Protective mechanism of gold nanoparticles on human neural stem cells injured by β-amyloid protein through miR-21-5p/SOCS6 pathway

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disorder with progressive memory loss in dementia. Gold nanoparticles (AuNPs) were reported beneficial for human neural stem cells (hNSCs) treated with Amyloid-beta (Aβ), but the neuroprotective mechanisms still are unknown. Cell Counting Kit-8 (CCK-8) was performed to detect hNSCs viability. The content of interleukin 6 (IL-6) and tumour necrosis factor-alpha (TNF-α) was analyzed by enzyme-linked immunosorbent (ELISA) assay. Immunocytochemistry was carried out to determinate Tuj-1 and glial fibrillary acidic protein (GFAP). The reactive oxygen species (ROS) and JC-1 assay kits were performed to detect ROS generation and mitochondrial membrane potential. miRNA array was used to systematically detect the differential miRNAs. Dual-luciferase reporter assay was applied to verify the targeting relationship between miR-21-5p and the suppressor of cytokine signalling 6(SOCS6). Quantitative PCR (qPCR) and Western blot assessments were also used to detect related gene expression intracellularly or in the supernatant. The pro-inflammation IL-6 and TNF-α were significantly decreased in the AuNPs co-treatment group. AuNPs could ameliorate neural stem cell differentiation inhibition due to Aβ accumulation. AuNPs co-treatment repressed the high expression of total tau (T-tau), phosphorylated tau (P-tau), and Aβ protein caused by the Aβ treatment. The apoptosis rate of hNSCs induced by Aβ was inhibited by AuNPs co-treatment and the expression of proteins associated with apoptosis was also reduced in AuNPs co-treatment group. Aβ-induced decreased mitochondrial membrane potential and mitochondria in the hNSCs were damaged, while AuNPs co-treatment showed a protective effect on mitochondrial membrane potential. Co-treatment with AuNPs significantly increased dynamin-related protein 1 (DRP1), nuclear respiratory factor 1 (NRF1), and mitochondrial transcription factor A (TFAM) mRNA levels. AuNPs may improve mitochondrial function impairment due to Aβ by elevating mitochondrial membrane potential, upregulating regulators of mitochondrial biogenesis, and inhibiting ROS production. hNSCs transfected with miR-21-5p inhibitor reversed AuNPs mediated cytoprotection induced by Aβ. miR-21-5p was involved in AuNPs protecting against Aβ-induced cell toxicity by reduced mitochondrial function. Overexpression of miR-21-5p contributes to enhancing the effect of cytoprotection of AuNPs. MiR-21-5p direct targeting SOCS6 and overexpression SOCS6 exerted opposite effects on hNSCs compared with miR-21-5p mimic. AuNPs can protect hNSCs from Aβ injury and decrease mitochondrial damage by regulating the miR-21-5p/SOCS6 pathway.

Introduction

AD is a chronic progressive neurodegenerative disease. Pathogenesis is related to its specific factors, genetic conditions, and environmental influences (Zamolodchikov et al. 2022). Aβ peptide is produced predominantly in endosomes and its release from neurons is modulated by synaptic activity, presynaptically and postsynaptically (Verges et al. 2011). Aβ accumulation may play an important role in beginning the AD pathological process (Long and Holtzman 2019). T-tau, P-tau, and the 42-amino acid form of Aβ are the most frequently used as AD-related biomarkers (Oyarzún et al. 2021). At present, drug development based on the Aβ hypothesis still showed big challenges, which may be attributed to the

following two reasons: one is that the intervening time may be too late, as the Aβ plaque has already formed and the toxic form of Aβ has already caused nerve damage at that time; the other is that abundant soluble Aβ oligomers existed before the deposition of Aβ fibrils is increasingly believed as the most toxic form of Aβ (Guan et al. 2021; Wen and Shen 2021).

AuNPs are nanocarriers for intracellular drug or gene delivery. They have the characteristics of low toxicity, large specific surface area, easy controllable surface assembly, and high specificity (Chatterjee et al. 2021). Anand et al. (Anand et al. 2021) found that AuNPs surface-functionalized with a plant-based amino acid mimosine can attenuate Aβ fibrillization and neuronal toxicity. AuNPs were labelled to study AD and other neurodegenerative, neurovascular, and other neuropsychiatric diseases (Perets et al. 2019). Javed et al. (Javed et al. 2019) reported that casein-coated-gold nanoparticles can cross the blood-brain barrier of zebrafish larvae and elicit toxicity of Aβ42. So, this study investigated the mechanism of AuNPs in eliminating Aβ toxicity to provide a reference for Alzheimer's disease treatment and diagnosis.

MicroRNAs (miRNAs) are short non-coding RNAs involved in regulating gene expression and changes in their expression have been associated with plenty of human diseases (Laffont and Rayner 2017). MiRNAs have been investigated as potential biomarkers for AD diagnosis, prognosis, and therapeutic for their involvement in multiple brain signalling pathways (Zhao et al. 2020). MiR-21 has been shown to regulate microglia/astrocytes activation and link to neuroinflammatory signalling (Liang and Wang 2021). Li et al. (Li et al. 2019) showed that miR-21-5p in neuronal exosomes was increased and inhibited neuronal autophagy by targeting RAB11A. miRNAs modulation acts as a key part of AD development, which has been identified across many studies. Moreover, Dong et al. (Dong et al. 2022) investigated that AuNPs-horseradish peroxidase as signal amplification probes showed great potential in clinical analysis and disease diagnosis based on miRNA. Therefore, in this study, we investigated the mechanism of AuNPs treatment in alleviating Aβ toxicity to hNSCs to provide new biological targets and protocols for AD diagnosis and treatment.

