Mesenchymal stem cell-derived exosomes inhibit Aβ1-42 induced microglia polarization by TLR2/MYD88/NF-κB pathway

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Abstract

Background

In recent years, more and more research evidence indicates that the causes of various neurodegeneration diseases are related to neuroinflammation in the brain, Aβ1–42 may be a key factor in the development of neuroinflammation in neurodegenerative diseases. Therefore, it is urgent to find an effective and targeted alleviation of neuroinflammation caused by Aβ1–42.

Methods

Our study found that exogenous administration of Aβ1–42 has a very obvious polarizing effect on microglia in the brain compared with the PBS-treated group. After the intervention of Aβ1–42, we obtained the mesenchymal stem cells derived exosome by ultracentrifugation. Microglia were treated with MSC-Exo in vivo and in vitro.

Results

The MSC-Exo were found have the effect of inhibiting microglial polarization in vivo and in vitro experiments. It was further found that its target gene was TLR2 on the surface of microglial cells to inhibit the expression of the downstream protein MYD88/NF-κB and inhibit the microglia polarization and the development of neuroinflammation.

Conclusion

MSC-Exo can significantly inhibited the Aβ1–42 induced microglia polarization by TLR2/MYD88/NF-κb pathway.

Background

Neuroinflammation is an important pathogenic factor affecting neurotrauma and neurodegenerative diseases[1]. In recent years, more and more research evidences have shown that the causes of death in a variety of neurological diseases are related to neuroinflammation in the brain, even if the main cause is not inflammation. After an acute neurological event (whether it is a traumatic disease or a non-traumatic disease), inflammatory markers increase in the brain and the whole body within a few minutes[2]. This process can be harmful, but it also promotes repairment of brain. Similarly, chronic neurological diseases, such as Alzheimer's disease and chronic traumatic encephalopathy, will have long-term and continuous neuroinflammation[3]. The chronic damage of neurons is accumulating and increasing. The regulatory cells of neuroinflammation are derived from the glial cells in brain. Microglia are the most important neuroimmune cells[4].
Microglia were first recognized as a unique cell population in the central nervous system a century ago. For a long time, they were mainly considered to be phagocytes, responsible for removing debris during the development of the central nervous system and disease[5]. Recently, advances in imaging and genetics, as well as the advent of single-cell technology, have provided new insights into the more complex and fascinating biology of microglia[6, 7]. Confirm the existence of microglia and better define their function in health and disease[8, 9]. Current studies believe that microglia are classified into M1 and M2 types, and some studies believe that they have transition state Mtran[10]. Generally, M1 microglia are considered to be pro-inflammatory cells that exacerbate neuroinflammation. M2 type microglia are cells that inhibit neuroinflammation[11]. Therefore, the polarization direction of microglia is an important factor determining the development of inflammation. Microglia are scavenger cells in the brain, which are extremely sensitive to various exogenous and endogenous neurotoxic substances. Among them are Aβ and other pathological proteins involved in the late stage of acute and chronic neuroinflammation.

β Amyloid (amyloid-β, Aβ) is a polypeptide containing 39–43 amino acids. It was produced by the proteolysis of amyloid precursor protein (APP) by β- and γ-secretase[12]. It can be produced by a variety of cells and circulate in the blood, cerebrospinal fluid and interstitial fluid[13]. Most of it binds to chaperone molecules and a few exist in a free state. The most common subtypes of Aβ in the human body are Aβ1–40 and Aβ1–42. In human cerebrospinal fluid, the content level of Aβ1–40 is 10 times higher than that of Aβ1–42. In human blood, the content level of Aβ1–40 is 10 times higher than that of Aβ1–42. Aβ1–42 are more toxic and easier to aggregate, thus forming the core of Aβ precipitation, triggering neurotoxic effects[14, 15]. More and more studies have proved that microglia have a clear immune response to Aβ[16]. In addition, activated microglia can carry and transport Aβ, continuously aggravating Aβ deposition and inflammation[17].

Mesenchymal stem cells are a kind of pluripotent stem cells. They have all the common characteristics of stem cells, that is self-renewal and multi-differentiation ability, and they have the most clinical applications[18]. However, the application of mesenchymal stem cells is mainly the direct differentiation and repair of cells, and there are problems in which the injection cannot be accurately positioned, and there is a certain immune rejection reaction, which limits the application of mesenchymal stem cells[19]. Therefore, it is necessary to explore a safer, more effective and more targeted methods of using mesenchymal stem cells.

