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Exosomal microRNA-22-3p influences oxidative stress injury and neuronal apoptosis in Alzheimer's disease *via* targeting KDM6B

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Abstract. This research was intended to unravel the effects of exosomal microRNA-22-3p (miR-22-3p) secreted from bone marrow mesenchymal stem cells (BMSCs) on oxidative stress damage and neuronal apoptosis in Alzheimer's disease (AD) *via* KDM6B. BMSCs and BMSCs-derived exosomes (BMSCs-Exos) were obtained and identified. An *in vitro* AD model was established. The levels of miR-22-3p and KDM6B were tested. Cell viability, ROS levels, MDA content and SOD levels, as well as apoptosis levels, were evaluated. The targeting relationship between miR-22-3p and KDM6B was validated. BMSC-Exos, miR-22-3p mimic and KDM6B siRNA advanced the cell viability, attenuated cell apoptosis, and ameliorated the oxidative stress injury of Aβ1-42-induced HT22 cells, including the decrease in the content of ROS and MDA and the increase in SOD activity. miR-22-3p inhibitor and KDM6B overexpression lentivirus advanced the decrease of cell viability, aggravated cell apoptosis, and promoted oxidative stress injury in Aβ1-42-induced HT22 cells, including the increase of ROS and MDA content and the decrease of SOD activity. miR-22-3p mimic combined with oe-KDM6B reversed the effects caused by miR-22-3p mimic alone. miR-22-3p targeted KDM6B. BMSC-Exos-derived miR-22-3p suppresses KDM6B expression to alleviate oxidative stress damage and apoptosis in AD neurons.

Key words: Alzheimer's disease — MicroRNA-22-3p — Lysine-specific demethylase 6B — BMSCs — Exosome — Oxidative stress — Neuronal apoptosis

Introduction

Alzheimer's disease (AD) is the most frequent neurodegenerative reason for dementia, which contributes to marked individual morbidity and mortality, as well as economic influence on the health care system (Rostagno 2022). Risk factors for AD consist of age, obesity, family history, diabetes, hypertension, apolipoprotein Eε4 genotype, hypercholesterolemia, low education level, along with traumatic brain injury (Cummings et al. 2019). Multiple therapeutic strategies have been discovered for several decades, while no curative treatment has been found, and the priority remains prevention (Passeri et al. 2022). At present, it is imperative to shift the research focus to the early stage of accurate assessment of increasingly preclinical AD (Tan et al. 2014).

Exosomes function as carriers for intercellular communication and as drug transportation platforms to attain achievements in treating central nervous system diseases (Vanherle et al. 2020). Especially, mesenchymal stem cells (MSCs)-derived exosomes are revealed to improve learning and memory abilities, reduce Aβ plagues and inhibit inflammation in AD (Cui et al. 2019). Bone marrow mesenchymal stem cells (BMSCs)-induced exosomes are equipped with the ability to improve the devastating changes caused by AD (Hassan et al. 2020). Delivered by exosomes, microRNAs are implied to function in neural remodeling, angiogenesis and neurogenesis (Reza-Zaldivar

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et al. 2018). Of the members of microRNAs, down-regulated microRNA-22-3p (miR-22-3p) is involved in the pathogenesis of AD, and its up-regulation can decrease Aβ peptides and ameliorate AD symptoms (Ji et al. 2019). Also, hsa-miR-22-3p, together with other microRNAs, can be employed as a biomarker of the detection for AD (Guo et al. 2017). Moreover, exosomal miR-22 is evidenced to function in cardioprotection such as apoptosis and stress responses (Moghaddam et al. 2019). Lysine-specific demethylase 6B (KDM6B) or JMJD3, is a histone H3 Lys27 demethylase that counteracts multiple polycomb-mediated transcription repression (Zhang et al. 2015). As we know, KDM6B demethylase can activate neuronal gene expression to promote postnatal and adult brain neurogenesis (Park et al. 2014). Interestingly, KDM6B is expressed in neurons and is considered a crucial promoter of neuronal apoptosis (Zhang et al. 2018). The independent actions of miR-22-3p and KDM6B are explored in previous articles, but inadequate studies have concentrated on the interactions of miR-22-3p and KDM6B in AD. In view of this, this paper was implemented to unmask the reciprocal interactions of exosomal miR-22-3p and KDM6B in oxidative stress damage and neuronal apoptosis of AD.

