Geniposide protection against AD\(\beta_{1-42}\) toxicity correlates with mTOR inhibition and enhancement of autophagy

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Overactivation of the PI3-K/Akt/mTOR signaling pathway and inhibition of autophagy in the brain are involved in Alzheimer’s disease. The present paper’s goal was to explore the potential mechanisms of geniposide to protect against Alzheimer’s disease. We treated the human neuroblastoma SH-SY5Y cell line with AD\(\beta_{1-42}\) as an Alzheimer’s disease in vitro model to explore the potential mechanisms of geniposide to protect against Alzheimer’s disease. Further, SH-SY5Y cells damaged by AD\(\beta_{1-42}\) were treated with geniposide. Akt/mTOR-related proteins and autophagy-associated proteins were measured to reveal the molecular mechanisms by which geniposide protects against AD\(\beta_{1-42}\)-induced toxicity. Results showed that Akt and mTOR’s geniposide inhibited phosphorylation induced by AD\(\beta_{1-42}\), enhanced expression of the LC3II/LC3I ratio, and Atg7 and Beclin1 expression and inhibited expression of p62 induced by AD\(\beta_{1-42}\). Our results lead us to hypothesize that inhibition of the Akt/mTOR signaling pathway and autophagy enhancement are fundamental molecular mechanisms for geniposide to protect against AD\(\beta\) toxicity.

Keywords
Geniposide; PI3K; Akt; mTOR; Autophagy; AD\(\beta\); Neurodegeneration; Alzheimer’s disease

1. Introduction

Aging is a pivotal factor for numerous diseases, including neurodegeneration, obesity, diabetes, cardiovascular diseases, and metabolic disorders [1, 2]. Growth factors, nutrients, and energy metabolism are pivotal factors for cell growth, development, and proliferation. Activation of the mechanistic target of rapamycin (mTOR) promotes cell growth in response to favorable environmental cues and is viewed as a master regulator of this response [3]. Many studies have shown that mTOR signaling dysregulation is involved in age-related diseases, including neurodegenerative diseases, diabetes, metabolic disorders, and cancer [4]. mTOR signaling networks stimulate the synthesis of nucleotides, proteins, and lipids and block autophagic catabolic response at the post-translational and transcriptional levels [5]. The PI3-K/Akt/mTOR signaling pathway is widely regarded as a central signaling axis to regulate cell growth and proliferation, crucial metabolism processes, apoptosis, and secretion [6]. Protein kinase B (PKB, also known as Akt) performs its action as a central intersection between phosphoinositide 3-kinase (PI3-K) and mTOR by phosphorylating various substrates. Considering its crucial role in regulating vital cellular functions, dysregulation of PI3-K/Akt/mTOR is a critical molecular event in mental illnesses [7]. Specifically, abnormalities in PI3-K/Akt/mTOR signaling are involved in Alzheimer’s disease (AD) [8]. Overactivation of PI3-K/Akt/mTOR signaling in the brain is regarded as an early pathogenic event in AD and an essential candidate for pathophysiological processes activated by AD\(\beta\)-amyloid [8]. Evidence gathered also indicates that insulin and IGF-1 can rescue and normalize the aberrant PI3-K/Akt/mTOR signaling and protect against AD’s physiopathologic processes [9].

Recent studies focused on the regulators of longevity and health span showed that strategies to delay aging are therapeutic strategies for aging-related diseases such as AD [10, 11]. mTOR inhibition [12] and autophagy enhancement [13] are regarded as crucial regulators of longevity and health span, as well as the novel therapeutic strategies for aging-associated diseases. mTOR functions as a nutrient sensor by regulating “protective” autophagy programs [14]. Interestingly, activation of the mTOR signaling pathway is related to AD [15]. The inhibition of mTOR is being developed into a novel AD therapy [16].