Exosomes are spherical vesicles ranging from 30 to 150 nm, which are released into the extracellular space through various cell types and regulate various cell function[20]. Similar to microvesicles, exosomes also transport many biological molecules to cells of different lineages. Together, they are often called extracellular vesicles, which can be distinguished not only by their size, but also by their origin. Contrary to the microvesicles that fall off the plasma membrane, exosomes are formed by the inward sprouting of the restricted membrane of late endosomes, and encapsulate cellular proteins, RNA and miRNAs from the cytoplasm in non-cellular cells. Excreted outside the cell. Exosomes are widely used in neurological diseases. Studies have found that exosomes are effective in treating neurological trauma, chronic traumatic encephalopathy, Alzheimer's disease and other neuroinflammation-related diseases[21,
The substances and effects of exosomes are mainly determined by their source. Therefore, exosomes derived from mesenchymal stem cells have become safer and more targeted drugs that can replace stem cell transplantation[23].

Our research focus on the effect of Aβ1–42 on the polarization of microglia and the use of mesenchymal stem cell-derived exosomes for interventional treatment. We found that injection of Aβ1–42 into the lateral ventricle can significantly cause the polarization of microglial cells and the administration of exosomes derived from mesenchymal stem cells can effectively alleviate the activation of microglia and inhibit the occurrence and development of neuroinflammation. After biosynthesis analysis and in vitro and in vivo experiments, we have confirmed that MSC-Exo can inhibit the polarization of microglia and the release of neuroinflammatory factors maybe through the TLR2/MYD88/NF-KB signaling pathway.

Materials And Methods

All experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Bethesda, MD, USA) and approved by the Tianjin Medical University Animal Care and Use Committee.

Animals

Adult male C57BL/6 mouse (aged 8 weeks, weighing 20-25g) and male Sprague-Dawley (SD) rats (aged 3-4 weeks, weighing 45-55g) were purchased from the Chinese Academy of Military Science (Beijing, China). Animals were raised in a controlled environment with enough food and water on a 12-h light/dark cycle. All experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Bethesda, MD, USA) and approved by the Tianjin Medical University Animal Care and Use Committee.

Cell culture

For BMMSCs isolation, SD mouse were sacrificed and the hind limbs were dissected from the trunk of the body. Remove the muscle and connective tissue from both the tibia and the femur. Cut the ends of the tibia and femur just below the end of the marrow cavity and flush the marrow using a 27-gauge needle attached to a 10-ml syringe containing culture medium (DMEM/F12, Gibco, San Diego, USA) until shaft turns white. The cells were cultured in DMEM/F12 supplemented with 10% fetal bovine serum (FBS, BI) [24].Differentiation potential of MSCs was assessed by evaluating the commitment toward osteogenic and adipogenic lineages.Antibodies against CD29, CD34, CD45, CD90, RT1A, and RT1B (BioLegend, San Diego, CA, USA) were used for phenotype identification by flow cytometry. The 2nd or 3rd passage of BMMSCs were assessed for meeting the minimum criteria established by the Mesenchymal and Tissue Stem Cell Committee of International Society for Cellular Therapy. Exosome isolation was performed for third passage BMMSCs.BV2 microglial cells were purchased from China Infrastructure of Cell Line Resources (Beijing, China) and cultured in DMEM/F12 culture medium containing 10% Exo-depleted FBS,
100 U/ml penicillin, and 100 mg/ml streptomycin (Thermo Fisher Scientific). Cells were both cultured at 37 °C in humidified atmosphere of 5% CO2[25].

In vivo animal model and drug administration

Briefly, before the injection, β-Amyloid (1-42) and HiLyte Fluor™ 488-labeled β-Amyloid (1-42) (AnaSpec, San Jose, CA) were reconstituted by adding 50 µl 1%NH4OH, then the peptide solution was diluted to approximately 1 mg/ml with PBS. Mouse were i.c.v. injected with 3µL Aβ1-42 peptide (3 µg/3 µl) or an equivalent volume of the PBS using a 10µl Hamilton Syringe (Bregma: -1.0 mm, Midline: ±1.5 mm, Depth: 2.5 mm). [26]After 3 days, each mouse was treated with 10ul PBS or MSC-Exo(4ug/ul) intranasally. For immunofluorescence staining, the mouse was sacrificed by trans cardiac perfusion with PBS followed by 4% paraformaldehyde. The samples were fixed, dehydrated and embedded in the optimum cutting temperature medium (Sakura, Torrance, CA, USA). After that, mouse coronal sections were cut using a cryostat, and the tissue sections were stored at -20 °C. For qRT-PCR and Western Blot, the cerebral cortex and hippocampus were acquired from the injected side brain. The brain tissue was stored at -80°C for subsequent experiments.