Materials and Methods

Ethics statement

All the experimental protocols got approval from the Animal Ethics Committee of Zhuhai College of Science and Technology, and the experimental process was followed by the Chinese Animal Protection and Use Guidelines.

Culture and identification of BMSCs

Separation of BMSCs was conducted following a previously described method (Xiao et al. 2020). C57BL/6 mice (the Animal Laboratory of Zhuhai College of Science and Technology, Guangdong, China) were euthanized under aseptic conditions, and the femur and tibia were obtained. The bone marrow was flushed with DMEM (Gibco, New York, USA), and the bone marrow cells were centrifuged for 3 min. After that, the cells were purified with DMEM encompassing 10% fetal bovine serum (FBS, Gibco), as well as 1% 100 U/ml penicillin and streptomycin (Invitrogen, Waltham, USA). Cells were cultivated at 37°C with 5% CO_2 and subsequently passaged when cells reached 70–80% confluence, and BMSCs at three passages were utilized for subsequent assays.

BMSC phenotype was assessed by flow cytometry (Chen et al. 2018) with specific antibodies against cell surface markers (CD29, CD90, CD45, and CD34; eBioscience, USA). BMSC was positive for CD29 and CD90 but negative for CD45 and CD34.

Extraction and identification of BMSC-derived exosomes (BMSC-Exos)

Exosomes were isolated, followed by purification by ultracentrifugation (Lin et al. 2022). In short, when reaching 70–80% confluence, cells were cultured in an exosome-free fresh medium encompassing 10% FBS (available from SBI Biosciences, Palo Alto, CA, USA). Upon 48-h incubation, MSC-conditioned medium was harvested and centrifuged at 2000×*g* for 20 min to remove debris and cells. After that, the supernatant was obtained and then transferred to a new sterile tube, followed by centrifugation at 10,000×*g* for 15 min for the removal of residual cell debris. Next, the supernatant was filtered using a 0.22 μm pore sterile filter (from Millipore, Billerica, MA, USA), followed by ultracentrifugation at 110,000×*g* and 4°C for 70 min to acquire exosome precipitates, which were resuspended and kept at −80°C. Exosomal protein content was tested by a protein assay kit (available from Thermo Scientific, Rockford, IL, USA).

To identify exosomes, TEM (Hitachi, Tokyo, Japan) and Western blot were implemented to evaluate the morphological size and specific surface markers (CD63 and TSG101) of isolated exosomes, respectively.

Cell culture

The mouse hippocampal neuronal cell line, i.e., HT22 cells (No. CL-0697, Procell, Hubei, China), were cultivated in 10% FBS-contained DMEM (Gibco), together with 1% 100 U/ml streptomycin and penicillin, and the cells were cultivated at 37°C under 5% CO_2.

Preparation of Aβ1-42 oligomers and neuronal precursor cell processing

Aβ1-42 was oligomerized and characterized as described previously (Xia et al. 2022). Briefly, the Aβ1-42 oligomer (P9001, Beyotime, Shanghai, China) was dissolved in hexafluoroisopropanol (HFIP, Sigma-Aldrich, USA) at 1 mg/ml. HFIP was evaporated using a steady stream of nitrogen. The resulting precipitate was resuspended in DMSO under the manufacturer's instructions and the buffer was renewed with Tris-EDTA with the application of a 5 ml HiTrap desalting column (from GE Healthcare, USA). The eluted peptides' concentration was assessed using the Bradford method (available from Bio-Rad, California, USA). The eluted peptides were aggregated for 2 h at ambient temperature. Prior to the treatment, serum-free DMEM was used instead of a culture medium. HT22 cells were cultivated in a neuromaintenance medium with 5 mM Aβ1-42 for 48 h.

Exosome uptake

To label exosomes, exosomes were first resuspended with the green fluorescent dye PKH67 (Sigma-Aldrich) before 5 min of incubation, followed by terminating with 2% bovine serum albumin. After that, exosomes were subjected to 1-h ultracentrifugation at 100,000×*g* for the removal of the unbound dye. HT22 cells were subjected to 24-h cultivation with 100 μg/ml PKH67-labeled exosomes, followed by 15-min 4% paraformaldehyde fixation and 5-min DAPI staining. The internalization of PKH67-labeled exosomes by HT22 was captured with a fluorescence microscope (from Leica, Wetzlar, Germany) (Hu et al. 2022).

