- Lanni C, Mazzucchelli M, Porrello E, Govoni S, Racchi M. Differential involvement of protein kinase C alpha and epsilon in the regulated secretion of soluble amyloid precursor protein. *European journal of biochemistry* 2004; **271**(14): 3068-3075.
- Benito E, Barco A. CREB's control of intrinsic and synaptic plasticity. *Trends in neurosciences* 2010; **33**(5): 230-240.
- Pugazhenth S, Wang M, Pham S, Sze CI, Eckman CB. Downregulation of CREB expression in Alzheimer's brain and in A $\beta$ -treated rat hippocampal neurons. *Molecular neurodegeneration* 2011; **6**(1): 60.
- Sedighi M, Baluchnejadmojarad T, Afshin-Majd S, Amiri M, Aminzade M, Roghani M. Anti-aging Klotho Protects SH-sy5y cells against amyloid  $\beta$ 1-42 neurotoxicity: involvement of Wnt1/pCREB/Nrf2/HO-1 signaling. *Journal of molecular neuroscience* 2021; **71**(1): 19-27.
- Amiri M, Braid N, Aminzadeh M. Protective effects of fibroblast growth factor 21 against amyloid-beta1-42-induced toxicity in SH-SY5Y cells. *Neurotoxicity research* 2018; **34**: 574-583.
- Filigrana R, Civiero L, Ferrari V, Codolo G, Greggio E, Bubacco L, Beltrami M, Bisaglia M. Analysis of the catecholaminergic phenotype in human SH-SY5Y and BE(2)-M17 neuroblastoma cell lines upon differentiation. *PLoS one* 2015; **10**(8): e0136769.
- Yeo ETY, Wong KWL, See ML, Wong KY, Gan SY, Chan EWL. Piper sarmentosum Roxb. confers neuroprotection on beta-amyloid (A $\beta$ )-induced microglia-mediated neuroinflammation and attenuates tau hyperphosphorylation in SH-SY5Y cells. *Journal of ethnopharmacology* 2018; **217**: 187-194.
- Kornelius E, Lin CL, Chang HH, Li HH, Huang WN,

- Yang YS, Lu YL, Peng CH, Huang CN. DPP-4 inhibitor linagliptin attenuates A $\beta$ -induced cytotoxicity through activation of AMPK in neuronal cells. *CNS neuroscience and therapeutics* 2015; **21**(7): 549-57.
21. Ni R, Kindler DR, Waag R, Rouault M, Ravikumar P, Nitsch R, Rudin M, Camici GG, Liberale L, Kulic L, Klohs J. FMRI reveals mitigation of cerebrovascular dysfunction by bradykinin receptors 1 and 2 inhibitor noscapine in a mouse model of cerebral amyloidosis. *Frontiers in aging neuroscience* 2019; **11**:27.
  22. Tansey MG, McCoy MK, Frank-Cannon TC. Neuroinflammatory mechanisms in Parkinson's disease: potential environmental triggers, pathways, and targets for early therapeutic intervention. *Experimental neurology* 2007; **208**(1): 1-25.
  23. Gupta P, Sil S, Ghosh R, Ghosh A, Ghosh T. Intracerebroventricular A $\beta$ -induced neuroinflammation alters peripheral immune responses in rats. *Journal of molecular neuroscience* 2018; **66**(4): 572-586.
  24. Gemma C, Bickford PC. Interleukin-1 $\beta$  and Caspase-1: Players in the regulation of age-related cognitive dysfunction. *Reviews in the neurosciences* 2007; **18**(2): 137-48.
  25. Tian Y, Chen KY, Liu LD, Dong YX, Zhao P, Guo SB. Sevoflurane exacerbates cognitive impairment induced by A $\beta$ 1-40 in rats through initiating neurotoxicity, neuroinflammation, and neuronal apoptosis in rat hippocampus. *Mediators of inflammation* 2018; Article ID 3802324.
  26. Inestrosa NC, DeFerrari GV, Garrido JL, Alvarez A, Olivares GH, Barria MaI, Marcelo achacon B. Wnt signaling involvement in  $\beta$ -amyloid-dependent neurodegeneration. *Neurochemistry international* 2002; **41**(5): 341-344.