Exosome preparation and identification

The collected cell culture supernatants were used to isolate exosomes by ultracentrifugation as described previously [25]. Briefly, the medium was removed free cells by centrifugations at 300g for 10 min. Subsequently, the supernatants were spun at 2000g for 10 minutes at 4 °C to remove cell debris, and at 10,000 g for 30 minutes at 4 °C to remove cell pellets. Next, the supernatant was filtered through a 0.22 mm filter (Millipore-Sigma, USA) to remove dead cells and particles larger than 200 nm. After that, the exosomes were harvested by ultracentrifugation at 100,000g for 70 min in a swing rotor (SW32Ti, XPN-100, Beckman, California, USA). All centrifugation steps were performed at 4°C. After the supernatant was discarded, the precipitation was resuspended with PBS pellets and stored at 4°C temporarily (<24 h) for further experiments. The total protein content of exosome was quantified by bicinchoninic acid (BCA) assay (Solarbio, Beijing, China). For the identification of exosomes, transmission electron microscope (TEM, HT8700; Hitachi, Tokyo, Japan) was used to observe the morphology of isolated particles. Briefly, twenty microliters of the sample were applied to a carbon coated formvar film that was attached to a metal sample grid. The grid was incubated with 50 ul of 2% phosphotungstic acid for 2 minutes at room temperature. After drying with filter paper, the sample was examined by TEM. Further, western blot analysis the biomarkers of exosomes including CD63, HSP70 and TSG101(1:1000, Abcam, USA). The size distribution of the particles in the particles was measured and analyzed using Nano Particle Tracking (NTA).

MSC-Exo Labeling and administration

For tracking studies, exosomes were labelled with PKH67 (green, Sigma-Aldrich, USA) and PKH26 (red, Sigma-Aldrich, USA) according to the manufacturer’s protocol. Briefly, 4µl PKH67 or PKH26 dye was mixed with exosome suspension in diluent C and incubated for 10 min at 37 °C. The labelling reaction
was stopped by adding 20 ml chilled PBS. Labelled exosomes were ultracentrifuged at 100,000×g for 70 min, washed with PBS, ultracentrifuged again at 100,000×g and the pellet were resuspended in PBS. For in vitro experiments, BV2 cells were treated with 10μg/ml exosomes for 12 h. For in vivo experiments, mouse was intranasally administered with exosome at a concentration of 4ug/ul per nostril alternately[27-29]. Each mouse was treated three days with 10μL exosome once a day.

**Preparation of brain extracts**

Mouse were i.c.v. injected with 3μL Aβ1-42 peptide (3μg/3μl) or an equivalent volume of the PBS using a 10μl Hamilton Syringe (Bregma: -1.0 mm, Midline: ±1.5 mm, Depth: 2.5 mm)[30]. After 3 days, each mouse was treated with an equivalent volume(10ul*4ug/ul) PBS or MSC-Exo intranasally. For immunofluorescence staining, the mouses were sacrificed by trans cardiac perfusion with PBS followed by 4% paraformaldehyde. The samples were fixed, dehydrated and embedded in the optimum cutting temperature medium (Sakura, Torrance, CA, USA). After that, mouse coronal sections were cut using a cryostat, and the tissue sections were stored at -20 ℃. For qRT-PCR and Western Blot, the cerebral cortex and hippocampus were acquired from the injected side brain. The brain tissue was stored at -80℃ for subsequent experiments.

**Western blot**

The total protein extracted from cell and brain tissue was for western blotting analysis as we previously described[25, 31]. The protein concentration was measured using the BCA Protein Assay Kit (Solarbio, China). Briefly, normalized protein samples were subjected to SDS acrylamide gel treatment, and then transferred to nitrocellulose membranes (Millipore, MA, USA). 5% skim milk in Tris Buffered saline Tween (TBST) were used to block nonspecific staining at room temperature for 2 h and incubated with primary antibodies(Table 1) overnight at 4℃ including TLR4(1:500; Cell Signaling Technology, USA), TLR2(1:500; Cell Signaling Technology, USA),GM130(1:1000; Abcam, UK), iNOS (1:1000; Cell Signaling Technology, USA), p65(1:1000; Abcam, UK), p-p65(1:1000; Cell Signaling Technology, USA), CD63 (1:1000; Abcam, UK), CD81(1:1000; Abcam, UK), Hsp70(1:1000; Abcam, UK), Myd88(1:1000; Cell Signaling Technology, USA), Caspase-3(1:1000; Abcam, UK) and IL-1β(1:1000; Cell Signaling Technology, USA), GAPDH (1:1000; Cell Signaling Technology, USA) was used as the internal control. After that, membranes were washed with TBST for three times and then incubated with horseradish peroxidase-conjugated secondary antibodies (ZSGB-BIO, China) at room temperature for 1 h. Membranes were scanned with an imaging system (Bio-Rad, Hercules, CA, USA) and analyzed were performed using Image J 7.0 software (National Institutes of Health, USA).