Cell transfection

Transfection of BMSCs and HT22 cells was performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). This included the use of miR-22-3p mimic (50 nM), miR-22-3p inhibitor (100 nM), and miRNA negative control (mimic NC, inhibitor NC, 50 nM). Cells were incubated for 48 h. Additionally, HT22 cells were transfected with small interfering (si) RNA targeting KDM6B (si-KDM6B, 100 nM; Genepharma, Shanghai, China) or with an overexpressing lentivirus (oe-KDM6B, MOI = 10). Control groups received si-NC or oe-NC, respectively. Cells were incubated for 48 hours. For combined treatment with Aβ1-42 and exosomes, HT22 cells were treated with 5 mM Aβ1-42 for 48 h after transfection, or co-incubated with 100 μg/ml BMSC-Exo for 24 h (Liu H et al. 2022).

CCK-8 assay

Cell viability was assayed with the CCK-8 assay kit (available from Dojindo, Kumamoto, Japan). In short, cells were inoculated into 96-well plates at a density of 5000 cells/well and then incubated and stimulated for 48 h at the indicated treatment concentrations. After that, 10 μl of CCK-8 reagent was appended to every well before incubating for 3 h at 37°C. Lastly, absorbance was measured at 450 nm by a microplate reader (Xu et al. 2021).

Oxidative stress assay

Oxidative stress was estimated based on reactive oxygen species (ROS) production, malondialdehyde (MDA) levels and superoxide dismutase (SOD) activity. Each sample consisted of 3 replicates in 3 wells for three independent experiments (Meng et al. 2022).

Flow cytometry

Cell apoptosis was processed with flow cytometry under the requirements of the annexin V/propidium iodide (PI) staining kit (Beyotime). In short, cells were processed, collected, as well as incubated with 500 μl of binding buffer containing 5 μl of FITC-annexin V and 5 μl of PI at ambient temperature devoid of light, and then processed with an Accuri C6 flow cytometer (BD Biosciences, USA) (Dong et al. 2022).

Western blot

Total proteins were extracted using RIPA lysis buffer (Beyotime) with the protease inhibitor PMSF and quantified using the BCA protein assay kit (Thermo Scientific, Rockford, IL, USA). Equal amounts of proteins were separated and transferred to a PVDF membrane (Millpore, Billerica, CA, USA). After that, the membranes were incubated overnight at 4°C with the following primary antibodies: rabbit anti-CD63 (1:1000; ab217345, Abcam, Cambridge, MA, USA), rabbit anti-TSG101 (1:2000; 28283-1-AP, Proteintech, Hubei, China), rabbit anti-Calnexin (1:5000; 10427-2-AP, Proteintech), rabbit anti-KDM6B (1:1000; ab169197, Abcam), as well as rabbit anti-GAPDH (1:5000, 10494-1, Proteintech), followed by 2-h cultivation with secondary antibodies of goat anti-rabbit IgG (SA00001-2, Proteintech). The bands were visualized by an enhanced chemiluminescence detection kit (from Thermo Fisher Scientific, Waltham, USA) (Wei et al. 2020).

RT-qPCR

The extraction of the total RNA from cells or exosomes was achieved using Trizol or Trizol LS (Invitrogen), respectively. cDNA was then synthesized with the ProtoScript® First Strand cDNA Synthesis Kit (from New England Biolabs, Beverly MA, USA) to synthesize cDNA, which was then quantified with FastStart™ Universal SYBR® Green Master (Sigma-Aldrich). For miRNAs, microRNA first-strand synthesis and miRNA quantitation kits were used for cDNA synthesis and quantification (Takara, Dalian, China). GAPDH or U6 was adopted as an internal reference and calculated using the $2^{-\Delta\Delta Ct}$ method (Meng et al. 2022). Three replicates were set up in 3 wells for each sample to complete a total of three independent biological experiments. The primer sequences are exhibited in Table 1.