Materials And Methods

Cell Culture and Transfection

StemProÒ human neural stem cells (hNSCs) (H9-derived) were purchased from ThermoFisher (USA). The medium complete was used for the optimal growth and expansion of hNSCs and to maintain the undifferentiated state of hNSCs as previously described (Mcc et al. 2021). The cells were divided into four groups, blank control, Aβ group with 5 μM Aβ1-42 (cat: P2000022, PLlabs, Canada) treatment for 24 h, AuNPs (Yaphank, USA) group with 10 ppm AuNPs treatment for 24 h, 10 ppm AuNPs were added into Aβ group for another 24 h. hNSCs were transfected with miR-21-5p inhibitor, inhibitor-NC, miR-21-5p mimic and mimic-NC using Lipofectamine 2000 (cat: no. 11668019, Invitrogen, USA). inhibitor-NC, inhibitor, miR-21-5p mimic and mimic-NC were purchased from Sangon Biotech (Shanghai). The sequence of NC and inhibitor were as follows: miR-21-5p inhibitor, 5’-UCAACAUCAGUCUGAUAAGCUA-3’; inhibitor negative

control, 5’-CAGUACUUUUGUGUAGUACAAA-3’; miR-21-5p mimic, 5’-AACAUCAGUCUGAUAAGCUAUU-3’; mimic-NC, 5’-UUGUACUACACAAAAGUACUG-3’.

CCK-8 Assay

CCK-8 kit (cat: AR1160, Boster, China) was used to detect cell viability. According to the manufacturers’ instructions, 20 μL CCK-8 solution was added to the incubation medium for 1 h and the absorbance was measured at 450 nm.

Flow Cytometry

Apoptosis was measured by flow cytometry in glioma cells. hNSCs were fixed with absolute ethanol overnight at 4 °C. Then, the cells were incubated with the propidium iodide (PI, cat: 40711ES10, Yeasen, China) for 0.5 h. Annexin-VFITC/PI Apoptosis Detection Kit (cat: KA3805, Abnova, USA) was employed to analyze apoptosis.

Immunocytochemically Staining

The immune-cyto staining was performed as described previously (Mehazri et al. 2021). The cultures were maintained for 7 days with medium changes every 72 h. Cultures were analyzed immunocytochemically for the class III beta-tubulin (Tuj-1) and GFAP.

Microarray-based Gene Expression Profiling

The total RNA was extracted using Trizol RNA extraction reagent (cat.no.by15596018, Invitrogen, USA). RNA integrity was checked by an Agilent Bioanalyzer 2100 system (Agilent Technologies, USA). After qualified total RNA and the microarray experiment was performed as described previously (Bishop et al. 2017).

ELISA

IL-6 and TNF-α levels in hNSCs were detected using ELISA Kits. Levels of IL-6 and TNF-α in culture supernatants were then assayed by enzyme-linked immunosorbent assay (IL-6, cat.no.BMS213-2, TNF-α, cat. no.88-7324, Invitrogen) according to the manufacturer’s instructions.

JC-1 Assay

JC-1 mitochondrial membrane potential detection kit (Beyotime, USA) was used to evaluate the mitochondrial membrane potential. For this purpose, hNSCs were incubated with JC-1 staining solution in 24-well plates for 20 min at 37 ℃. Nikon Ti-S fluorescence microscope was used to observe cells' fluorescence.

ROS Measure

The ROS assay kit (Beyotime, China) was used to detect intracellular ROS. All operations were carried out strictly by the kit instructions.

Dual-luciferase Reporter Assay

The SOCS6 3'UTR wild-type (WT) plasmids and the mutant-type (MUT) plasmids were constructed and were transfected with miR-21-5p mimic and mimic NC into hNSCs. The activities of firefly luciferase were measured using the Dual-Luciferase Reporter Assay System (Promega) after 48 h.

Western Blot

The total protein of the cells was extracted using sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (SDS-PAGE). And 5% skimmed milk powder was used to seal the membrane for 2 h, the membrane was incubated overnight at 4 °C with an antibody for the corresponding gene. Antibodies against T-Tau, P-Tau, Aβ, caspase-3, caspase-9, Bax, Bcl-2 and SOCS6 were purchased from Proteintech (USA). Using the Rio-Rad gel imaging analysis system, the grey band value was calculated using β-actin as the internal reference.

qPCR

RNA was extracted from cells using an RNA extraction kit (cat: FAVRE 002, Seebio, China). A total of 40 cycles, including 95 °C for 30 s, 95 °C for 5 s, 60 °C for 34 s, 70 °C for 10 s, and 95 °C for 15 s, were performed. A blank control and an internal reference (β-actin) control were used for comparison. After the reaction, the gene expression level was analyzed. The primers used are summarized in Table 1:

Table 1

Statistical Analysis

Experimental data were analyzed using SPSS 22.0, the measurement data were expressed as `x ± s, a *t*-test was used for comparison between two groups, and one-way analysis of variance was used for comparison between multiple groups. A significance level set at P 0.05 was considered significant for all the tests.

Results

Nanogold Promoted Cell Viability and Inhibited Inflammatory Response in Aβ-Treated hNSCs

Cell viability was analyzed by the CCK-8 assay and the results found that Aβ inhibited hNSCs viability and co-treatment with AuNPs significantly enhanced the activity of hNSCs. Proliferation did not significantly increase over time and we optimized 24 h as the treatment time in subsequent experiments (Fig. 1a). The concentrations of IL-6 and TNF-α were measured by ELISA kit and the level of IL-6 and TNF-α remarkably increased after treatment with Aβ. This pro-inflammation IL-6 and TNF-α were significantly decreased in the AuNPs co-treatment group (Fig. 1b,c).

Tuj-1 is the neural marker and GFAP is the glial marker. Immunohistochemistry was performed to analyze the expression of Tuj-1 and GFAP in hNSCs. AuNPs increased the decrease in Tuj-1 and GFAP expression induced by Aβ in the hNSCs and increased the number of Tuj-1 and GFAP-positive cells (Fig. 1d-f). The results suggested that AuNPs could ameliorate neural stem cell differentiation inhibition due to Aβ accumulation.

The level of T-Tau, P-Tau, and Aβ could reflect the tau and Aβ deposition in cells to some extent; moreover, Aβ affects Tau kinase activity or localization resulting in tau and Aβ toxicity in neurons (Kim et al. 2018).