Autophagy is a critical molecular mechanism in mediating the lifespan-extending effects of dietary restriction and mTOR inhibition [17]. Autophagy is a normal cellular process in which the lysosome degrades older cytosolic components due to nutrient deprivation [18]. Many studies have
shown that damage due to autophagy occurs at the early stages of the AD process. Studies also showed that autophagy performs a pivotal role in the production and metabolism of Aβ and AD progress [19]. As the self-degrading process, autophagy is key in maintaining cellular homeostasis. Defects in autophagy homeostasis are considered pivotal pathogenesis in shortening lifespan and promoting multifarious aged-related diseases, including obesity, insulin resistance, diabetes, dementia, atherosclerosis, and neoplasm. Preclinical evidence supports autophagy modulators’ therapeutic promise to treat obesity and metabolic diseases [20]. Recent work has shown that glucagon-like peptide-1 (GLP-1)-based therapeutic approaches may positively affect autophagy in perivascular adipose tissue, thus improving obesity-related endothelial dysfunction [21]. To explore the effects of GLP-1 in GLP-1/insulin/insulin-like growth factor-1 (IGF-1) signaling pathway and the autophagic process, Candeias et al. [22] evaluated the effect of GLP-1 GLP-1 mimetics, exendin-4 (Ex-4) on insulin, and IGF-1, their downstream signaling and autophagic markers in brain of the T2D rats [22]. The results showed that Ex-4 protects T2D rats against hyperglycemia; insulin resistance enhances GLP-1 and IGF-1 levels in brain cortical and subsequent signaling pathways. Ex-4 also regulated autophagy markers (as mTOR, PI3K class III, LC3 II, Atg7, p62, LAMP-1, and Parkin).

Geniposide is a traditional Chinese medicine monomer isolated from the herb Gardenia jasminoides. Its extensive pharmacological effects, including anti-diabetes, anti-inflammation, antioxidation, neuroprotection, and anti-asthma, have been noted [23]. The protective effects of geniposide in neurodegenerative diseases have been of keen interest. A glucagon-like peptide-1 receptor (GLP-1R)-the dependent mechanism-protected geniposide [24, 25]. Further, activation of PI3K/AKT signaling may also involve a geniposide-induced protective effect [26]. Li et al. [27] showed that although geniposide was a useful bioactive substance in treating AD, its toxicity was apparent at a dose higher than 50 mg/kg/d. Dinda et al. [28] reviewed the therapeutic potential of plant iridoids, including geniposide, in AD and Parkinson’s disease. Plant iridoids exhibit the property of retarding the process of neurodegeneration in AD and Parkinson’s disease. Geniposide performed its protective effects after passing the blood-brain barrier [29]. Plant iridoids, including geniposide, can ameliorate AD by increasing the expression of PPAR-γ, and α-secretase, insulin-degrading enzyme, nephrilysin, and decreasing the levels of Aβ oligomers (Aβ42) deposited in brain neurons. The molecular mechanism has been extensively explored. It is suggested that plant iridoids, including geniposide, may: 1. Decrease expression of GSK-3β and its receptor gene; 2. Improve the lysosomal autophagy process by increasing the expression of LC3II, Beclin-1, and cathepsin B genes for the clearance of Aβ and neurofibrillary tangles (NFT); 3. Enhanced expression of transporter proteins, such as P-glycoprotein and low-density lipoprotein receptor-related protein-1, for the clearance of Aβ load from brain across the blood-brain barrier; 4. Enhanced expression of PPAR-γ and ApoE proteins for clearance of Aβ in ApoE mediated pathway from the brain. Further, plant iridoids may decrease cognitive impairment by enhancing the expression of synaptic proteins, such as SNAP-25, BDNF, PSD-95, GAP-43 and SYP, to improve learning memory ability in AD. Some of those plant iridoids, including geniposide, may improve the expression of TH-positive neurons, GDNF, and Bcl-2 proteins by increasing the levels of antioxidant enzymes, such as GSH-PX and SOD, and down-regulate insulin/IGF signaling by activating MEK. Furthermore, geniposide may enhance the expression of autophagy-related LAMP-2A protein for clearance of LB from dopaminergic neurons in the PD brain via improving the lysosomal autophagy process.

