Protective mechanism of gold nanoparticles on human neural stem cells injured by β-amyloid protein

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Abstract

BACKGROUND AND PURPOSE

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.

METHODS

Cell Counting Kit-8 (CCK-8) was performed to detect hNSCs viability. The content of interleukin 6 (IL-6) and tumor 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. Quantitative PCR (qPCR) and Western blot assessments were also used to detect related gene expression intracellularly or in the supernatant.

RESULTS

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 the 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 level. 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.

CONCLUSION

AuNPs can protect hNSCs from Aβ injury by up-regulating miR-21-5p and protecting mitochondrial function.

1. 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 challenge, 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 labeled 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 signaling pathways (Zhao et al. 2020). MiR-21 has been shown to regulate microglia/astrocytes activation and link to neuroinflammatory signaling (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.

2. Materials And Methods

2.1. Cell culture and transfection

StemPro® 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 and inhibitor-NC using Lipofectamine 2000 (cat: no. 11668019, Invitrogen, USA). NC and inhibitor were purchased from Sangon Biotech (Shanghai). The sequence of NC and inhibitor were as follows: miR-21-5p inhibitor, 5'-UCAACAUCAGUCUGAUAAGCUA-3'; and inhibitor negative control, 5'-CAGUACUUUUGUGUAGUACAAA-3'.

2.2. CCK-8 assay

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

2.3. 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.

2.4. 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.

2.5. 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).

2.6. 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.

2.7. 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 °C. Nikon Ti-S fluorescence microscope was used to observe cells' fluorescence.

2.8. ROS measure

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

2.9. 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, and Bcl-2 were purchased from Proteintech (USA). Using the Rio-Rad gel imaging analysis system, the gray band value was calculated using β-actin as the internal reference.

2.10. 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
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

2.11. Statistical analysis

Experimental data were analyzed using SPSS 22.0, the measurement data were expressed as $\bar{x} \pm s$, a *t*-test was used for comparison between two groups, and a 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.

3. Results

3.1. 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.

3.2. AuNPs inhibited hNSCs apoptosis induced by Aβ treatment

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

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.

3.3. AuNPs improved mitochondrial function impairment due to Aβ

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 was observed in AuNPs co-treatment group (Fig. 3A). These data suggested that Aβ induced decreased mitochondrial membrane potential and mitochondria in the hNSCs were damaged, while AuNPs co-treatment showed the 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. 3B-D).

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. 3E, 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.

3.4. AuNPs protect hNSCs injury induced by Aβ associated with upregulation of miR-21-5p expression

The study analyzed the difference of miRNAs in Aβ and AuNPs treatment. miRNA microarray analysis revealed that there exist 8 common genes aberrantly expressed (Fig. 4A). 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. 4B). hNSCs were transfected with miR-21-5p inhibitor and NC inhibitor, and treated with Aβ and AuNPs, respectively. qPCR was used to determinate the miR-21-5p expression, which was significantly lower than that of hNSCs transfected with inhibitor NC (Fig. 4C). 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. 4D and E). CCK-8 results showed that cell proliferation was significantly reduced by miR-21-5p inhibition and enhanced by AuNPs treatment (Fig. 4F). As shown in Fig. 4G, transfection with miR-21-5p inhibitor significantly promoted the apoptosis of hNSCs compared with the NC group (Fig. 4G and H). These data implied that transfected with miR-21-5p inhibitor reversed AuNPs mediated cytoprotection induced by Aβ.

3.5. AuNPs activated miR-21-5p upregulated, which protected hNSCs from Aβ damage is associated with mitochondrial function

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. 5A-C). 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. 5D). 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. 5E). The results described above suggested that miR-21-5p was involved in AuNPs protection against Aβ-induced cell toxicity by reduced mitochondrial function.

4. 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. Fourthly, The miR-21-5p was unregulated in AuNPs treated cells, while the effect of AuNPs treatment on hNSCs was reversed after transfection with miR-21-5p inhibitor. Finally, inhibited miR-21-5p expression in hNSCs also reduced mitochondrial function induced by Aβ accumulation.

AD is the most common form of dementia and causes problems with memory, thinking, and behavior. 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, antibodies that promote Aβ clearance, and then none of them have 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-KB-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. The present results showed that miR-21-5p was unregulated 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) had 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 by regulating miR-21-5p.