The protein expression of T-Tau, P-Tau, and Aβ was measured by Western blot and the results showed that AuNPs co-treatment represses the high expression of these proteins caused by Aβ (Fig. 1g-j). These data suggested that AuNPs can improve the cytotoxicity induced by Aβ accumulation.

Fig. 1.

AuNPs Inhibited hNSCs Apoptosis and Improved Mitochondrial Function Induced by Aβ Treatment

Apoptosis analysis of hNSCs was determined by flow cytometry and the results found that Aβ treatment led to an apoptosis rate significantly increased, while AuNPs co-treatment with AuNPs reduced the rate of apoptosis in hNSCs (Fig. 2a and a).

Further analysis was conducted by qPCR and Western blot to evaluate the mRNA and protein expression of apoptosis markers including Bax, Bcl-2, caspase-3, and caspase-9 in hNSCs. It turned out to be the mRNA and protein expression of Bax, caspase-3 and caspase-9 showed significant increment in Aβ treatment, while these pro-apoptosis factors were down-regulated in conjunction with AuNPs treatment. The change of anti-apoptotic factors Bcl-2 showed the opposite trend in both Aβ and AuNPs treatment in hNSCs (Fig. 2c-k). These results indicated that apoptosis of hNSCs induced by Aβ was inhibited with AuNPs co-treatment and expression of proteins associated with apoptosis was also reduced in AuNPs co-treatment group.

Mitochondrial membrane potential was assessed by JC-1 dye (Cen et al. 2020). Aβ treatment decreased green fluorescence of JC-1 monomeric, which means lower mitochondrial membrane potential and higher mitochondrial membrane potential were observed in the AuNPs co-treatment group (Fig. 2l). These data suggested that Aβ induced decreased mitochondrial membrane potential and mitochondria in the hNSCs were damaged, while AuNPs co-treatment showed a protective effect on mitochondrial membrane potential. Mitochondrial dynamics proteins Drp1 and biogenesis protein NRF1 and TFAM were regulators of mitochondrial biogenesis. The key activators of mitochondrial biogenesis, DRP1, NRF1, and TFAM, were downregulated by 0.62-fold (P 0.01) and 0.47-fold (P 0.01) in Aβ treatment. However, co-treatment with AuNPs significantly increased DRP1, NRF1, and TFAM mRNA levels (Fig. 2m-o). Reactive oxygen species (ROS) production was assayed by ROS Kit. The levels of ROS in hNSCs were measured at 24 h after treatment with Aβ and AuNPs. As indicated in Fig. 2p, the generation of ROS was greatly increased in the Aβ treatment group and AuNPs co-treatment decreased the Aβ-induced increased mitochondrial

ROS production. The data described above suggest that AuNPs may improve mitochondrial function impairment due to Aβ by elevating mitochondrial membrane potential, upregulating regulators of mitochondrial biogenesis, and inhibiting ROS production.

Fig. 2.

AuNPs Protect hNSCs Injury Induced by Aβ by Upregulation of miR-21-5p Expression and Exert a Mitochondrial Protective Function

The study analyzed the difference between miRNAs in AD model cells and control cells. miRNA microarray analysis revealed that there exist 8 common genes aberrantly expressed (Fig. 3a). Further analyses identified that miR-21-5p, miR-132, miR-107, miR-369, miR-181c, and miR-212 were upregulated, while miR-34a and miR-138 were downregulated in hNSCs treated with AuNPs. Among these upregulated genes, miR-21-5p had the highest fold increase (4.78-fold), thus we analyzed miR-21-5p for subsequent experiments (Fig. 3b). hNSCs were transfected with miR-21-5p inhibitor and NC inhibitor, and treated with Aβ and AuNPs, respectively. qPCR was used to determine the miR-21-5p expression, which was significantly lower than that of hNSCs transfected with inhibitor NC (Fig. 3c). Then, an ELISA kit was used to detect the expression of inflammatory factors IL-6 and TNF-α. It turned out to be the AuNPs attenuated IL-6 and TNF-α levels in hNSCs and cells transfected with miR-21-5p inhibitor significantly increased the concentration of IL-6 and TNF-α (Fig. 3d and e). CCK-8 results showed that cell proliferation was significantly reduced by miR-21-5p inhibition and enhanced by AuNPs treatment (Fig. 3f). As shown in Fig. 3g, transfection with miR-21-5p inhibitor significantly promoted the apoptosis of hNSCs compared with the NC group (Fig. 3g and h). These data implied that transfected with miR-21-5p inhibitor reversed AuNPs mediated cytoprotection induced by Aβ.

hNSCs were transfected with either a miR-21-5p inhibitor or inhibitor-NC and qPCR was performed to analyze the expression of mitochondrial biogenesis regulators. qPCR showed that AuNPs treated with hNSCs enhanced the expression of Drp1, NRF1, and Tfam, while these genes' expression was reduced after hNSCs were transfected with miR-21-5p inhibitor (Fig. 3i-k). The JC-1 dye was used to assess membrane potential and findings confirmed that transfection with miR-21-5p inhibitor reduced the mitochondrial membrane potential compared with the inhibitor-NC groups (Fig. 3l). Next, we analyzed mitochondrial ROS production by ROS assay kit. Results found that compared with Aβ treatment, AuNPs co-treatment caused a significant decrease in ROS overproduction. Transfection of miR-21-5p increased ROS production in hNSCs (Fig. 3m). The results described above suggested that miR-21-5p was involved in AuNPs protection against Aβ-induced cell toxicity by reduced mitochondrial function.

Fig. 3.

AuNPs Exert Protective Cellular Effects Related to the miR-21-5p/SOCS6 Pathway

To obtain the intersection of predicted gene targets of miR-21-5p from starBase and TargetScan and significantly downregulated genes in GES198323, a Venn diagram analysis was carried out. The results

found that SOCS6 may be directly targeted by miR-21-5p (Fig. 4a). The binding site between miR-21-5p and SOCS6 was predicted by TargetScan (Fig. 4b). Dual-luciferase reporter assays indicated that miR-21-5p bound to 3'-UTR of SOCS6 and luciferase activity was decreased in miR-21-5p mimic and SOCS6 WT cotransfection group (Fig. 4c).