**Real-time quantitative PCR**

Total RNA from BV₂ cells and mouse brain tissue were performed using the Trizol reagent (Invitrogen, Carlsbad, CA). Both concentration and purity were estimated using a NanoDrop One Spectrophotometer (ThermoFisher Scientific). cDNA was synthesized using a cDNA reverse transcription kit (Tian Gen, China) on a CFX Connect Real-Time PCR Detection System (Bio-Rad, California, USA). GAPDH was used as an
internal control. The primer sequences are listed in Table 2. The data were analyzed with the $2 - \Delta \Delta \text{Ct}$ formula.

**Immunofluorescence**

The cell samples were fixed in 4% PFA for 20 min at room temperature, and animal samples were prepared as described previously[32]. For blocking nonspecific staining, the post-fixed samples were treated with 3% BSA for 30 min at 37°C. After that, the samples were incubated with primary antibodies overnight. The primary antibodies (Table 1) consisted of Iba-1 (1:400, Abcam, UK), iNOS (1:400, Cell Signaling Technology, USA) and Aβ1-42 (1:400, Abcam, UK). The next day, cells or sections were washed thrice in PBS, and samples subjected to treatment with secondary antibodies for 1 h at room temperature. DAPI (Abcam, UK) was used to probe cell nuclei.

**Nitric Oxide Synthase Assay**

Cellular inducible nitric oxide synthase (iNOS) activity was measured by Nitric Oxide Synthase Assay (Beyotime, China). Briefly, the BV2 cells were cultured at a density of 5000 cells/well in 96-well plates for 24 h, and then treated with LPS or Aβpeptide for 6 h. After that, the cells were collected and examined with a microplate reader at excitation and emission wavelengths of 495 and 515 nm, respectively.

**Cytotoxicity assay**

MTT [3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide] (0.5 mg/ml) was used to measure cell viability. In brief, BV2 cells were divided into four groups including control, control+MSC-Exo, Aβ1-42, Aβ1-42+MSC-Exo and then treated with MSC-Exo (10 μg/ml) and 1 μg/ml Aβ1-42 peptide for 12 h. Cells were stained with 200 μl MTT stock solution (5 mg/ml) to each well for 4 h. After that, the cells were dissolved with DMSO, and then transfer the solution to a 96-well plate. Optical density was examined with a microplate reader at excitation and emission wavelengths of 490 nm.

**Statistical analysis**

Statistical analysis was performed with Graph-Pad Prism 9 (GraphPad Software). All data are presented as means ± S.E.M. and were analyzed using Student’s t test (two groups), one-way ANOVA followed by Bonferroni’s multiple comparison test (more than two groups). Differences between means were considered statistically significant when $P < 0.05$. Animal weight was used for randomization and group allocation. No animals were excluded from analysis.

**Results**

1. **Aβ1-42 can promote M1 polarization of microglia and release of inflammatory factors in vitro**

In this part, we confirmed that Aβ1-42 can promote the activation of M1 type microglia and the release of inflammatory factors in vitro. In order to verify that Aβ1-42 is related to the M1 polarization of microglia,
green fluoresce labeled Aβ1-42 was added to BV2 microglia (Fig.1A). The results showed the co-localization of Aβ1-42 and M1 marker iNOS (Fig. 1B). In order to confirm that Aβ1-42 can induce the release of inflammatory factors, we tested the experimental group and control group by Western blot. iNOS were used to detect the M1 polarization of BV2 microglia. Compared with the control group, the expression of iNOS protein in LPS group (Fig.1C,1E), Aβ1-42(9ul)(Fig.1C,1E) group and Aβ1-42(15ul) group (Fig.1C,E) were apparently increased. Nf-κB(P-65) and Caspase-3 were used to detect the apoptosis of BV2 microglia. Compared with the control group, the expression of Nf-κB in LPS group (Fig.1C,1F), Aβ1-42(3ul) group(Fig.1C,1F), Aβ1-42(9ul)(Fig.1C,1F) group and Aβ1-42(15ul) group (Fig.1C,1F) were apparently increased. Compared with the control group, the expression of Caspase-3 protein in Aβ1-42(3ul) group(Fig.1C,1F), Aβ1-42(9ul)(Fig.1C,1F) group and Aβ1-42(15ul) group (Fig.1C,1F) were apparently increased. Similarly, The result of Nitric Oxide Synthase Assay is consistent with the above experiment results. Compared with control group, the relative activity of nitric oxide synthase (RFU) significantly increased in LPS group (Fig.1D) and Aβ1-42 group (Fig.1D). Similarly, compared with control group, the results of PCR show that Aβ1-42 group and LPS group significantly promote the expression of IL-1β(Supplementary Fig.1A), IL-6(Supplementary Fig.1B), Rela(Supplementary Fig.1D) and Nos2(Supplementary Fig.1F). Besides, the result of nitric oxide synthase test show that LPS (Fig.1G) and Aβ1-42(Fig.1G) can obviously promote the release the nitric oxide synthase. The above experimental results confirm that Aβ1-42 has the same effect as LPS in promoting microglia polarization and apoptosis.

