

# Mediation of insulin growth factor-1 in Alzheimer's disease and the mechanism of PRNP genetic expression and the PI3K/Akt signaling pathway

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**Abstract.** The aim of the study was to examine the mediation of insulin growth factor-1 (IGF-1) in Alzheimer's disease (AD), as well as the underlying mechanism of the PRNP genetic expression and PI3K/Akt signaling pathway. The A $\beta_{25-35}$ -incubated rat adrenal pheochromocytoma cell (PC12) *in vitro* was established, constituting the AD model. Different doses (0, 20, 40 and 80 ng/ml) of IGF-1 were used in PC12 cells and the level of PRNP mRNA was tested after 24 h using the quantitative PCR method and the level of APP protein was assessed using western blot analysis. PC12 cells were divided into the control group (PC12 cells without A $\beta_{25-35}$  treatment), model group (PC12 cells with A $\beta_{25-35}$  treatment), IGF-1 80 ng/ml group, IGF-1 80 ng/ml+PI3K inhibitor LY294002 25  $\mu$ mol/l group, and IGF-1 80 ng/ml+LY294002 50  $\mu$ mol/l group, whose PRNP mRNA level and Akt, pAkt and APP protein level were tested 24 h later. As the dose of IGF-1 increases, the expression levels of PRNP mRNA and APP protein were more highly expressed. The difference between them was significant ( $P<0.05$ ). In addition, regarding Akt protein, the expression levels of PRNP mRNA, APP protein and pAkt protein in the IGF-1 groups were significantly higher than those in the control and model groups. With the LY concentration increasing, the levels of expression of the three substances gradually decreased significantly ( $P<0.05$ ). In conclusion, IGF-1 can mediate the expression of the PRNP gene and APP protein through the PI3K/Akt signaling pathway, in a rat model.

life of elderly individuals (1). AD's most prominent neuro-histopathological characteristic is neuro-entanglement inside neurons and amyloid plaques *in vitro* (2). The incidence of AD is related to the deposition of  $\beta$ -amyloid (A $\beta$ ) peptides, a high level of phosphorylation of microtubule protein ( $\tau$  protein), ApoE4, neurofibrillary tangles and many other features (3). It has been found that prion protein was significantly involved in regulating the process of A $\beta$ -induced AD (4). Previous findings showed that the polymorphic coding by the PRNP gene of prion protein (PrPc) was important in the incidence of AD (5). However, there is no unified conclusion and the exact mechanism remains unclear.

Recent findings showed that the pathologic change in AD features was associated with the transferring disorder of signals mediated by insulin in brain and insulin growth factor-1 (IGF-1), an important neuro-nutrition factor (6). Indeed, IGF-1 and its receptors were prevalently expressed in the neuro system (7). IGF-1 affects the eradication of IGF-1 and the phosphorylation of  $\tau$  protein, which was related to the PI3K/Akt and mitogen-activated protein kinase (MAPK)/ERK1/2 pathway (8). However, the adjustment by IGF-1 to PRNP genetic expression remains unclear. Accordingly, the present study analyzes the mediation of IGF-1 in AD PRNP genetic expression and PI3K/Akt signaling pathway mechanism, through constructs of AD cell models *in vitro*.

## Introduction

Alzheimer's disease (AD), a neurodegenerative disease, is the most common type of dementia affecting the quality of

## Materials and methods

**Constructing of AD cell model *in vitro*.** The PC12 cells (AD cell model) induced by A $\beta_{25-35}$  were provided by Shanghai Jimian Bioengineering Co., Ltd. PC 12 cells were cultivated in DMEM cell-culture media (Solarbio Science & Technology Co., Ltd., Beijing, China) containing 10% heat-inactivated FBS (fetal bovine serum) and 1% mixture of penicillin and streptomycin in a 37°C incubator (Beijing Liyi Apparatus Factory) with 5% CO<sub>2</sub> and 100% humidity. When 80% of the cells merged, the cell-culture media were discarded and the digestion was proceeded with trypsin for cell passages. When the cells entered the logarithmic growth phase, the culture media were discarded and the cells were washed with PBS twice. The cell was cultured with DMEM without serum. After 24 h, DMSO was added at a final concentration of 0.1%. After a further 24 h, the supernatant

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liquid was collected and the concentrated protein was stored at  $-20^{\circ}\text{C}$ .