We constructed a damaged hNSCs model by treating cells with Aβ and then mimicking NC and miR-21-5p mimic were transfected in cells and the miR-21-5p expression was evaluated by qPCR, resulting in a significant increase in expression of miR-21-5p in miR-21-5p mimic group compared with the NC group. After AuNPs treated hNSCs which were transfected with miR-21-5p mimic, miR-21-5p expression was significantly enhanced (Fig. 4d). The results of Western blot showed that miR-21-5p mimic and AuNPs treatment co-suppressed SOCS6 expression (P 0.01) (Fig. 4e and f). Next, cell viability and apoptosis were evaluated by CCK-8 and flow cytometry. The cells transfected with miR-21-5p mimic increased cell viability and reduced apoptosis. Besides, cells treated with AuNPs promoted the effect of miR-21-5p mimic on cell viability and apoptosis (Fig. 4g-i). The observation outlined above indicates that in the presence of miR-21-5p, the protection effect of AuNPs treatment on hNSCs injured by Aβ was enhanced. The cytoprotective effects of AuNPs may be associated with upregulated miR-21-5p and downregulated SOSC6 expression.

Fig. 4.

AuNPs Through miR-21-5p/SOCS6 Pathway Protect hNSCs Injured by Aβ

Aβ-injured hNSCs cell model was constructed and then transfected with miR-NC, miR-21-5p mimic, pcDNA-SOCS6 or empty vector to verify the mechanism of AuNPs' protection effect. After AuNPs were treated in all groups of cells, the protein expression of SOCS6 was analyzed by Western blot and the results showed that miR-21-5p mimic inhibited SOCS6 expression significantly (Fig. 5a and b). Compared with that in the miR-NC group, miR-21-5p mimic robustly reduced the release of inflammatory cytokines, while overexpress of SOCS6 heightened the levels of inflammatory (IL-6 and TNF-α) (Fig. 5c and d). Overexpression of SOCS6 significantly reduced the cell viability and induced apoptosis, which had the opposite effect of miR-21-5p overexpression (Fig. 5e-g). SOCS6 overexpression reduced mitochondrial membrane potential and the mitochondrial in the cells were damaged (Fig. 5h). Additionally, the SOCS6 also resulted in a significant decrease in DRP1, NRF1, TFAM and elevated mitochondrial ROS levels (Fig. 5i-l). Overall, the above results suggest that AuNPs protect mitochondrial function and decrease the Aβ-induced apoptosis via miR-21-5p/SOCS6.

Fig. 5.

Discussion

The findings of this study demonstrated that AuNPs inhibited the expression of pro-inflammation factors and AD-related protein (T-Tau, P-Tau, and Aβ), moreover, AuNPs could ameliorate the inhibition of neural stem cell differentiation due to Aβ accumulation. Then, AuNPs increased mitochondrial membrane

potential, activated expression of Drp1, NRF1, and TFAM, and decreased ROS production in hNSCs. Thirdly, apoptosis of hNSCs induced by Aβ was inhibited with AuNPs co-treatment, and expression of proteins associated with apoptosis was also reduced in AuNPs co-treatment group. Then, the miR-21-5p was upregulated in AuNPs treated cells, while the effect of AuNPs treatment on hNSCs was reversed after transfection with miR-21-5p inhibitor. Inhibited miR-21-5p expression in hNSCs also reduced mitochondrial function induced by Aβ accumulation. Finally, AuNPs protect mitochondrial function and decrease the Aβ-induced apoptosis via miR-21-5p/SOCS6 pathway.

AD is the most common form of dementia and causes problems with memory, thinking, and behaviour. It is generally accepted that Aβ is the main toxic form, and there are multiple strategies for drug development against this hypothesis, including BACE1 inhibitors that inhibit Aβ production, and antibodies that promote Aβ clearance, and then none of them has been successfully marketed. The reason for this may be that the therapeutic modality is intervened too late, Aβ appears much earlier than the occurrence of clinical symptoms of AD, and toxic forms of Aβ have caused irreversible neurological damage when cognitive impairment appears (Wang et al. 2021). Therefore, the development of Aβ-labeled small molecules is important for the early diagnosis and treatment of AD.

The AuNPs preparation process is simple and controllable, allows a variety of functionalized modifications to the surface, and has the characteristics of good biocompatibility, high specific surface area, and low toxicity (Gao et al. 2015). AuNPs, as a new method, may have some advantages in eliminating Aβ-induced cytotoxicity in neurodegeneration studies. Kim et al. (Kim et al. 2017) suggested that AuNPs could act as an effective gene regulator and decrease the aggregation of Aβ. In addition, AuNPs have a protective effect on Aβ1-42-induced apoptosis, and the process is related to NF-κB-induced neuroinflammation (Ali et al. 2016). Muller et al. (Muller et al. 2017) also found that AuNPs have an important role in improving mitochondrial function, neuroinflammation as well as oxidative stress in diabetic rats. The results of this study revealed that AuNPs could ameliorate the cytotoxicity of hNSCs induced by Aβ enrichment, including decreasing apoptosis, promoting cell proliferation and differentiation, increasing mitochondria-related gene expression, and decreasing ROS production. This was consistent with previously reported results and AuNPs can improve the cytotoxicity induced by Aβ accumulation.