2. The bioinformatics analysis of Aβ1-42 treatment on microglia

In order to explore the target gene of Aβ1-42 in promoting the polarization of microglia, we download chip data GSE55627 on the Geo platform, and compared the changes in the genome of the primary microglia after the treatment of Aβ1-42 and the normal group. First, the distribution of differential genes was analyzed by using the OmicStudio tools at https://www.omicstudio.cn/tool (Figure 2A), GO and KEGG enrichment analysis was performed on the DAVID data platform (Figure 2B-C). The enrichment analysis of KEGG showed that the differential genes were in the TNF signal pathway and Toll-like receptor pathway which the enrichment is the most obvious (Figure 2D). The GO analysis results showed that in the biological process part, the differential genes were concentrated in the inflammatory response, and in the molecular biological function part, the differential genes were concentrated in factor activation (Figure 2E). Finally, by comprehensive analysis, we focus the signal pathway on the TLR2/MYD88/NF-κB(Figure 2F).

3. MSC-Exo can significantly inhibit the polarization of microglia in vitro

In this part, we verified the effect of MSC-Exo on inhibiting the polarization of microglia. First, we extracted and identified rat bone marrow mesenchymal stem cells(Supplementary Fig2A-E), then we identified the exosomes extracted by ultracentrifugation (Fig. 3D). The results of NTA showed that the peak range of the particles was between 30-150nm (Fig. 3A). The peak value is 85nm, which is in line with the diameter distribution of exosomes. Western blot was used to detect the expression of CD63, Tsg101, and HSP70.
Compared with the supernatant group, the expression of each protein in the exosome group is significantly increased (Fig. 3B). The results of transmission electron microscopy show that the particles we extracted are basically in about 100nm, the shape is round-shaped, which is fully in line with the standard shape of exosomes (Fig. 3C). Besides, the PKH67-labeled exosomes were added to the BV2 cells culture medium. Immunofluorescence was used to demonstrate that MSC-Exo (green fluorescence) were engulfed by BV2 cells (red fluorescence) (Fig. 3E).

Then, we divide the group into: control+PBS, control+MSC-Exo, Aβ1-42, Aβ1-42+MSC-Exo. The results of western blot show that compared with the Aβ1-42 treatment group, the Aβ1-42+MSC-Exo group significantly inhibited the expression of iNOS (Fig. 4A, C). The results of immunofluorescence show that iNOS+ positive cells in the MSC-Exo group were significantly reduced (Fig. 4B, E). The results of MTT show that compared with control group, the cytoactive of the Aβ1-42 group was significantly reduced. After exosomal treatment, the cytoactive of Aβ1-42+Exo was improved compared with the Aβ1-42 group. Besides, the PCR results showed the expression of related pro-inflammatory factors. After MSC-Exo treatment, there is a decrease expression of the pro-inflammatory factors IL-1β, IL-6, TNF-α, and increase expression of the anti-inflammatory factor IL-10 (Fig. 4G-I). Similarly, we tested the expression of Aβ1-42 (Supplementary Fig. 3A, 3B) and Mature-IL-1β (Supplementary Fig. 3A, 3D) by western blot. The above results confirm that MSC-Exo inhibits M1 polarization of microglia and regulates the release of related inflammatory factors in vitro.

**4. MSC-Exo can decrease the expression of TLR2/MYD88/Nf-κB in vitro.**

In order to confirm the expression of related signal pathways, we detected the expression of TLR2 and RELA in each group by PCR (Fig. 4L). At the same time, WB results confirmed the expression changes of related proteins. Compared with the Aβ1-42 treatment group, the expression of TLR2 and Nf-κB in MSC-Exo treatment group was significantly decreased (Fig. 4D, 4E). The above results are basically consistent with the results of the biometric analysis.