Table 1. The primer sequences for genes

	Primer sequence
miR-22-3p	F: 5'-AAGCTGCCAGTTGAAGAACTGT -3'
KDM6B	F: 5'-TGAAGAACGTCAAGTCCATTGTG -3'
	R: 5'-TCCCGCTGTACCTGACAGT-3'
U6	F: 5'-CGATACAGAGAAGATTAGCATGGC -3'
	R: 5'-AACGCTTCACGAATTTGGT-3'
GAPDH	F: 5'-CAAAATGGTGAAGGTCGGTGT-3'
	R: 5'-GAGGTCAATGAAGGGGTCGTT -3'

F, forward; R, reverse.

Dual luciferase assay

The pGL3 enhancer vector (available from Genscript, Nanjing, China) was cloned into the mouse KDM6B wild-type (WT)-3' untranslated region (3'UTR) or the KDM6B mutant (MUT)-3'UTR containing the miR-22-3p binding site. HEK293T cells (CL0005, Procell) were cultivated in 24-well plates for 24 h. The luciferase reporter vector was then subjected to co-transfection with the miR-22-3p mimic or the NC mimic under the instructions of Lipofectamine 2000 reagent (Invitrogen). At 48 h later, luciferase activity was examined with a dual luciferase reporter gene assay system (Zhang et al. 2021).

Statistical methods

Data were processed with GraphPad Prism 8.0 software. Measurement data were expressed as mean ± standard deviation, and then analyzed by the independent *t*-test, or one-way analysis of variance (ANOVA) with Tukey's *post hoc* test. $p < 0.05$ stood for statistical significance.

Results

BMSCs-Exos have protective effects on AD neurons

To unveil the influences of BMSCs-Exos on AD, we first isolated mouse BMSCs and characterized their surface markers by flow cytometry, which revealed high CD29 and CD90 expression as well as low CD34 and CD45 expression (Fig. 1A). Subsequently, BMSCs-Exos were isolated and characterized, and the exosomes were spherical and cup-shaped, with diameters distributed approximately 100 nm (Fig. 1B). Western blot analysis further unveiled that the exosomes exhibited specific biomarkers CD63 and TSG101 (Fig. 1C).

Next, in order to unravel the influence of BMSCs-Exos on the AD model, we first needed to determine whether BMSCs-Exos could be endocytosed by HT22 cells. We incubated PKH67-labeled BMSCs-Exos with HT22 cells for 24 h and observed that, as shown in Figure 1D, exosomes (green) aggregated around the nucleus (blue) of HT22 cells, reflecting the process of BMSCs-Exos internalization. Then, we constructed an *in vitro* AD model by treating HT22 cells with Aβ1-42 and added exosomes for intervention. CCK-8 assay results indicated that Aβ1-42 treatment decreased cell viability, while exosomes reversed cell viability in the AD model (Fig. 1E). In addition, Aβ1-42 treatment increased ROS levels and MDA content and decreased SOD viability in HT22 cells, and exosome treatment suppressed ROS levels and MDA levels and restored SOD viability (Fig. 1F–H). Similarly, flow cytometry results also disclosed that Aβ1-42 significantly increased apoptosis and exosome treatment decreased apoptosis (Fig. 1I).

It is indicated that BMSCs-Exos could alleviate oxidative stress injury and apoptosis in AD neurons.

BMSCs-Exos alleviate neuronal injury through miR-22-3p

As reported, miR-22-3p is down-regulated in brain tissues of AD patients (Xia et al. 2022), and miR-22-3p ameliorates apoptosis in Aβ1-42-treated HT22 cells and ameliorates AD in mice. Therefore, we further explored whether BMSCs-Exos could ameliorate Aβ1-42-induced HT22 cell injury through modulating miR-22-3p. First, we evaluated miR-22-3p expression in HT22 cells after incubation with exosomes. RT-qPCR results exhibited a significantly elevated miR-22-3p in HT22 cells after incubation with exosomes (Fig. 2A). After that, we treated BMSCs with miR-22-3p inhibitor, and RT-qPCR assay revealed that miR-22-3p in both BMSCs and their exosomes were notably decreased after using miR-22-3p inhibitor (Fig. 2B,C). In the meantime, the levels of miR-22-3p in HT22 cells were markedly decreased after incubation with BMSCs-Exos with miR-22-3p inhibitor treatment (Fig. 2D).