AuNPs are promising agents and materials for therapeutic applications such as drug delivery. In recent years, more and more attention has been paid to the role of miRNAs in the disease's progression. There have been several studies on AuNPs in AD drug therapy, but the mechanism of action of AuNPs on miRNA regulation during treatment is not yet clear. AuNPs nanomaterials, designed by Song et al. (Song et al. 2022), can accurately diagnose miRNA-125b and miR-361 in exosomes of AD patients and have great potential for AD clinical diagnosis as well as future dementia research and treatment fields. Gao et al. (Gao et al. 2021) found DNAzyme machines combined with AuNPs to detect specific miRNAs in living cells and this technology can accurately distinguish diseased cells from healthy cells and indicate a promising tool for cancer diagnosis and prognosis. Ven et al. (van der Ven et al. 2021) coupled AuNP and miRNAs mimics and improved drug delivery efficiency. Ng et al. (Ng et al. 2016) used AuNPs

intravenously to inject AD rats and found that miR-327 expression in rat cells showed differential regulation, and the process may be related to pulmonary inflammation. AuNP, coated with cargo DNA, can act as an effective delivery tool for anti-miRNA oligonucleotides (Kim et al. 2011). The present results showed that miR-21-5p was upregulated in AuNPs treated cells, while the effect of AuNPs treatment on hNSCs was reversed after transfection with miR-21-5p inhibitor. Differential miRNA expression may be the result of AuNPs playing a role in cells and in promoting cells against Aβ.

In addition to Aβ and tau protein being involved in the initiation and progression of AD, impaired mitochondrial bioenergetics and dynamics are likely major etiological factors in AD pathogenesis (Onyango et al. 2021). Several reports had demonstrated the neuroprotection effects of AuNPs in different aspects. Chiang et al. (Chiang et al. 2020) proved that AuNPs protect mitochondrial function and morphology from Aβ in hNCSs. This is consistent with our findings that in this study, we found that AuNPs protect mitochondrial function, consistent with up-regulation of miR-21-5p action, therefore, AuNPs in inhibiting Aβ aggregation and reducing Aβ cytotoxicity, may be related to regulating miR-21-5p. SOCS6 is not only involved in neuroinflammatory responses in AD, but also participate in the regulation of many cytokines. SOCS6 is one of the AD risk-associated proteins and is down-regulated during the treatment of AD with polyphenol compounds (Rose et al. 2021). It was reported that SOCS6 promoted mitochondrial fission and plays an important role in cardiomyocyte apoptosis (Zhang et al. 2022). SOCS6 took part in regulating mitochondrial function in the vascular endothelial cell after spinal cord injury (Ge et al. 2021). This is in line with the present study and AuNPs decrease mitochondrial damage and ROS production by regulating the miR-21-5p/SOCS6 pathway.

This study has shortcomings, only hNSCs cells are used for analytical studies, and subsequent studies will be conducted using animal tests to further improve the rigour. Besides, the research process regulated miR-21-5p expression by AuNPs and improved Aβ-induced hNSCs toxicity, especially on mitochondrial function regulation, while the miR-21-5p downstream targeted genes have not been deeply explored, which will be analyzed in subsequent studies. Our study is the first to show AuNPs can ameliorate Aβ-induced cytotoxicity in hNSCs, and the process is associated with miR-21-5p up-regulation and further acts through mitochondrial function regulation.

Conclusions

In conclusion, we proved AuNPs can protect hNSCs from Aβ injury by up-regulating miR-21-5p, increasing mitochondrial membrane potential, and up-regulating mitochondria-related genes. The unique potential and molecular mechanism of AuNPs in AD treatment were revealed.

Declarations

Authors' contributions – GW: Writing Original Draft and Supervision, XSh: Conceptualization and Data Curation, XS: Investigation, NW: Software and Validation, XW: Investigation, YG: Editing.

Competing interests – The authors declare that they have no conflict of interest.

Ethics approval – StemProÒ human neural stem cells (hNSCs) (H9-derived) were purchased from ThermoFisher (USA).

Data Availability statement – The data that support the findings of this study are available on request from the corresponding author [Guoqing Wang].

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References

Ali T, Kim MJ, Rehman SU, Ahmad A, Kim MO (2016) Anthocyanin-Loaded PEG-Gold Nanoparticles Enhanced the Neuroprotection of Anthocyanins in an Aβ1-42 Mouse Model of Alzheimer's Disease. Molecular Neurobiology

Anand BG, Wu Q, Karthivashan G, Shejale KP, Amidian S, Wille H, Kar S (2021) Mimosine functionalized gold nanoparticles (Mimo-AuNPs) suppress β-amyloid aggregation and neuronal toxicity. Bioact Mater 6(12):4491-4505 https://doi.org/10.1016/j.bioactmat.2021.04.029

Bishop JL, Thaper D, Vahid S, Davies A, Ketola K, Kuruma H, Jama R, Nip KM, Angeles A, Johnson F, Wyatt AW, Fazli L, Gleave ME, Lin D, Rubin MA, Collins CC, Wang Y, Beltran H, Zoubeidi A (2017) The Master Neural Transcription Factor BRN2 Is an Androgen Receptor-Suppressed Driver of Neuroendocrine Differentiation in Prostate Cancer. Cancer discovery 7(1):54-71 https://doi.org/10.1158/2159-8290.cd-15-1263

Cen X, Chen Y, Xu X, Wu R, He F, Zhao Q, Sun Q, Yi C, Wu J, Najafov A, Xia H (2020) Pharmacological targeting of MCL-1 promotes mitophagy and improves disease pathologies in an Alzheimer's disease mouse model. Nat Commun 11(1):5731 https://doi.org/10.1038/s41467-020-19547-6

Chatterjee T, Das G, Ghosh S, Chakrabarti P (2021) Effect of gold nanoparticles on the structure and neuroprotective function of protein L-isoaspartyl methyltransferase (PIMT). Sci Rep 11(1):14296 https://doi.org/10.1038/s41598-021-93752-1

Chiang MC, Nicol C, Cheng YC, Yen C, Huang RN (2020) Nanogold Neuroprotection in Human Neural Stem Cells Against Amyloid-beta-induced Mitochondrial Dysfunction. Neuroscience 435

Dong J, Yang H, Zhao J, Wen L, He C, Hu Z, Li J, Huo D, Hou C (2022) Sandwich-type microRNA biosensor based on graphene oxide incorporated 3D-flower-like MoS and AuNPs coupling with HRP enzyme signal amplification. Mikrochimica acta 189(1):49 https://doi.org/10.1007/s00604-021-05141-0

Gao N, Sun H, Dong K, Ren J, Qu X (2015) Gold-Nanoparticle-Based Multifunctional Amyloid-beta Inhibitor against Alzheimer's Disease.