**Group division.** Different doses of IGF-1 (0, 20, 40 and 80 ng/ml) were incubated in PC12 cells and the levels of PRNP mRNA and APP protein were tested 24 h later using quantitative PCR and western blotting, respectively. PC12 cells were divided into the control group (PC12 cells without  $\text{A}\beta_{25-35}$  treatment), model group (PC12 cells with  $\text{A}\beta_{25-35}$  treatment), IGF-1 80 ng/ml group, IGF-1 80 ng/ml+PI3K inhibitor LY294002 25  $\mu\text{mol/l}$  group, and IGF-1 80 ng/ml+LY294002 50  $\mu\text{mol/l}$  group. PRNP mRNA level and Akt, pAkt and APP protein levels were tested 24 h later. Cells disintegrated with RIPA lysis solution were centrifuged for 30 min in  $4^{\circ}\text{C}$  (10,000  $\times$  g), the supernatant liquid was removed and then stored separately at  $4^{\circ}\text{C}$ .

**Real-time quantitative PCR.** TRIzol, the RNA extraction reagent (Invitrogen, Waltham, MA, USA), dNTP, reverse transcriptase, Taq enzyme (Promega, Madison, WI, USA), PCR markers (Shanghai Huamei Bioengineering Co., Ltd). The main steps were as follows: Total RNA was extracted using the TRIzol one-step method. Total RNA (1  $\mu\text{g}$ ) was used for reverse transcription with Oligo18 as the primer. The reaction system was 20  $\mu\text{l}$ . Oligo18 was produced by Shanghai Boya Bioengineering Co., Ltd. The primers included: PRNP (F): 5'-AGGTGGTTCTCATTCTTGC-3', (R): 5'-GTGGCTTCTTTGGTTGTA-3', 307 bp; and internal reference GAPDH (F): 5'-ACCACAGTCCATGCCATCAC-3', (R): 5'-CACCACCCTGTTGCTGTA-3', 432 bp. The reaction conditions included pre-denaturation at  $95^{\circ}\text{C}$  for 5 min, denaturation in  $94^{\circ}\text{C}$  for 30 sec, annealing in  $58^{\circ}\text{C}$  for 30 sec (GAPDH: annealing at  $60^{\circ}\text{C}$  for 30 sec), extension at  $70^{\circ}\text{C}$  for 40 sec with 25 cycles in total (GAPDH: 28 cycles in total). The final extension lasted for 7 min at  $72^{\circ}\text{C}$ . After 20g/l agarose gel electrophoresis, UAP gel density scanner should be used to scan the electrophoretic band of the amplification products of the target and internal reference genes. The results should be demonstrated using the  $2^{-\Delta\Delta\text{C}_q}$  method.

**Western blot analysis.** Cells were reacted with RIPA lysis solution on ice for 30 min and vibrated intensely for 1 min every 5 min. The sample was centrifuged at  $4^{\circ}\text{C}$  for 20 min at 1,500  $\times$  g. The supernatant liquid was the total proteins. The quantity of protein was calculated using the Bradford method. The kit was provided by Jiangsu Biyuntian Bioengineering Science and Technology Research Institute (Jiangsu, China). Then SDS-PAGE gel electrophoresis was performed, followed by 50 V of stacking gel and 100 V to separate 100 V gel for 3 h in total. Proteins were then transferred to nitrocellulose membranes. Sealing liquid (5% skimmed milk) was added to the membranes at 0.1 ml/cm<sup>2</sup>. The membranes were incubated at room temperature for 4 h after being sealed by a welding machine. After discarding the blocking liquid, diluted primary antibodies (1:500 APP, Akt, pAkt and 1:2,000  $\beta$ -actin) were added and the membranes were incubated at  $37^{\circ}\text{C}$  for 1 h. Rabbit polyclonal APP antibody (dilution, 1:500; cat. no. ab95195), rabbit polyclonal Akt antibody (dilution, 1:500; cat. no. ab8805) and rabbit monoclonal pAkt antibody (dilution, 1:500; cat.