Song et al. [30] pretreated differentiated SH-SY5Y cells or primary hippocampal neurons with Schizandrol A and subsequently subjected the cells to β-amyloid peptides of 1-42 amino acids (Aβ1-42) and estimated the effect of Schizandrol A by testing its effects on cell viability, apoptosis, oxidative stress, and autophagy. Further, these investigators explored the molecular mechanism underlying this effect by treating cells with an mTOR inhibitor (rapamycin) and a PI3K inhibitor (LY294002) to analyze the role of the PI3K/AKT/mTOR pathway. Their results showed that Schizandrol A effectively inhibited Aβ1-42-triggered increases in apoptotic cell number and pro-apoptotic protein expression, reduction of viable cells, as well as alterations in markers of oxidative stress. Also, Schizandrol A enhanced LC3-II/LC3-I and Beclin-1 and reduced the expression of p62. At the molecular level, they showed Schizandrol A rescued the PI3K/AKT/mTOR-autophagy pathway dysregulation resulting from Aβ1-42 exposure.

Based on the overlapping functions between GLP-1 and mTOR inhibition, including energy balance, AD protection and diabetes treatment, we hypothesized in an earlier study that mTOR inhibition may mediate the protective effect of GLP-1 in AD [31]. Similarly, Jiang et al. [32] explored molecular mechanisms underlying the effect of GLP-1 to improve insulin signaling in ER-stressed adipocytes. These investigators showed GLP-1 directly modulated ER stress response, in part, by inhibiting the mTOR signaling pathway. Further, a study from our group showed that the downregulation of mTOR signaling and enhancement of autophagy in APP/PS1 mice mediated the effect of geniposide to protect against amyloid deposition and behavioral impairment [33].

In this paper, we test the hypothesis that mTOR inhibition and autophagic activity are key molecular events that control the protective effects of geniposide against Aβ in vitro.

2. Materials and methods
2.1 Chemicals and reagents

The SH-SYSY cell line’s human neuroblastoma was obtained from the Stem Cell Bank, Chinese Academy of Sciences. Geniposide (purity ≥ 98%) was purchased from

Volume 20, Number 1, 2021
Aladdin Bio-Chem Technology Company, LTD, Shanghai, PR China. Aβ1-42 (CAT: 1932-2-15, Peptide Sequence: Asp-Ala-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Ala-Glu-Asp-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala) was purchased from Qiangyao Biotechnology Company. Anti-NCiii antibody (CAT: L8918) was purchased from Sigma, USA. Anti-mTOR antibody (CAT: ab134903), anti-p-mTOR (Ser2448) antibody (CAT: ab109268), anti-Akt (Ser473) antibody (CAT: ab81283), anti-Beclin1 antibody (CAT: ab210498), anti-Akt antibody (CAT: ab238477), anti-Atg7 antibody (CAT: ab133528), anti-Bcl2 antibody (CAT: ab210498), and anti-P62 antibody (CAT: ab210498) were purchased from Abcam, UK. Anti-Bax antibody (CAT: BS6420), anti-Bcl2 antibody (CAT: BS70205), and anti-LC3II antibody (CAT: L8918) were purchased from Proteintech Group, Inc., Shanghai, PR China. Anti-Beclin1 antibody (CAT: bs13528) was purchased from Qiangyao Biotechnology Co., LTD, Shanghai, PR China. Anti-LC3II antibody (CAT: L8918) was purchased from Qiangyao Biotechnology Co., LTD, Shanghai, PR China. Aβ1-42 was prepared. Neurons were grouped into control; Aβ1-42, Aβ1-42 and geniposide, Aβ1-42 + geniposide separately. Anti-β-actin antibody and Anti-β-actin IgG were purchased from Bioworld Technology Company, Shanghai, PR China. Fetal bovine serum was purchased from Cellmax technology Bioworld Technology Company, Shanghai, PR China. 2.2 Cell culture