Gao Y, Zhang S, Wu C, Li Q, Shen Z, Lu Y, Wu Z (2021) Self-Protected DNAzyme Walker with a Circular Bulging DNA Shield for Amplified Imaging of miRNAs in Living Cells and Mice. ACS nano https://doi.org/10.1021/acsnano.1c04260

Ge X, Tang P, Rong Y, Jiang D, Lu X, Ji C, Wang J, Huang C, Duan A, Liu Y, Chen X, Chen X, Xu Z, Wang F, Wang Z, Li X, Zhao W, Fan J, Liu W, Yin G, Cai W (2021) Exosomal miR-155 from M1-polarized macrophages promotes EndoMT and impairs mitochondrial function via activating NF-κB signaling pathway in vascular endothelial cells after traumatic spinal cord injury. Redox biology 41:101932 https://doi.org/10.1016/j.redox.2021.101932

Guan P, Cao L, Yang Y, Wang P (2021) Calcium Ions Aggravate Alzheimer's Disease Through the Aberrant Activation of Neuronal Networks, Leading to Synaptic and Cognitive Deficits. Frontiers in molecular neuroscience 14:757515 https://doi.org/10.3389/fnmol.2021.757515

Javed I, Peng G, Xing Y, Yu T, Zhao M, Kakinen A, Faridi A, Parish CL, Ding F, Davis TP, Ke PC, Lin S (2019) Inhibition of amyloid beta toxicity in zebrafish with a chaperone-gold nanoparticle dual strategy. Nat Commun 10(1):3780 https://doi.org/10.1038/s41467-019-11762-0

Kim DK, Park J, Han D, Yang J, Kim A, Woo J, Kim Y, Mook-Jung I (2018) Molecular and functional signatures in a novel Alzheimer's disease mouse model assessed by quantitative proteomics. Mol Neurodegener 13(1):2 https://doi.org/10.1186/s13024-017-0234-4

Kim HY, Lee D, Ryu KY, Choi I (2017) A gold nanoparticle-mediated rapid in vitro assay of anti-aggregation reagents for amyloid β and its validation. Chemical Communications 53(32)

Kim J, Yeom J, Ko J, Han M, Lee K, Na S, Bae J (2011) Effective delivery of anti-miRNA DNA oligonucleotides by functionalized gold nanoparticles. Journal of biotechnology 155(3):287-292 https://doi.org/10.1016/j.jbiotec.2011.07.014

Laffont B, Rayner KJ (2017) MicroRNAs in the Pathobiology and Therapy of Atherosclerosis. The Canadian journal of cardiology 33(3):313-324 https://doi.org/10.1016/j.cjca.2017.01.001

Li D, Huang S, Zhu J, Hu T, Han Z, Zhang S, Zhao J, Chen F, Lei P (2019) Exosomes from MiR-21-5p-Increased Neurons Play a Role in Neuroprotection by Suppressing Rab11a-Mediated Neuronal Autophagy In Vitro After Traumatic Brain Injury. Medical science monitor : international medical journal of experimental and clinical research 25:1871-1885 https://doi.org/10.12659/msm.915727

Liang Y, Wang L (2021) Inflamma-MicroRNAs in Alzheimer's Disease: From Disease Pathogenesis to Therapeutic Potentials. Frontiers in cellular neuroscience 15:785433 https://doi.org/10.3389/fncel.2021.785433

Long JM, Holtzman DM (2019) Alzheimer Disease: An Update on Pathobiology and Treatment Strategies. Cell 179(2):312-339 https://doi.org/10.1016/j.cell.2019.09.001

Mcc A, Cjbnb C, Chldef G, Sjc H, Cy I, Rnh J (2021) Nanogold induces anti-inflammation against oxidative stress induced in human neural stem cells exposed to amyloid-beta peptide. Neurochemistry International 145

Mehazri L, Shpungin S, Bel S, Nir U (2021) Loss of Fer Jeopardizes Metabolic Plasticity and Mitochondrial Homeostasis in Lung and Breast Carcinoma Cells. Int J Mol Sci 22(7) https://doi.org/10.3390/ijms22073387

Muller AP, Ferreira GK, Pires AJ, Silveira GDB, Souza D, Brandolfi J, Souza C, Paula M, Silveira P (2017) Gold nanoparticles prevent cognitive deficits, oxidative stress and inflammation in a rat model of sporadic dementia of Alzheimer's type. Mater Sci Eng C Mater Biol Appl 77(AUG.):476-483

Ng CT, Li JJ, Balasubramanian SK, You F, Yung L, Bay BH (2016) Inflammatory changes in lung tissues associated with altered inflammation-related microRNA expression after intravenous administration of gold nanoparticles in vivo. Acs Biomaterials Science & Engineering:acsbiomaterials.6b00358

Onyango I, Bennett J, Stokin G (2021) Mitochondrially-Targeted Therapeutic Strategies for Alzheimer's Disease. Current Alzheimer research https://doi.org/10.2174/1567205018666211208125855

Oyarzún MP, Tapia-Arellano A, Cabrera P, Jara-Guajardo P, Kogan MJ (2021) Plasmonic Nanoparticles as Optical Sensing Probes for the Detection of Alzheimer's Disease. Sensors 21(6) https://doi.org/10.3390/s21062067

Perets N, Betzer O, Shapira R, Brenstein S, Angel A, Sadan T, Ashery U, Popovtzer R, Offen D (2019) Golden Exosomes Selectively Target Brain Pathologies in Neurodegenerative and Neurodevelopmental Disorders. Nano letters 19(6):3422-3431 https://doi.org/10.1021/acs.nanolett.8b04148