Table I. Intervention result of different-dose IGF.

Groups	PRNP mRNA	APP protein
0	0.0243 $\pm$ 0.0055	0.08 $\pm$ 0.02
20 ng/ml	0.1156 $\pm$ 0.0322	0.16 $\pm$ 0.09
40 ng/ml	0.2478 $\pm$ 0.0483	0.33 $\pm$ 0.10
80 ng/ml	0.4316 $\pm$ 0.0695	0.75 $\pm$ 0.18
F-value	15.632	13.428
P-value	<0.001	<0.001

IGF, insulin growth factor.

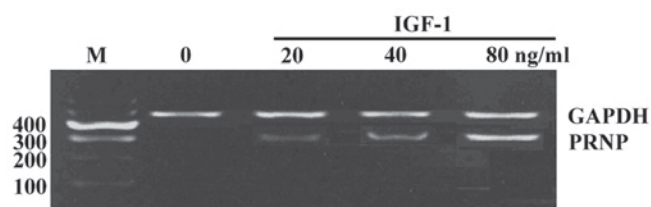


Figure 1. PRNP mRNA expression detected by RT-PCR in groups using different concentrations of IGF-1. IGF-1, insulin growth factor-1.

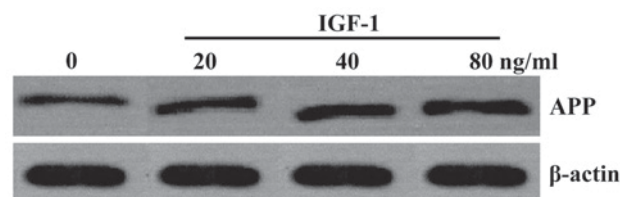


Figure 2. APP protein expression detected by western blot analysis in groups using different concentrations of IGF-1. IGF-1, insulin growth factor-1.

no. ab81283) were all purchased from Abcam (Cambridge, MA, USA). The membranes were washed with TBST three times for 10 min. The goat anti-rat secondary antibody (dilution, 1:2,000; cat. no. ab6721; Abcam) was added. Then the membrane was incubated in  $37^{\circ}\text{C}$  for 1 h. The membranes were further washed three times with TBST for 10 min. The result was revealed with ECL staining and scanned. Image analysis software IPP6.0 was used for gray analysis.

**Statistical analysis.** Data of the study were analyzed using SPSS19.0 software (Chicago, IL, USA). Quantitative data were presented as means  $\pm$  standard deviation. Comparison among groups was analyzed with single-factor ANOVA analysis.  $P < 0.05$  indicated that the difference was statistically significant.

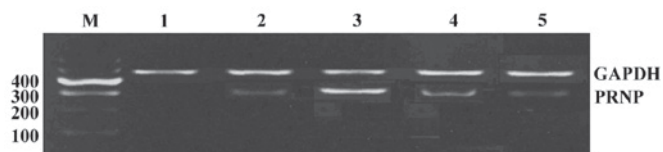
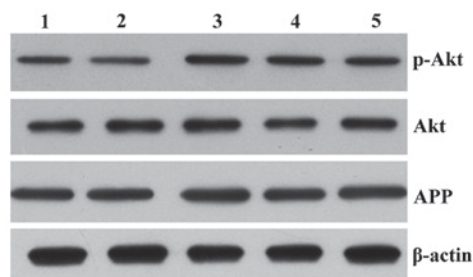
## Results

**Intervention of different-dose IGF.** As the dose of IGF-1 increased, the expression levels of PRNP mRNA and APP protein were highly expressed in a significant manner ( $P < 0.05$ ) (Table I, and Figs. 1 and 2).

Table II. Results of the intervention by PI3K inhibitor.