SH-SY5Y cells (ATCC CRL-2266, Shanghai, PR China) were cultured in DMEM/F-12 medium containing streptomycin (100 μg/mL), penicillin (100 U/mL), and 10% heat-inactivated fetal bovine serum at 37 °C in a humidified incubator based on 5% CO2 and 95% air. Aβ1-42 was dissolved in 100% 1, 1, 1, 3, 3-hexafluoro-2-propanol (HFIP) to a concentration of 1 mg/mL. This solution was incubated at 37 °C for 12 min at RT and then pipetted and stored at -80 °C. The HFIP/Aβ1-42 solution was subsequently dried down in a gentle stream of nitrogen, and the dried Aβ1-42 was resuspended in 1 mM DMSO. The preparation was incubated for 12 min at RT and then pipetted and stored at -80 °C. Before use, the preparation was rapidly thawed, utilizing 0.1 M PBS, and a final Aβ1-42 concentration of 20 μM was prepared. Neurons were grouped into control; Aβ1-42 treatment, the only treatment of geniposide, and Aβ1-42 + geniposide treatment.

2.3 Cell viability (MTT) assay

The viability of SH-SY5Y cells was measured utilizing a 3, (4, 5-dimethylthiazol-2-yl) 2, 5-diphenyltetrazolium bromide (MTT) assay. Before analysis, SH-SY5Y cells were seeded into 96-well dishes, and the cell density was adjusted to 5,000 cells/well and incubated for 24 h before treatment. For selecting an appropriate concentration of Aβ1-42, the cells were treated with different concentrations of Aβ1-42 (0, 5, 10, 20, 40 μM). Apparent cytotoxicity was seen in cells treated by 20, 40 μM Aβ1-42 and the concentration of 20 μM Aβ1-42 was selected to conduct our study. Where indicated, cells treated with 20 μM Aβ1-42 were also treated with different concentrations of geniposide (0, 5, 10, 20, 40 μM).

SH-SY5Y cells in various treatment groups (Aβ1-42 only, geniposide only, and Aβ1-42 and geniposide) were treated 24 h. After this, MTT was added to the culture media (0.5 mg/mL final concentration) and incubated for 4 h at 37 °C in a CO2 incubator. The culture medium was mixed with extraction buffer, and then absorbance was measured at 490 nm after an overnight incubation utilizing a microplate absorbance reader (Bio-Rad Instruments). Untreated cells were used as controls, and cell viability was calculated using the formula:

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\text{Cell viability} = \frac{A_{\text{sample}}}{A_{\text{control}}} 
\]

where A is absorbance.

2.4 Western blot

SH-SY5Y cells were lysed with RIPA protein lysis buffer containing 1 mM PMSF (Beiyotime Biotechnology, Shanghai, PR China) for 30 minutes after washing with cold PBS. Total proteins in the supernatant were quantified using a BCA protein assay (Beiyotime Biotechnology, Shanghai, PR China) after centrifugation of the cell lysate at 12000 r/min for 20 min at 4 °C. Proteins were subsequently resolved in 10% sodium dodecyl sulfate-polyacrylamide (SDS-PAGE) gels (Beiyotime Biotechnology, Shanghai, PR China) and transferred to polyvinylidene differuoride membranes (Beiyotime Biotechnology, Shanghai, PR China). Membranes were incubated in 5% BSA (TBST) for 2 h at room temperature and after that were incubated with primary antibodies against Anti-NCiii, mTOR, p-mTOR, Akt, p-Akt, Atg7, Beclin1, and P62 overnight at 4 °C. Membranes were subsequently washed and treated with horseradish peroxidase-conjugated secondary antibody (1 : 5000) for 2 h at room temperature. Proteins were visualized utilizing an enhanced chemiluminescence method, and β-actin was used as a loading control.