**5. Aβ1-42 can promote M1 polarization of microglia in vivo**

In order to verify the results of in vitro experiments, stereotactic injection device were used to inject Aβ1-42 with green fluorescent labeled into the lateral ventricle (Fig. 5A). Immunofluorescence showed the distribution range of green fluorescence (Fig. 5B) and the expression of Aβ1-42 in cortex and hippocampus after injected 1 day, 3 day and 14 day (Fig. 5C). Besides, the results of Immunofluorescence and western blot showed that compared with control group, the Aβ1-42 group significantly increase the expression of iNOS (Fig. 6A, 6B, 6C, 6H).

**6. MSC-Exo nasal administration can inhibit the polarization of microglia through the TLR2/MYD88/Nf-κB pathway**

Three days after the injection of Aβ1-42 into the lateral ventricle, we administered MSC-Exo to the mouse by nasal injection. Three days after treatment, the mRNA and protein in brain tissues were taken for PCR
and WB analysis. Frozen section of brain tissue was used for Immunofluorescence. PCR results showed that the exosomes intervention group significantly reduced the expression of IL-1β and TNF-α, and increased the expression of IL-10 (Fig. 6I-L). At the same time, the expression of related pathway genes and proteins were detected. Compared with the normal group, the Aβ1-42 group significantly increased the expression of TLR2, MYD88 and P-p65 protein (Fig. 6A, 6E-G). Compared with the Aβ1-42 group, Aβ1-42+MSC -Exo group significantly reduced the expression of the above proteins (Fig. 6A, 6E-G). The above results confirmed that TLR2/MYD88/NF-κB may be an important pathway for MSC-Exo to inhibit microglia activation and neuroinflammation.

**Discussion**

Our research has found that in vivo and in vitro experiments have confirmed that exogenous administration of Aβ1–42 can promote the M1 polarization of microglia, and MSC-Exo can inhibit the effect of Aβ1–42 in promoting microglia polarization and reduce the release of inflammatory factors. Through Geo-shared chip biometric analysis and further confirmatory experiments, it is confirmed that the specific pathway of this effect is: TLR2/MYD88/NF-κB. Based on our research, we can have a closer understanding of the polarization of microglia caused by Aβ1–42, and have a deeper exploration of the mechanism by which MSC-EXO inhibits the polarization of glial cells and the development of neuroinflammation (Fig. 7). It has played a guiding role in the treatment of neuroinflammation and nerve damage caused by chronic degenerative diseases.

Aβ1–42 is a very important protein involved in neurodegenerative diseases, especially Alzheimer's disease[33, 34]. A large number of previous studies focused on the study of Aβ1–42 on neuronal damage and cognitive dysfunction. With the deepening of research, more and more studies have found that neuroinflammation is also an important factor in the development of neurodegenerative diseases[35]. In a variety of brain injuries including traumatic brain injury and chronic traumatic encephalopathy, Aβ participates in the occurrence and development of neuroinflammation in the acute phase, especially in the chronic phase[36]. The specific mechanism of action is still not very clear. Our research has verified the effect of Aβ1–42 in promoting the polarization of microglia both in vivo and in vitro. In vitro experiments found that this effect has a concentration-related effect of lipopolysaccharide, and the evidence of activation of related pathways was obtained through in vivo and in vitro experiments of bioinformatics analysis.

Mesenchymal stem cells (MSCs) have strong potential for self-renewal and multi-directional differentiation, as well as low immunogenicity and immunomodulatory properties. They are the most promising research objects[37, 38]. Bone marrow mesenchymal stem cells (BMSCs) have significant tissue regeneration potential and can secrete signal molecules. For example, neurotrophic factors, growth factors and cytokines play a role in tissue regeneration and injury protection through paracrine action[39]. In recent years, a large number of experiments have verified the effect of MSC cell transplantation on Aβ metabolism and glial cell polarization in the brain, and some clinical trials have begun to progress, but the use of mesenchymal stem cells still has certain limitations[40, 41]. The first is the source of cells and
the contamination of foreign bodies in the process of culturing and cryopreservation brings a certain risk of infection in the body. Some patients have symptoms such as fever and arrhythmia, and then the targeted injection of certain organs (heart and brain and other important organs). There is a certain degree of difficulty, and it is difficult for cell therapy to reach a specific target location that needs to be treated. The discovery of exosomes provides a safer and more targeted option for MSC research. As same as our research, some studies have found that MSC-Exo can alleviate the occurrence and development of inflammation in cardiac reperfusion injury, which can be regulated by the polarization of astrocytes in the brain alleviates the neuroinflammation caused by spinal cord injury[42]. In addition, it also has a more obvious therapeutic effect in the research of skin injury and bronchitis[43, 44]. Compared with exosomes derived from other cells, the exosomes derived from mesenchymal stem cells have a single composition, a more obvious therapeutic effect, and are easier to obtain in vitro and mass-produced[45, 46]. For the use of mesenchymal stem cell exosomes, we used exosomes extracted from untreated simple cells. In the current study, some experimenters also used exosomes obtained after intervention with other substances, such as interferon gamma[47–49]. As with other related secretion-promoting substances, whether the use of activated exosomes is more beneficial to the treatment of diseases remains to be verified. The changes in the contents of activated exosomes still need to be further clarified, so we chose to use simple exosomes derived from processed mesenchymal stem cells. Toll-like receptors (TLR) are an important class of protein molecules involved in non-specific immunity (natural immunity), and they are also a bridge between non-specific immunity and specific immunity. At present, there are different experimental results on the activation of TLR receptors of microglia by Aβ[50]. The most studied is the activation of TLR2 and TLR4[51, 52], and some studies believe that it is the co-activation of multiple TLR receptors[53–55]. So it needs to be closer to explore the synergy of receptor activation.