  27. Vitolo OV, Sant'Angelo A, Costanzo V, Battaglia F, Arancio O, Shelanski M. Amyloid  $\beta$ -peptide inhibition of the PKA/CREB pathway and long-term potentiation: Reversibility by drugs that enhance cAMP signaling. *Proceedings of the national academy of sciences* 2002; **99**(20): 13217-13221.
  28. Alkon DL, Sun M-K, Nelson TJ. PKC signaling deficits: a mechanistic hypothesis for the origins of Alzheimer's disease. *Trends in pharmacological sciences* 2007; **28**(2): 51-60.
  29. Hölscher C. Potential role of glucagon-like peptide-1 (GLP-1) in neuroprotection. *CNS and neurological disorders drug targets* 2012; **26**(10): 871-882.
  30. Thomas L, Eckhardt M, Langkopf E, Tadayyon M, Himmelsbach F, Mark M. (R)-8-(3-Amino-piperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methyl-quinazolin-2-ylmethyl)-3,7-dihydro-purine-2,6-dione (BI 1356), a Novel xanthine-based dipeptidyl peptidase 4 inhibitor, has a superior potency and longer duration of action compared with other dipeptidyl peptidase-4 inhibitors. *Journal of pharmacology and experimental therapeutics* 2008; **325**(1): 175-182.
  31. Wang WY, Tan MS, Yu JT, Tan L. Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease. *Annals of translational medicine* 2015; **3**(10): 136.
  32. Spooren A, Kolmus K, Laureys G, Clinckers R, De Keyser J, Haegeman G, Gerlo S. Interleukin-6, a mental cytokine. *Brain research reviews* 2011; **67**(1-2): 157-183.
  33. Nakamura Y, Hasegawa H, Tsuji M, Oguchi T. Linagliptin inhibits lipopolysaccharide-stimulated interleukin-6 production, intranuclear p65 expression, and p38 mitogen-activated protein kinase phosphorylation in human umbilical vein endothelial cells. *Renal replacement therapy* 2019; **2**(1): DOI: 10.1186/s41100-016-0030-6.
  34. Inestrosa NC, Varela-Nallar L. Wnt signaling in the nervous system and in Alzheimer's disease. *Journal of molecular cell biology* 2014; **6**(1): 64-74.
  35. Inestrosa NC, Varela-Nallar L. Wnt signalling in neuronal differentiation and development. *Cell and tissue research* 2015; **359**(1): 215-223.
  36. De Strooper B, Vassar R, Golde T. The secretases: enzymes with therapeutic potential in Alzheimer disease. *Nature reviews neurology* 2010; **6**(2): 99-107.
  37. Quintanilla RA, Muñoz FJ, Metcalfe MJ, Hirschfeld M, Olivares G, Godoy JA, Inestrosa NC. Trolox and 17  $\beta$ -estradiol protect against amyloid  $\beta$ -peptide neurotoxicity by a mechanism that involves modulation of the Wnt signaling pathway. *Journal of biological chemistry* 2005; **280**(12): 11615-11625.
  38. Gough S. Dipeptidyl peptidase-4 inhibitors. *Handbook of Incretin-Based Therapies in Type 2 Diabetes*. Switzerland: ADIS; 2016.
  39. Angelopoulou E, Piperi C. DPP-4 inhibitors: a promising therapeutic approach against Alzheimer's disease. *Annals of translational medicine* 2018; **6**(12): 255.
  40. Gault VA, Lennox R, Platt PR. Sitagliptin, a dipeptidyl peptidase-4 inhibitor, improves recognition memory, oxidative stress and hippocampal neurogenesis and upregulates key genes involved in cognitive decline. *Diabetes obesity and metabolism* 2015; **17**(4): 403-413.
  41. DeFerrari G, Chacon M, Barria A, Garrido J, Godoy J, Olivares G, Reyes AE, Alvarez A, Bronfman M, Inestrosa NC. Activation of Wnt signaling rescues neurodegeneration and behavioral impairments induced by  $\beta$ -amyloid fibrils. *Molecular psychiatry* 2003; **8**(2): 195-208.