The protein bands were visualized using the Chemi-Doc XRS + imaging system (Bio-Rad). The Western blots were subjected to quantification of the protein band density using the Image Pro.

2.5 Statistical analyses

The results were expressed as mean ± SD. A one-way ANOVA analysis was used to determine statistical significance. The contrast between multiple groups was performed by one-way ANOVA based on SPSS 19.0 software, and the differences observed were further analyzed by the least significant difference (LSD)-t-test. A P-value of less than 0.05 was considered statistically significant.

3. Results

3.1 Geniposide reverses loss of cell viability induced by Aβ1-42 in SH-SY5Y cells

To investigate the effect of Aβ1-42 on SH-SY5Y cells, an MTT assay was conducted to quantify cell viability. Results indicated a concentration-dependent effect of Aβ1-42 on cell viability (Fig. 1A). Lower doses of Aβ1-42 (5 and 10 μM) did not affect cell viability, whereas higher concentrations of Aβ1-42 (20 and 40 μM) had measurable effects on cell viability. Aβ1-42 (20 μM) treatment significantly decreased the cell viability to 61.8 ± 4.1% versus control (100%).
Based on these findings, 20 μM concentrations of Aβ1–42 were selected for further study. Treatment of SH-SY5Y cells with various concentrations of geniposide did not affect the cells’ viability versus untreated controls (Fig. 1B). However, a concentration-dependent relationship of geniposide to protect against lost cell viability following Aβ concentration-dependent relationship of geniposide to protect against lost cell viability following Aβ concentration-dependent relationship of geniposide to protect against lost cell viability following Aβ concentration-dependent relationship of geniposide to protect against lost cell viability following Aβ concentration-dependent relationship of geniposide to protect against lost cell viability following Aβ treatment of geniposide and control. Cell treated with 20 μM Aβ1–42 for 24 hours. Cell viability was measured utilizing an MTT assay. Values were denoted as mean ± SD. ***P < 0.001, *P < 0.05 vs. control. *P < 0.05, **P < 0.01, ***P < 0.001 vs. Aβ1–42 treatment.

3.2 Geniposide protects against Aβ1–42 by downregulating mTOR signaling

mTOR signaling was upregulated in the SH-SY5Y cells treated by Aβ1–42. Phospho-AKT (Ser473)/AKT ratio increased from 0.370 ± 0.087 in control to 0.748 ± 0.131 in SH-SY5Y cells treated by Aβ1–42 (Fig. 2A), and the phospho-mTOR (Ser2448)/mTOR ratio increased from 0.476 ± 0.076 in control to 0.907 ± 0.160 in SH-SY5Y cells treated with Aβ1–42 (Fig. 2B).

Treatment of SH-SY5Y cells with geniposide only did not influence mTOR signaling as the phospho-AKT (Ser473)/AKT, and phospho-mTOR (Ser2448)/mTOR ratios were restored to near control levels with geniposide, indicating that treatment of SH-SY5Y cells by geniposide only did not influence autophagy-related signaling. Geniposide did reverse the inhibition of autophagy induced by Aβ1–42. Specifically, geniposide treatment increased the level of LC3-II/LC3-I ratio to 0.317 ± 0.066 (Fig. 3A), Beclin1 expression to 0.310 ± 0.075 (Fig. 3B), and Atg7 to 0.705 ± 0.247 (Fig. 3D). Similarly, geniposide treatment decreased the expression of p62 to 0.506 ± 0.155 (Fig. 3C).