Further, we constructed an *in vitro* AD model and treated it with exosomes carrying low levels of miR-22-3p, and CCK-8 assay disclosed that cell viability was notably reduced in the Aβ1-42+Exos-miR-22-3p inhibitor group *versus* the Aβ1-42+Exos-inhibitor NC group (Fig. 2E). Similarly, we assessed the oxidative stress level and apoptosis level, and found that the cellular ROS level, MDA content, and apoptosis ratio were notably increased and SOD viability was markedly decreased in the Aβ1-42+Exos-miR-22-3p inhibitor group in contrast to the Aβ1-42+Exos-inhibitor NC group (Fig. 2F–I).

The above results imply that BMSCs-Exos attenuate oxidative stress injury and apoptosis in neurons *via* modulating miR-22-3p.

KDM6B in neurons is a target of miR-22-3p

To reveal the downstream regulatory targets of miR-22-3p, we subsequently searched the TargetScan database to forecast the downstream genes of miR-22-3p, and identified a site where miR-22-3p and KDM6B bind to each other (Fig. 3A). Therefore, we implemented a dual luciferase assay, which demonstrated that the miR-22-3p mimic targeted the 3'UTR region of KDM6B (Fig. 3B). Afterwards, we examined the levels of KDM6B after exosomal treatment of the AD model. RT-qPCR and Western blot results indicated a notable increase in KDM6B expression in the Aβ1-42+PBS-treated group relative to the Control group, and a significant decrease in the levels of KDM6B in the Aβ1-42+Exos group *versus* the Aβ1-42+PBS group (Fig. 3C,D). In addition, functional