Groups	PRNP mRNA	APP protein	Akt protein	pAkt protein
Control	0.0056±0.0012	0.02±0.01	0.24±0.06	0.06±0.01
Model	0.0312±0.0078	0.07±0.02	0.22±0.05	0.15±0.04
IGF-1	0.4867±0.0152	0.72±0.13	0.23±0.07	0.88±0.16
IGF-1+LY 25 $\mu$ mol/l	0.2516±0.0123	0.53±0.10	0.29±0.10	0.63±0.13
IGF-1+LY 50 $\mu$ mol/l	0.1028±0.0257	0.39±0.08	0.27±0.12	0.42±0.11
F-value	19.654	16.527	2.306	20.325
P-value	<0.001	<0.001	0.548	<0.001

IGF, insulin growth factor.

Figure 3. PRNP mRNA expression of PC12 cells after different treatments detected by RT-PCR. Lane 1, control group; lane 2, model group; lane 3, IGF-1 group; lane 4, IGF-1+LY 25  $\mu$ mol/l group; and lane 5, IGF-1+LY 50  $\mu$ mol/l group. IGF-1, insulin growth factor-1.Figure 4. AKT and APP protein expression of PC12 cells after different treatments detected by western blot analysis. Lane 1, control group; lane 2, model group; lane 3, IGF-1 group; lane 4, IGF-1+LY 25  $\mu$ mol/l group; and lane 5, IGF-1+LY 50  $\mu$ mol/l group. IGF-1, insulin growth factor-1.

**Results of the intervention by PI3K inhibitor.** Regarding Akt protein, the levels of expression of PRNP mRNA, APP protein and pAkt protein in the IGF-1 groups were significantly higher than those in the control and model groups. With the increase in LY concentrations, the levels of expression of the three substances gradually decreased, which was statistically significantly ( $P<0.05$ ) (Table II, and Figs. 3 and 4).

## Discussion

*PRNP* gene is a single copy gene located at the short arm of chromosome 13 (5). In the 5' end activation domain, there are two SP1 transcription factors binding sites but no TATA boxes determining the starting site for transcription, which is a typical housekeeping gene structure. Such a gene is very conservative and there is little difference between species. The open reading frame of men's gene is included in a complete

exon. The gene of rat and sheep consists of 3 exons. The study on the 3' end gene shows different poly(A) areas in this domain for different categories, which can impact the speed of protein formation and further the incidence of disease (9). Prion protein genes are expressed in the central neuro-system of animals and peripheral tissues, but its expression features such as when and where it is expressed remain unclear. A study on gene rat demonstrated that the levels of expression of *PRNP* gene is positively correlated with the body's susceptibility to prion (10). Prion protein can function as membrane-anchored glycoprotein in the incidence of AD. It can function as the receptor of signal modular and induce the pathway inside of the cell, by combining with A $\beta$  oligomer *in vitro*, and further becomes toxic and contains neuroprotective effects by impacting A $\beta$  formation. In fact, due to the structural features of prion protein and the complexity of glycosylation modifications, it has multiple functions, two of which can coexist and exert a certain impact on the body (11).

IGF-1R is a member of the receptor tyrosine kinase family. Phosphorylated by IGF-1, it activates and stimulates the transcription of insulin receptor substrate series and other series of signals, including the MAPK and PI3K/PKB pathways (12). Adlerz *et al* found that IGF-1 stimulates  $\alpha$  secretase to lessen A $\beta$  formation (13). A previous result confirmed in PC12 cells by Zhang *et al* also found that IGF-1 treatment significantly decreased the  $\beta$  secretase (BACE-1) mRNA and protein levels, in order to reduce A $\beta$  formation, which was related to the PI3K/Akt and MAPK/ERK1/2 signaling pathways (14). The disorder of the signaling pathway was involved in the incidence of AD. Wang *et al* used A $\beta_{25-35}$  protein to induce the damaged PC12 cells to construct  $\tau$  protein over-phosphorylation cell model and carried out IGF-1 intervention (15). Those authors found that IGF-1 can hinder the apoptosis of PC12 cells and  $\tau$  protein phosphorylation, which was achieved through the PI3K/Akt signaling pathway. Our data showed that as the dose of IGF-1 increased, the expression of PRNP mRNA and APP protein were significantly increased. Regarding the Akt protein, the levels of expression of PRNP mRNA, APP protein and pAkt protein in the IGF-1 groups were significantly higher than those in the control and model groups. The increase in LY concentrations led to the expression of the three substances gradually decreasing, a difference that was statistically significant ( $P<0.05$ ). Altogether, the data suggest that IGF-1 may mediate the expression of *PRNP* gene

and APP protein through the PI3K/Akt signaling pathway, in the rat model.