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 rigor. 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.

4. 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.

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Figures

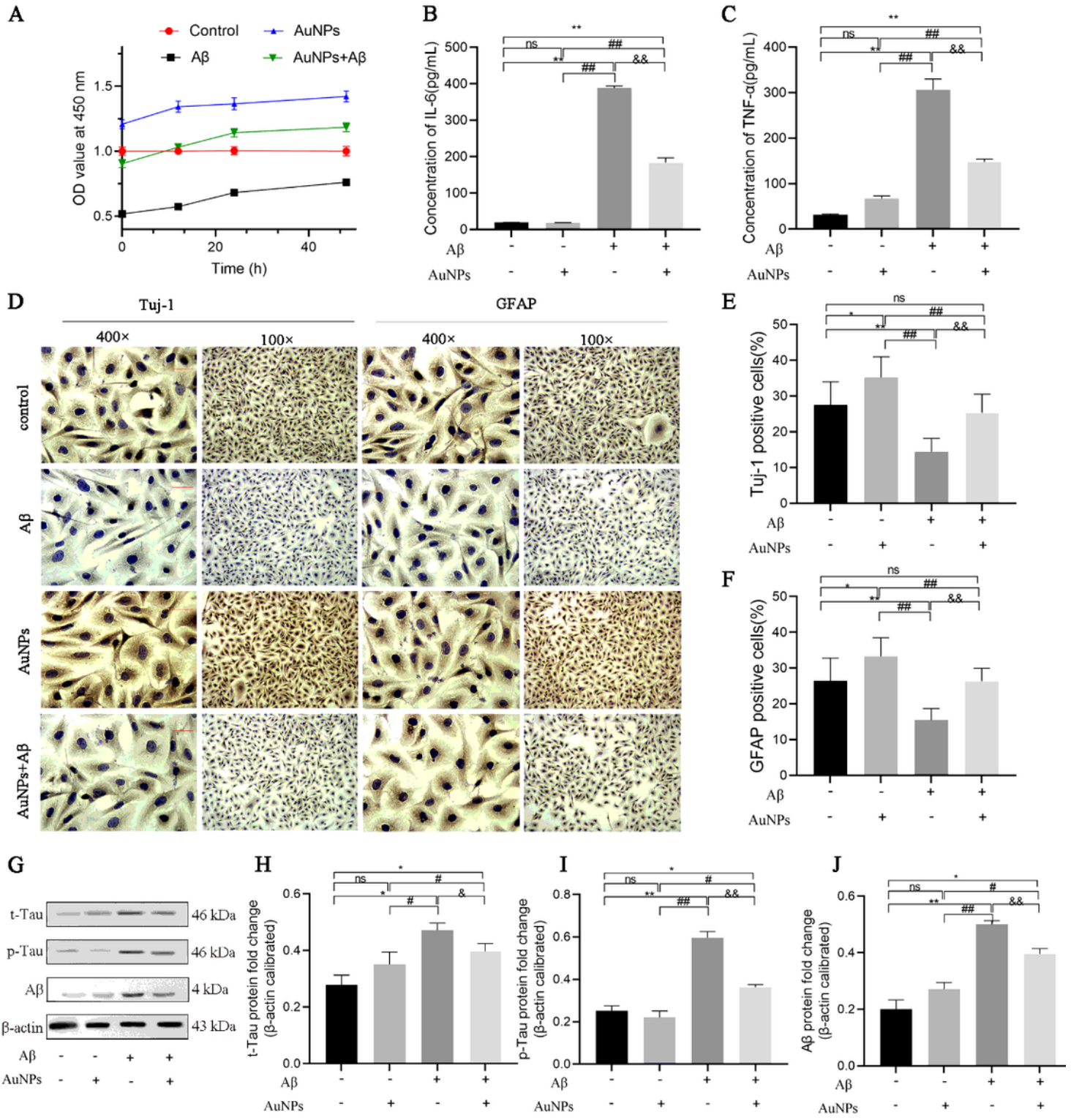

Figure 1

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 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 mean±SD of 3 independent experiments.