3.4 Geniposide protects against Aβ1–42 by inhibiting Apoptosis

Apoptosis was activated in the SH-SY5Y cells following treatment with Aβ1–42. The Bax/Bcl-2 ratio was increased after a 24 hours treatment with Aβ1–42 (1.864 ± 0.333) versus control (0.391 ± 0.194) (Fig. 4). However, geniposide alone did not influence the Bax/Bcl-2 ratio in SH-SY5Y (0.421 ± 0.140) cells treated with only geniposide. In contrast, geniposide blunted apoptosis activation induced by Aβ1–42 as the Bax/Bcl-2 ratio fell dramatically to 0.499 ± 0.185 in SH-SY5Y cells treated with geniposide and Aβ1–42 (Fig. 4).

In sum, data gathered during this study provides evidence that geniposide can protect against the toxic effects of Aβ1–42 by inhibiting mTOR. Evidence supporting this conclusion comes from the observations that phospho-AKT (Ser473)/AKT and phospho-mTOR (Ser2448)/mTOR ratios were restored to near control levels with geniposide, and geniposide enhanced autophagy by increasing the LC3-II/LC3-I ratio, increasing expression of Beclin 1, Atg7, and inhibiting expression of p62. Finally, we observed that geniposide blunted the apoptotic response to Aβ1–42, as evidenced by measuring the Bax/Bcl-2 ratio.
Fig. 2. Changes in mTOR signaling in SH-SY5Y cells treated with Aβ$_{1-42}$ and geniposide. Western blot analysis was conducted to measure the phospho-AKT (Ser473)/AKT ratio, phospho-mTOR (Ser2448)/mTOR ratios in treated SH-SY5Y cells. Geniposide inhibited increases in phospho-AKT (Ser473)/AKT ratio and phospho-mTOR (Ser2448)/mTOR ratios induced by Aβ$_{1-42}$. β-actin was used as an internal control. All results are presented as the mean ± SD (n = 6). *P < 0.05, **P < 0.001 vs. control. ##P < 0.01, ###P < 0.001 vs. Aβ$_{1-42}$ treatment.

4. Discussion

A prior study showed that geniposide-mediated protection against pathological hallmarks of AD and behavioral impairment correlates with downregulation of mTOR signaling and enhanced autophagy in APP/PS1 double transgenic mice [33, 34]. In the present study, we sought to determine the mechanism by which geniposide prevents Aβ-associated toxicity. Considering that geniposide can activate the glucagon-like-1 receptor (GLP-1R) and adenylyl cyclase (AC)/cAMP signaling pathways and promotes insulin secretion and inhibition of protein kinase A (PKA) [35], we hypothesized that geniposide prevents Aβ toxicity by inhibiting the PI3-K/Akt/mTOR signaling pathway, and enhances the autophagy as an agonist of the GLP-1 receptor. Our results showed that geniposide protected SH-SY5Y cells against lost cell viability induced by Aβ$_{1-42}$. Furthermore, we showed mTOR signaling was upregulated in the SH-SY5Y cells treated by Aβ$_{1-42}$. phospho-AKT (Ser473)/AKT ratio and the phospho-mTOR (Ser2448)/mTOR ratio increased in SH-SY5Y cells treated with Aβ$_{1-42}$. Geniposide reversed mTOR signaling upregulation induced by Aβ$_{1-42}$. The phospho-AKT (Ser473)/AKT and phospho-mTOR (Ser2448)/mTOR ratios were reversed after geniposide treatment. This finding implies that inhibition of the PI3-K/Akt/mTOR pathway may be a pivotal molecular event controlling geniposide’s ability to prevent the toxic effects of Aβ.