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## References

1. De-Paula VJ, Radanovic M, Diniz BS and Forlenza OV: Alzheimer's disease. *Subcell Biochem* 65: 329-352, 2012.
2. DeMichele-Sweet MA and Sweet RA: Genetics of psychosis in Alzheimer disease. *Curr Genet Med Rep* 2: 30-38, 2014.
3. Cramer PE, Cirrito JR, Wesson DW, Lee CY, Karlo JC, Zinn AE, Casali BT, Restivo JL, Goebel WD, James MJ, *et al*: ApoE-directed therapeutics rapidly clear  $\beta$ -amyloid and reverse deficits in AD mouse models. *Science* 335: 1503-1506, 2012.
4. Perneczky R and Kurz A: Diagnosis and therapy of Alzheimer's disease: what's important for the family doctor?. *MMW Fortschr Med* 150: 42-45, quiz 46, 2008 (In German).
5. Bate C and Williams A: Amyloid- $\beta$ -induced synapse damage is mediated via cross-linkage of cellular prion proteins. *J Biol Chem* 286: 37955-37963, 2011.
6. Freude S, Schilbach K and Schubert M: The role of IGF-1 receptor and insulin receptor signaling for the pathogenesis of Alzheimer's disease: from model organisms to human disease. *Curr Alzheimer Res* 6: 213-223, 2009.
7. White MF: Regulating insulin signaling and beta-cell function through IRS proteins. *Can J Physiol Pharmacol* 84: 725-737, 2006.
8. Kim SJ, Winter K, Nian C, Tsuneoka M, Koda Y and McIntosh CH: Glucose-dependent insulintropic polypeptide (GIP) stimulation of pancreatic beta-cell survival is dependent upon phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB) signaling, inactivation of the forkhead transcription factor Foxo1, and down-regulation of bax expression. *J Biol Chem* 280: 22297-22307, 2005.
9. Laurén J, Gimbel DA, Nygaard HB, Gilbert JW and Strittmatter SM: Cellular prion protein mediates impairment of synaptic plasticity by amyloid-beta oligomers. *Nature* 457: 1128-1132, 2009.
10. Westergard L, Christensen HM and Harris DA: The cellular prion protein (PrP(C)): its physiological function and role in disease. *Biochim Biophys Acta* 1772: 629-644, 2007.
11. Kellett KA and Hooper NM: Prion protein and Alzheimer disease. *Prion* 3: 190-194, 2009.
12. Moloney AM, Griffin RJ, Timmons S, O'Connor R, Ravid R and O'Neill C: Defects in IGF-1 receptor, insulin receptor and IRS-1/2 in Alzheimer's disease indicate possible resistance to IGF-1 and insulin signalling. *Neurobiol Aging* 31: 224-243, 2010.
13. Adlerz L, Holback S, Multhaup G and Iverfeldt K: IGF-1-induced processing of the amyloid precursor protein family is mediated by different signaling pathways. *J Biol Chem* 282: 10203-10209, 2007.
14. Zhang H, Gao Y, Dai Z, Meng T, Tu S and Yan Y: IGF-1 reduces BACE-1 expression in PC12 cells via activation of PI3-K/Akt and MAPK/ERK1/2 signaling pathways. *Neurochem Res* 36: 49-57, 2011.
15. Wang PJ, Zhang Y, Song RR and Shen DF: Study of the protection and mechanism of IGF-1 on tau protein hyperphosphorylation in PC12 cells induced by Abeta(1-40). *Sichuan Da Xue Xue Bao Yi Xue Ban* 41: 960-964, 2010 (In Chinese).