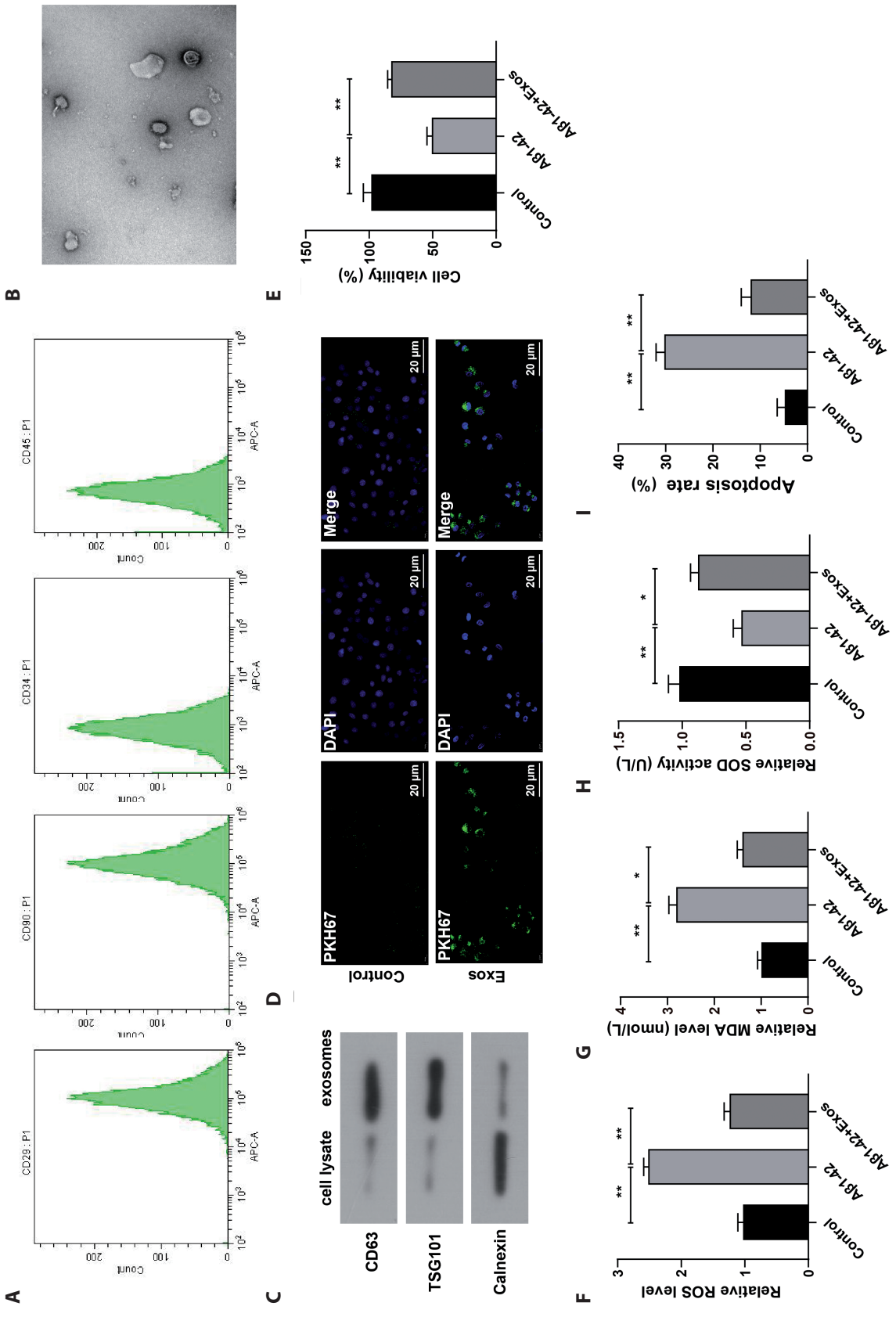

Figure 1. BMSCs-Exos have protective effects on AD neurons. **A.** Flow cytometry was utilized to evaluate BMSC cell surface markers CD29, CD90, CD34, and CD45. **B.** Representative TEM images of BMSCs-Exos, scale bar = 100 nm. **C.** Western blot was implemented to assess exosome marker proteins CD63 and TSG101. **D.** Representative images of neuronal internalization of PKH67-labeled exosomes, green: exosomes, and blue: nuclei, scale bar = 20 μm. **E.** CCK-8 assay for cell viability. Cellular ROS levels (**F**), MDA levels (**G**) and SOD activity (**H**) were detected to assess oxidative stress. **I.** Degree of apoptosis was tested by flow cytometry. * $p < 0.05$, ** $p < 0.01$. (For color figure see online version).

experiments disclosed that transfection of miR-22-3p mimic contributed to decreased KDM6B levels, while miR-22-3p inhibitor caused the opposite impact (Fig. 3E–G).

It is suggested that miR-22-3p in BMSCs-Exos can target and restrain KDM6B expression in neuronal cells.

KDM6B advances neuronal oxidative stress injury and apoptosis

In our previous results, we observed that KDM6B levels were remarkably increased in cells after Aβ1-42 induction (Fig. 3C,D). To determine the role of KDM6B in AD, we further treated cells with siRNA and found that si-KDM6B significantly decreased Aβ1-42-induced KDM6B levels (Fig. 4A,B). In addition, we detected that in the presence of si-KDM6B, the cell viability of Aβ1-42-induced neurons was increased, ROS levels and MDA levels were decreased, SOD activity was increased, and apoptosis was reduced (Fig. 4C–G).

Overall, the above evidence suggests that KDM6B promotes neuronal oxidative stress injury and apoptosis in AD.