Rose K, Barlock B, DaSilva N, Johnson S, Liu C, Ma H, Nelson R, Akhlaghi F, Seeram N (2021) Anti-neuroinflammatory effects of a food-grade phenolic-enriched maple syrup extract in a mouse model of Alzheimer's disease. Nutritional neuroscience 24(9):710-719 https://doi.org/10.1080/1028415x.2019.1672009

Song S, Lee JU, Jeon MJ, Kim S, Sim SJ (2022) Detection of multiplex exosomal miRNAs for clinically accurate diagnosis of Alzheimer's disease using label-free plasmonic biosensor based on DNA-Assembled advanced plasmonic architecture. Biosensors and Bioelectronics 199:113864 https://doi.org/https://doi.org/10.1016/j.bios.2021.113864

van der Ven C, Tibbitt M, Conde J, van Mil A, Hjortnaes J, Doevendans P, Sluijter J, Aikawa E, Langer R (2021) viaControlled delivery of gold nanoparticle-coupled miRNA therapeutics an injectable self-healing hydrogel. Nanoscale https://doi.org/10.1039/d1nr04973a

Verges DK, Restivo JL, Goebel WD, Holtzman DM, Cirrito JR (2011) Opposing synaptic regulation of amyloid-β metabolism by NMDA receptors in vivo. The Journal of neuroscience : the official journal of the

Society for Neuroscience 31(31):11328-11337 https://doi.org/10.1523/jneurosci.0607-11.2011

Wang P, Yang P, Qian K, Li Y, Xu S, Meng R, Guo Q, Cheng Y, Cao J, Xu M, Lu W, Zhang Q (2021) Precise gene delivery systems with detachable albumin shell remodeling dysfunctional microglia by TREM2 for treatment of Alzheimer's disease. Biomaterials 281:121360 https://doi.org/10.1016/j.biomaterials.2021.121360

Wen L, Shen L (2021) Effect of Surface-Chelated Cu on Amyloid-β Peptide Fibrillation. Langmuir : the ACS journal of surfaces and colloids https://doi.org/10.1021/acs.langmuir.1c02322

Zamolodchikov D, Duffield M, Macdonald L, Alessandri-Haber N (2022) Accumulation of high molecular weight kininogen in the brains of Alzheimer's disease patients may affect microglial function by altering phagocytosis and lysosomal cathepsin activity. Alzheimer's & dementia : the journal of the Alzheimer's Association https://doi.org/10.1002/alz.12531

Zhang P, Guan P, Ye X, Lu Y, Hang Y, Su Y, Hu W (2022) SOCS6 Promotes Mitochondrial Fission and Cardiomyocyte Apoptosis and Is Negatively Regulated by Quaking-Mediated miR-19b. Oxid Med Cell Longev 2022:1121323 https://doi.org/10.1155/2022/1121323

Zhao Y, Jaber V, Alexandrov PN, Vergallo A, Lista S, Hampel H, Lukiw WJ (2020) microRNA-Based Biomarkers in Alzheimer's Disease (AD). Front Neurosci 14:585432 https://doi.org/10.3389/fnins.2020.585432

Tables

Table 1 The primers used in qPCR analysis.

Gene	Forward	Reverse
Bax	GTCCACCAAGAAGCTGAGCGAGT	TCCACGGCGGCAATCATCC
Bcl-2	ACATCGCCCTGTGGATGACT	CTGGGGCCGTACAGTTCCACAA
caspase-3	AGCACCTGGTTACTATTCCTG	TAAATTCTAGCTTGTGCGCGTA
caspase-9	GTGAACTTCTGCCGTGAGTCC	AGCACCATTTTCTTGGCAGTCA
Drp1	CAAAACCCATTCCAATTATGCC	GAGCAGATAGTTTTCGTGCAACA
NRF1	TTCGGAAACTTCGAGCCAC	CTTGTACTTACGCACCACA
Tfam	GCTCAGAACCCAGATGCAA	TTCTTTATATACCTGCCACTCCG
miR-21-5p	CTCAACTGGTGTCGTGGAGT	TCGGCAGGTAGCTTATCAGACTGAT
U6	ACGATACAGAGAAGATTAGCATGG	AAATATGGAACGCTTCACGAA
β-actin	TCCTCCTGAGCGCAAGTACTCC	CATACTCCTGCTTGCTGATCCAC

Figures

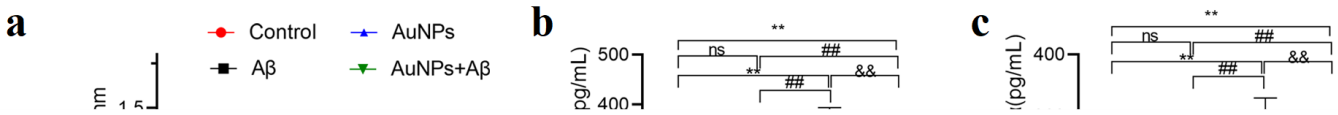

Figure 1

Nanogold promoted cell viability and inhibited inflammatory response in Aβ-treated hNSCs. **a** Cell viability detected by CCK-8. **b,c** IL-6 and TNF-α expression were measured by ELISA. **d** Immunohistochemistry analysis of Tuj-1 and GFAP in hNSCs. **e-f** Quantitative analysis of Tju-1 positive cells and GFAP positive cells. **g** The expression of T-Tau, P-Tau, and Aβ was detected by Western blot. **h-j** Quantification analysis of the Western blot data for T-Tau, P-Tau, and Aβ.

Compared with the control group, $^{*}P$<0.05, $^{**}P$<0.01. Compared with the AuNPs group, $^{\#}P$<0.05, $^{\#\#}P$<0.01. Compared with the Aβ group, $^{\&}P$<0.05, $^{\&\&}P$<0.01. ns means no significance. Values are the mean±SD of 3 independent experiments.