Autophagy is a primary physiologic function for clearing abnormal proteins within mammalian cells and contributes to protein homeostasis and neuronal health. An autophagy deficit is found in early AD pathogenesis, and autophagy plays a critical role in the formation and metabolism of Aβ [31]. In the present study, we assessed autophagy by measuring the LC3-II/LC3-1 ratio, as well as Atg7, p62, and Beclin1 expression utilizing western blotting in SH-SY5Y cell lines treated with Aβ$_{1-42}$. Our results showed that geniposide protected against the cellular damage induced by Aβ$_{1-42}$ in SH-SY5Y cells. Further, we showed that geniposide reversed the LC3-II/LC3-1 ratio and repression of Atg7 and Beclin1 induced by Aβ$_{1-42}$ and reversed the expression of p62 enhanced by Aβ$_{1-42}$ in SH-SY5Y cells. The cytosolic form of LC3-1 is converted to the phosphatidylethanolamine-conjugated form (LC3-II) and binds to autophagosomes’ membranes [36]. Thus, the LC3-II/LC3-1 ratio is an often-used marker for autophagy in various tissues, including the brain [37]. We observed a decrease of the LC3-II/LC3-1 ratio after treatment of SH-SY5Y cells with Aβ$_{1-42}$, which suggests that Aβ damages the brain by, in part, inhibiting autophagy. The ratio was reversed after the treatment by geniposide, indicating that geniposide protects against AD by enhancing autophagy. Atg7 is an E1-like activating enzyme that is down-regulated during aging [38] and is needed for the autophagic conjugation system and formation of autophagosomes [39]. Sim-
Fig. 3. Changes in autophagy-related proteins in SH-SY5Y cells. Western blot analysis of the LC3-II/LC3-I ratio, Beclin1, Atg7, and p62 expression was quantified by western blot. Geniposide increased the LC3-II/LC3-I ratio and Beclin1 and Atg7 expression and decreased the expression of p62 induced by Aβ1–42. β-actin was used as an internal control. All results are presented as the mean ± SD (n = 6). * P < 0.05, ** P < 0.01, *** P < 0.001 vs. control. *P < 0.05, **P < 0.01, ***P < 0.001 vs. Aβ1–42 treatment.

Fig. 4. Changes in apoptosis-associated proteins in SH-SY5Y cells. Quantitative western blot analyses of Bax and Bcl-2 expression were conducted. β-actin was used as an internal control. All results are presented as the mean ± SD (n = 6). ***P < 0.001 vs. control. ***P < 0.001 vs Aβ1–42 treatment.
Fig. 5. Molecular mechanism for geniposide protection. Geniposide performs its protection against Aβ_{1–42} toxicity by inhibiting mTOR and enhancing autophagy. Geniposide reverses increase of AKT and mTOR induced by Aβ_{1–42}. Geniposide reverses a decrease in the LC3-II/LC3-I ratio, decreases expression of Beclin 1 and Atg, and increases expression of p62 induced by Aβ_{1–42}. Geniposide also blunts the Aβ_{1–42}-induced apoptotic response by reducing the Bax/Bcl-2 ratio.

In summary, we speculate that mTOR inhibition and enhancement of autophagy induced by mTOR inhibition may be a critical molecular event in geniposide mitigating Aβ-induced toxicity.

Abbreviations

Aβ, β-amyloid; Aβ_{0}, Aβ oligomers; AC, adenyl cyclase; AD, Alzheimer’s disease; Atg7, autophagy-related gene 7; Ex-4, exendin-4; GLP-1, glucagon-like peptide-1; GLP-1R, glucogen-like peptide-1 receptor; HFIP, 1, 1, 1, 3, 3‐hexafluoro‐2‐propanol; IGF-1, insulin-like growth factor-1; mTOR, mechanistic target of rapamycin; NFT, neurofibrillary tangles; P13K, phosphoinositide 3-kinase; PKA, protein kinase A; PKB (also known as Akt), Protein kinase B; RT, room temperature.

Author contributions

Dong-Xing Liu, Yan-fang Chang were involved in the design and execution of the experimental job and the statistical analysis of the data. Di Zhang and Wei-min Hu contributed to the statistical analysis of the data and manuscript writing. Xiao-hui Wang and Lin Li were involved in the design and execution of the study. All authors contributed to the development of the manuscript and reviewed and approved the final version of the manuscript.
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