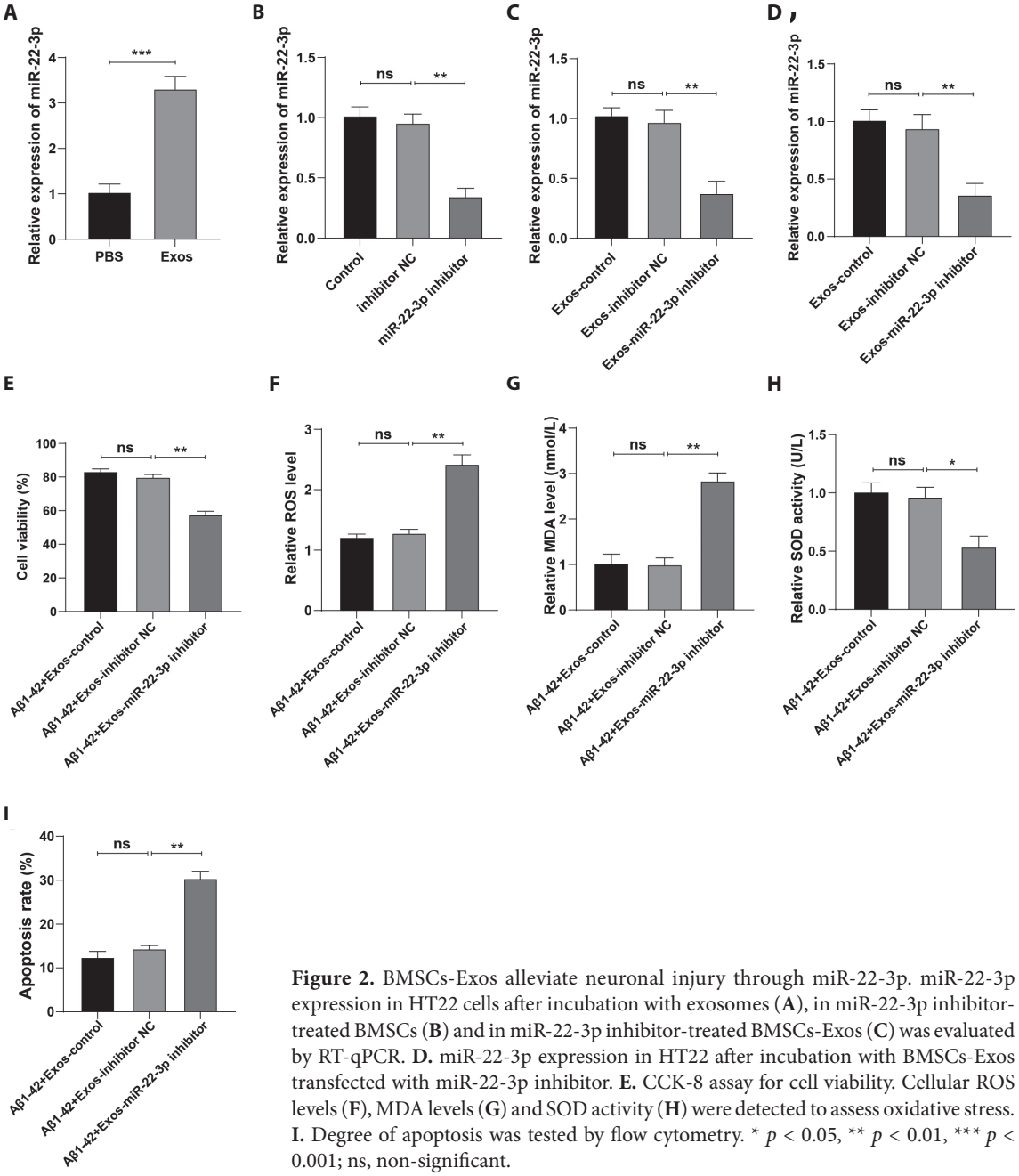

Figure 2. BMSCs-Exos alleviate neuronal injury through miR-22-3p. miR-22-3p expression in HT22 cells after incubation with exosomes (**A**), in miR-22-3p inhibitor-treated BMSCs (**B**) and in miR-22-3p inhibitor-treated BMSCs-Exos (**C**) was evaluated by RT-qPCR. **D.** miR-22-3p expression in HT22 after incubation with BMSCs-Exos transfected with miR-22-3p inhibitor. **E.** CCK-8 assay for cell viability. Cellular ROS levels (**F**), MDA levels (**G**) and SOD activity (**H**) were detected to assess oxidative stress. **I.** Degree of apoptosis was tested by flow cytometry. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; ns, non-significant.