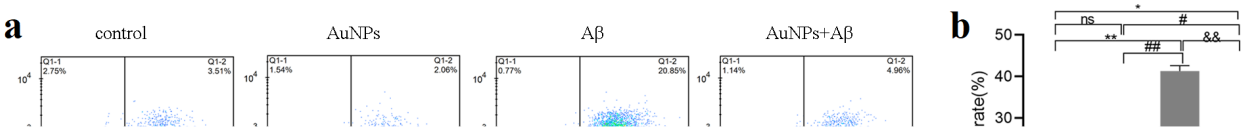

Figure 2

AuNPs inhibited hNSCs apoptosis induced by Aβ treatment. **a** hNSCs apoptosis was examined through flow cytometry. **b** Apoptosis rate of hNSCs at 24 h. **c-f** Quantitative qPCR of Bax, Bcl-2, caspase-3, and caspase-9. **g** Representative images of Western blot are shown. **h-k** Quantitative analysis of relative protein expression of Bax, Bcl-2, caspase-3, and caspase-9. **l** Quantitative analysis of JC-1 fluorescence value. qPCR analysis of the mRNA expression of **m** DRP1, **n** NRF1, and **o** TFAM. **p** Quantification of relative ROS generation.

Compared with the control group, $^{*}P$ 0.05, $^{**}P$ 0.01. Compared with the AuNPs group, $^{\#}P$ 0.05, $^{\#\#}P$ 0.01. Compared with the Aβ group, $^{\&}P$ 0.05, $^{\&\&}P$ 0.01. ns means no significance. Values are the mean±SD of 3 independent experiments.

Figure 3

AuNPs protection hNSCs injury induced by Aβ was associated with upregulation of miR-21-5p expression. **a** Venn diagram shows the differential expression of miRNAs in AD model cells and control cells. **b** qPCR was used to analyze the abnormal miRNA expression treated with AuNPs. **c** Transfection efficiency was verified by qPCR after transfected with miR-21-5p inhibitor-NC and miR-21-5p inhibitor after 24 h. The cells were treated with Aβ and AuNPs for 24 h; **d,e** The concentration of IL-6 and TNF-α was measured by ELISA kit. **F** Cell viability detected by CCK-8. **g** hNSCs apoptosis was examined through flow cytometry. **h** Apoptosis rate of hNSCs at 24 h. qPCR analysis of the mRNA expression of **i** DRP1, **j** NRF1, and **K** TFAM. **L** Quantitative analysis of JC-1 red/green fluorescence value. **M** Quantification of relative ROS generation.

Compared with the Aβ-NC group, $^{*}P$ 0.05, $^{**}P$ 0.01. Compared with the Aβ-inhibitor group, $^{\#}P$ 0.05, $^{\#\#}P$ 0.01. Compared with the Aβ-AuNPs-inhibitor group, $^{\&}P$ 0.05, $^{\&\&}P$ 0.01. ns means no significance. Values are the mean±SD of 3 independent experiments.

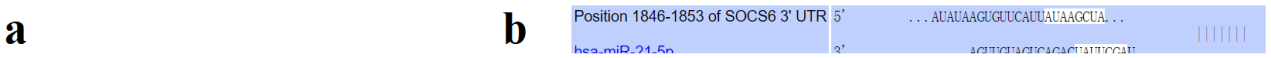

Figure 4

AuNPs exert protective cellular effects related to the miR-21-5p/SOCS6 pathway. **a** Venn diagram shows the overlapping of StarBase and TargetScan predicted target genes of miR-21-5p and significantly downregulated genes in GES198323. **b** Binding site of miR-21-5p and SOCS6 was predicted through TargetScan. **c** Dual-luciferase reporter assay. **d** The expression level of miR-21-5p analyzed by qPCR. **e**

SOCS6 expression was examined by Western blot. **f** Quantitative analysis of the Western blot results. **g** hNSCs activity was determined by CCK-8. **h-i** flow cytometry analysis of apoptotic cells.

Compared with the mimic-NC group, $^{*}P$<0.05, $^{**}P$<0.01. Compared with the miR-21-5p mimic group, $^{\#}P$<0.05, $^{\#\#}P$<0.01. Compared with the AuNPs-mimic NC group, $^{\&}P$<0.05, $^{\&\&}P$<0.01. ns means no significance. Values are the mean±SD of 3 independent experiments.

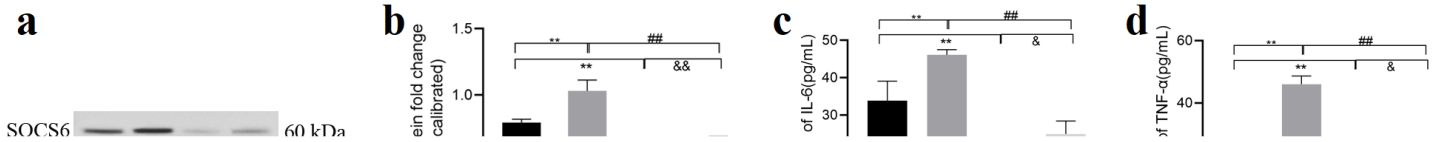

Figure 5

AuNPs through miR-21-5p/SOCS6 pathway protect hNSCs injured by Aβ. **a** The expression of SOCS6 analyzed by Western blot. **b** Quantitative analysis of the Western blot results. Inflammatory cytokines **c** IL-6 and **d** TNF-α were determined using ELISA kits. **e** cell viability was determined by CCK-8. **f-g** The cell apoptosis evaluation by flow cytometry. **h** quantitative analysis of JC-1 fluorescence value. qPCR analysis of the mRNA expression of **i** DEP1, **j** NRF1, and **k** TFAM. **l** Quantification of relative ROS generation.

Compared with the miR-NC-oe NC group, $^{*}P$ 0.05, $^{**}P$ 0.01. Compared with the miR-NC-oe SOCS6 group, $^{\#}P$ 0.05, $^{\#\#}P$ 0.01. Compared with the miR-21-5p mimic-oe NC group, $^{\&}P$ 0.05, $^{\&\&}P$ 0.01. ns means no significance. Values are the mean±SD of 3 independent experiments.

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