There are several deficiencies in our research. The first is the use of exogenous Aβ1–42 to interfere with cells and brain tissues, which may deviate from the amount, time and range of Aβ1–42 produced by itself, and then Toll like receptors is affected by it. The choice of the body may have other receptors activated at the same time, and the result of the joint action. Our experimental results are simply limited to specific changes at a certain point in time. The changes in TLR2 are the most obvious. In addition, the research on the role of the specific contents of MSC-Exo is not perfect and needs to be further carried out in future experiments.

**Conclusion**

In conclusion, this study not only demonstrates the effect of Aβ1–42 on microglia polarization, but also verified the mechanism of treatment of MSC-Exo on neuroinflammation induced by Aβ1–42. In subsequent studies, we will focus on exploring the specific substance in MSC-Exo acts on microglia. In addition, we will also study the effect of MSC-Exo in neuronal cells and astrocytes after Aβ1–42 treatment.
Abbreviations

AD: Alzheimer’s disease; MSC: Mesenchymal stem cell; Exo: Exosome; Aβ: amyloid β-protein; TLR: Toll-like Receptor; FBS: Fetal Bovine Serum; Iba-1: ionized calcium binding adaptor molecule-1; iNOS: Inducible nitric oxide synthase; NF-κB: nuclear factor kappa-B; MYD88: myeloid differentiation factor 88; DAPI: 4',6-diamidino-2-phenylindole; DMSO: Dimethyl sulfoxide; PBS: Phosphate Buffered Saline; PVDF: Poly(vinylidene fluoride); RFU: Relative fluorescence unit; TEM: Transmission Electron Microscope; ICV: Intracerebroventricular injection; RIPA: Radio-Immunoprecipitation Assay; NTA: Nanoparticle tracking analysis; Tsg101: Tumor susceptibility gene 101; HSP70: Heat shock protein 70.

Declarations

Acknowledgments

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Authors’ contributions

Ping Lei and Zhenyu Yin were responsible for study design. Zhenyu Yin and Mengtian Guo developed methodology. Zhenyu Yin, Mengtian Guo, Dong Wang, Zhaoli Han, Tianpeng Hu, Yan Wang, Xiangyang Xiong, Xintong Ge, Lu Wang, Shishuang Zhang, Yan Zuo, Dai li, Jing Zhao, Jinwen Yu and Wenzhu Li carried out the experiments. Fanglian Chen provided technical support. Zhenyu Yin and Mengtian Guo interpreted the results, performed data analysis, and prepared the figures and tables. Zhenyu Yin and Mengtian Guo wrote the manuscript. Ping Lei supervised the study. All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.
Competing interests

We declare no conflict of interest in this manuscript.

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Tables

Table1. Sequences of Real-time PCR
### Table 2. The list of antibodies

| Antibody | Manufacturer | Catalogue No. | Application | Dilution |
|----------|--------------|---------------|-------------|----------|
| iNOS     | CST          | 13120         | WB          | 1:1000   |
| TLR2     | CST          | 13744         | WB          | 1:1000   |
| P65      | CST          | 8242          | WB          | 1:1000   |
| P-P65    | CST          | 3033          | WB          | 1:1000   |
| MYD88    | CST          | 4283          | WB          | 1:1000   |
| GAPDH    | CST          | 2118          | WB          | 1:1000   |
| Iba1     | abcam        | ab5076        | IF          | 1:400    |
| Aβ1-42   | abcam        | ab201060      | WB/IF       | 1:1000/1:200 |
| CD63     | abcam        | ab59479       | WB          | 1:1000   |
| HSP70    | abcam        | ab2787        | WB          | 1:1000   |
| TSG101   | abcam        | ab133586      | WB          | 1:1000   |