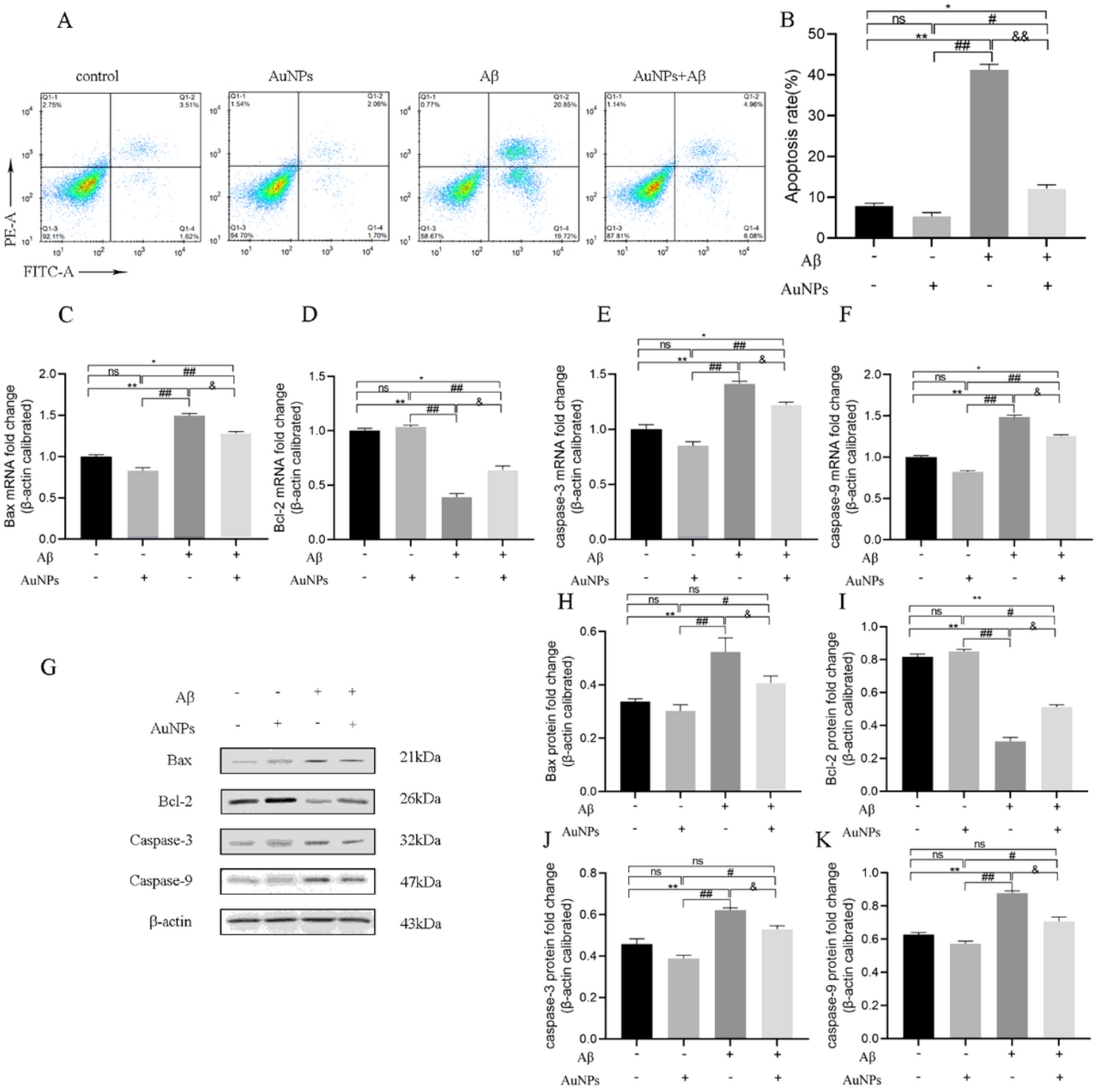

Figure 2

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.

Compared with the control group, *P<0.05, ** P<0.01. compared with 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 mean±SD of 3 independent experiments.

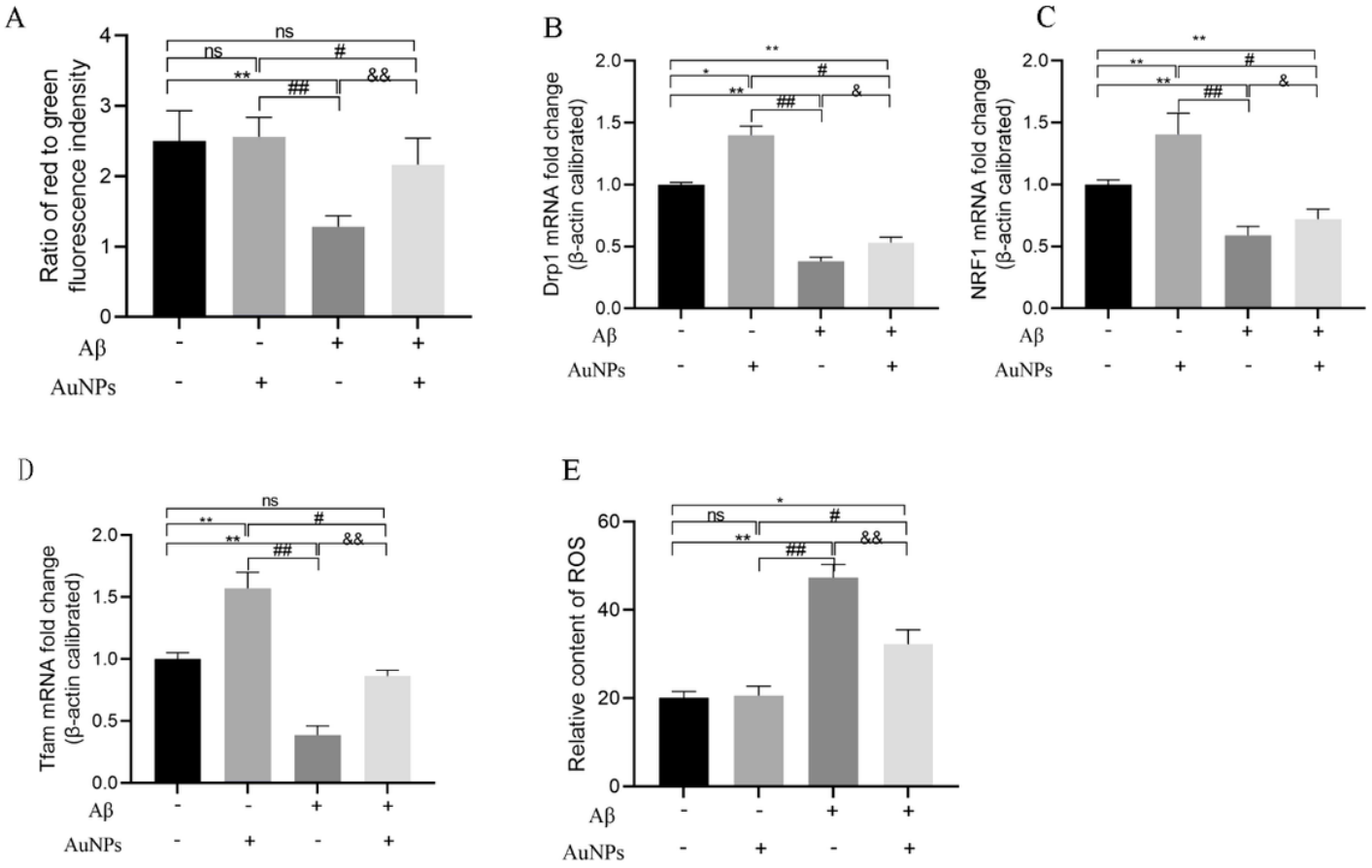

Figure 3

AuNPs improved mitochondrial function impairment due to Aβ, A. Quantitative analysis of JC-1 red/green fluorescence value (Mitochondrial membrane potential was detected by JC1 staining); qPCR analysis the mRNA expression of B. Drp1, C. NRF1, and D. Tfam; E. Quantification of relative ROS generation.

Compared with the control group, *P<0.05, ** P<0.01. compared with 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 mean±SD of 3 independent experiments.