### Figures
Figure 1

Aβ1-42 promote the polarization and apoptosis of BV2 microglia in vitro. BV2 cells labeled for Iba1, DAPI, HiLyte FluorTM 488-labeled Aβ1-42, Merged and Enlarged (A), Scale bar: A, 50 µm. M1 microglia labeled for iNOS, DAPI, HiLyte FluorTM 488-labeled Aβ1-42, Merged and Enlarged (B) Scale bar: B, 50 µm. Compared with the control group, the expression of iNOS protein in LPS group (C, E, **p < 0.01), Aβ1-42 (9ul) (C, E, **p < 0.01) group and Aβ1-42 (15ul) group (C, E, ***p < 0.001) were apparently increased. Compared with the control group, the expression of Nf-κB protein in LPS group (C, F, *p < 0.05), Aβ1-42 (3ul) group (C, F, ***p < 0.001), Aβ1-42 (9ul) (C, F, ***p < 0.001) group and Aβ1-42 (15ul) group (C, F, ****p < 0.0001) were apparently increased. Compared with the control group, the expression of Caspase-3 protein in Aβ1-42 (3ul) group (C, F, **p < 0.01), Aβ1-42 (9ul) (C, F, ****p < 0.0001) group and Aβ1-42 (15ul) group (C, F, ****p < 0.0001) were apparently increased. The results of nitric oxide synthase test. Compared with control group, the nitric oxide synthase significantly increased in LPS group (D, **p < 0.01) and Aβ1-42 group (D, **p < 0.01). Data are means ± S.E.M., n = 3.
Figure 2

The bioinformatics analysis of GEO data GSE55627. Differential gene distribution of Aβ1-42 group versus Control group (A, LogFC > 1.5 or LogFC < -1.5, P value < 0.05) PPI network analyzed by String and hubgenes analyzed by Cytoscape (B, C) GO and KEGG analyzed by DAVID (D, E) Cell signal pathways analyzed by DAVID (F)
Figure 3

The isolation, identification and staining of MSC-derived exosomes. The schematic diagram of ultracentrifugation for MSC-derived exosomes(D) The size distribution of the MSC-derived exosomes determined by a NanoSight tracking analyzer. The peak diameter of the exosomes was 101.0 ± 4.0 nm (A). The western blot analysis of characteristic biomarkers for exosomes, including HSP70, TSG101 and CD63. They were all more highly expressed in the PC12 cell-derived exosomes (Exo) than in the supernatant (Sup). (B) The PC12 cell-derived exosomes were observed by transmission electron microscope and showed round-shaped with a size range of 30–150nm (C) Scale bar: C, 500 nm.
Figure 4

MSC-Exo can alleviate microglia polarization and neuroinflammation caused by Aβ1-42 in vitro. Immunoblotting (A) and quantitative data (C-E) of iNOS, TLR2, P-65 and P-P65 in BV2 microglia after Aβ1-42 and MSC-Exo treatment. Immunofluorescence staining (A) and quantitative data (C) of iNOS in BV2 microglia after Aβ1-42 and exosome treatment. The MTT assay quantitative data in different group (G). The RT-PCR quantitative data of IL-1β, IL-6, IL-10, TNF-α, and RELA (H-L) in different group. Data are means ± S.E.M., n = 3. p < 0.05 (*), p < 0.01 (**), p < 0.001 (***) , p < 0.0001 (****).
Figure 5

Lateral ventricle injection of Aβ1-42 in C57BL/6 mouse. Lateral ventricle injection pattern of HiLyte FluorTM 488-labeled Aβ1-42(A). The distribution of Aβ1-42 in the brain after lateral ventricle injection (B). Co-localization of Aβ1-42 and Iba1 at different time points (C).
Figure 6

MSC-Exo can alleviate the M1 polarization of microglia caused by Aβ1-42 in vivo through TLR2/MYD88/NF-κb pathway. Immunofluorescence staining(B) and quantitative data (H) of iNOS in BV2 microglia after Aβ1-42 and exosome treatment. Immunoblotting (A) and quantitative data (C-G) of iNOS, TLR2, P-P65, P65 and MYD88 in BV2 microglia after Aβ1-42 and exosome treatment. The RT-PCR quantitative data of IL-1β, IL-6, IL-10, TNF-α and RELA(I–M) in different group. Data are means ± S.E.M., n = 3. p < 0.05 (*), p < 0.01 (**), p < 0.001 (***), p < 0.0001 (****).
Figure 7

The mechanism of MSC-Exo inhibit the polarization of microglia. MSC-Exo inhibit the polarization of microglia by the TLR2/MYD88/NF-κB pathway.

Supplementary Files

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