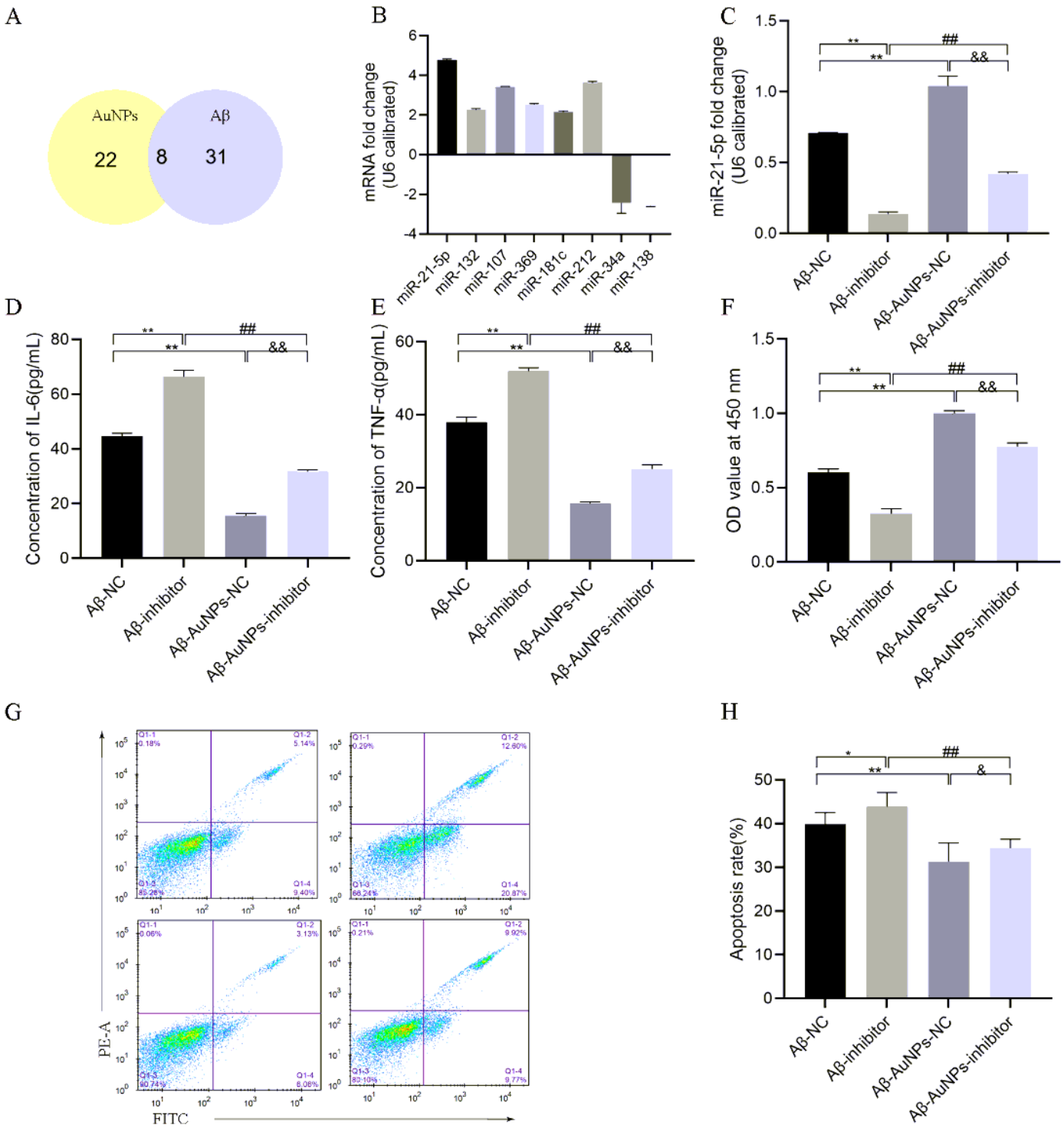

Figure 4

AuNPs protection hNSCs injury induced by Aβ was associated with upregulation of miR-21-5p expression, A. Venn diagram shows the global differential expression of miRNA in Aβ and AuNPs-treated hNSCs; 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.

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 mean±SD of 3 independent experiments.

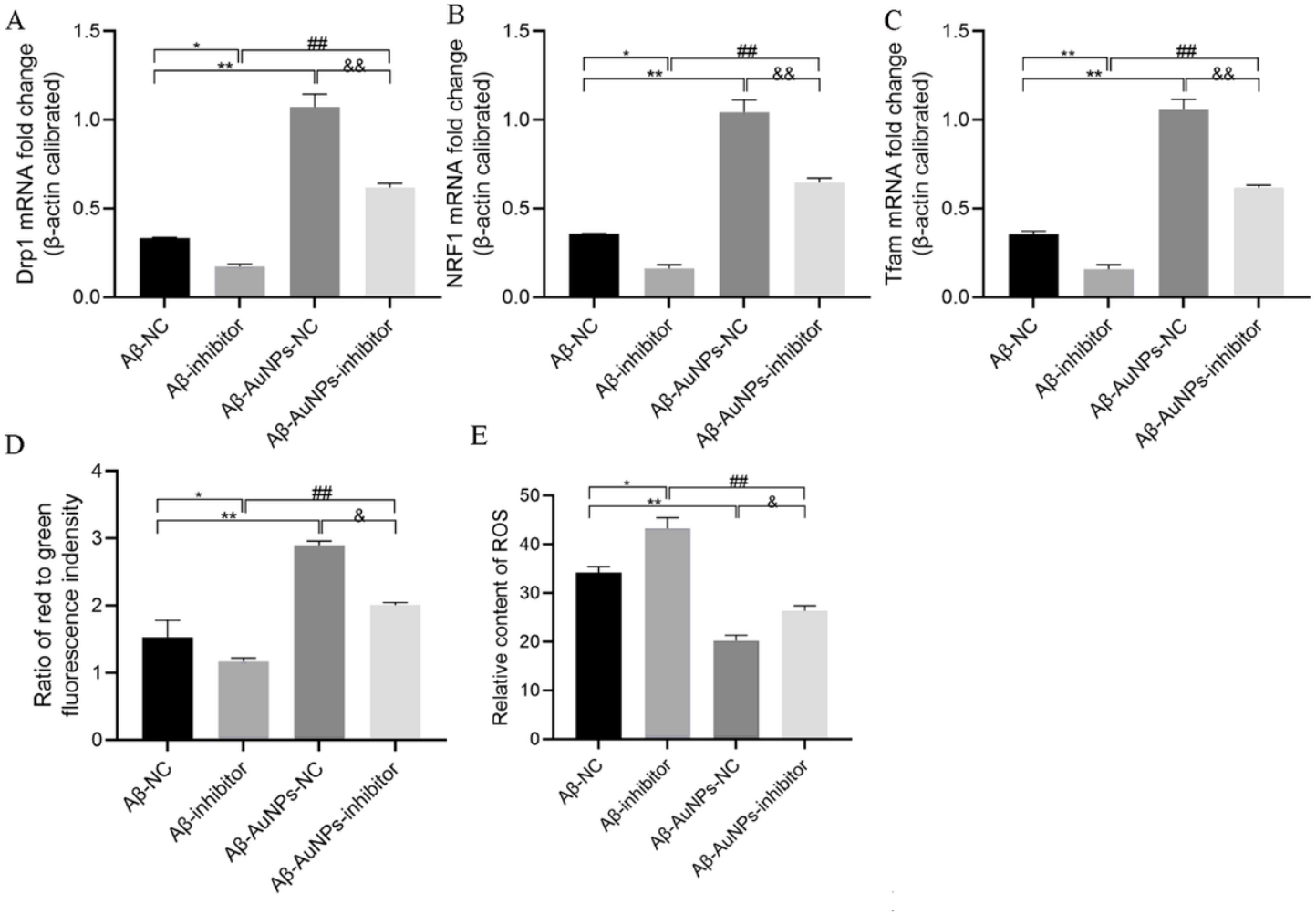

Figure 5

AuNPs activated miR-21-5p upregulated and protected hNSCs from Aβ damage is associated with mitochondrial function, qPCR analysis the mRNA expression of A. Drp1, B. NRF1, and C. Tfam; D. Quantitative analysis of JC-1 red/green fluorescence value; E. 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 mean±SD of 3 independent experiments.

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