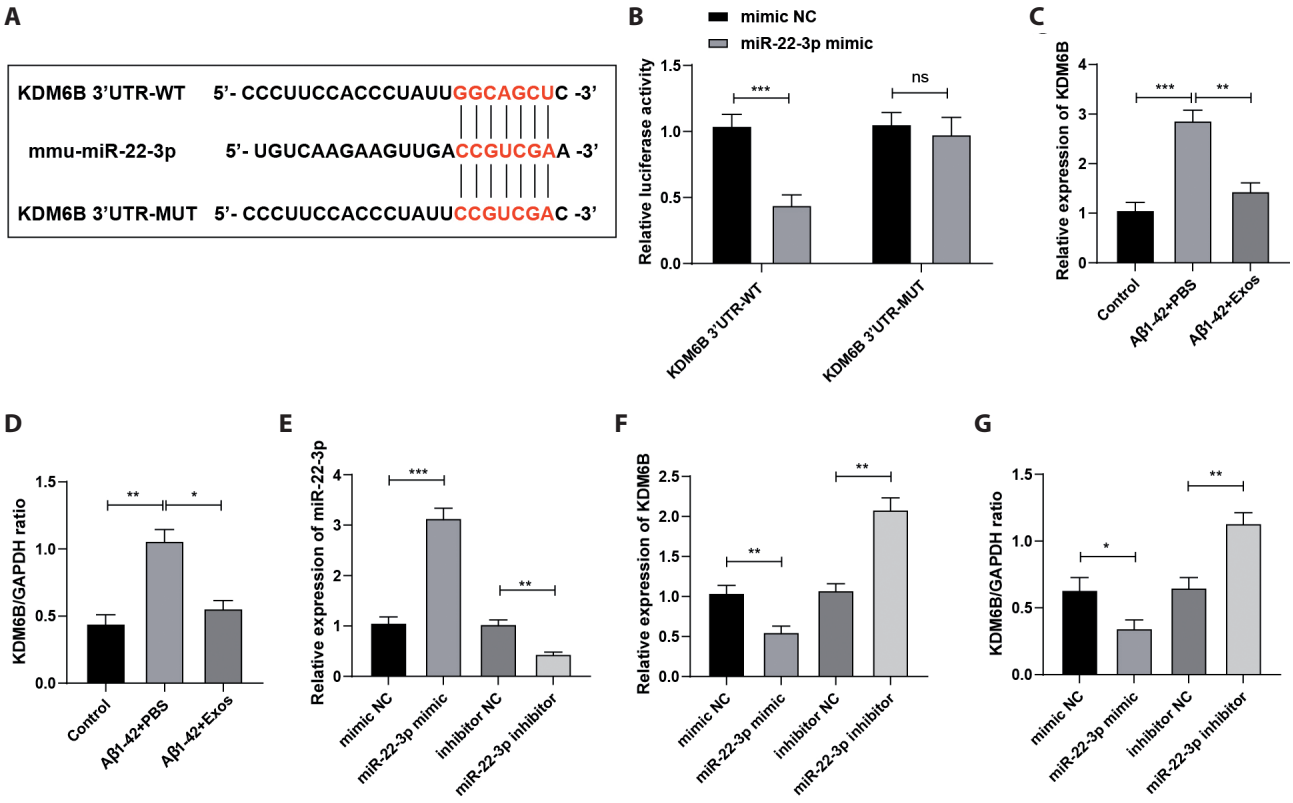

Figure 3. KDM6B in neurons is a target of miR-22-3p. **A.** Specific binding sites of KDM6B and miR-22-3p predicted by TargetScan database. **B.** Dual luciferase reporter gene assay for the target relationship between KDM6B and miR-22-3p. **C.** KDM6B expression in neurons after Aβ1-42 or exosome treatment was evaluated by RT-qPCR. **D.** Protein expression of KDM6B in neurons after Aβ1-42 or exosome treatment was determined by Western blot. **E.** miR-22-3p expression in neurons transfected with miR-22-3p mimic or inhibitor was evaluated by RT-qPCR. **F.** KDM6B expression in neurons after transfected with miR-22-3p mimic or inhibitor was tested by RT-qPCR. **G.** Protein expression of KDM6B in neurons after transfection with miR-22-3p mimic or inhibitor was determined by Western blot. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

BMSC-Exo attenuates oxidative stress damage and apoptosis in neurons through the miR-22-3p/KDM6B axis

To test the direct relationship between BMSC-Exos and KDM6B, we treated each group of cells with Aβ1-42 and exosomes and simultaneously transfected neurons with either oe-KDM6B or miR-22-3p mimic, which indicated that, relative to the Exos+mimic NC+oe-NC treatment, miR-22-3p mimic treatment notably elevated miR-22-3p expression and reduced KDM6B expression in neurons, whereas oe-KDM6B treatment elevated KDM6B expression with no impact on miR-22-3p expression; moreover, the combination of miR-22-3p mimic and oe-KDM6B treatment restored the influence of treatment alone on cellular KDM6B expression (Fig. 5A–C). Further, we found that compared to the Exos+mimic NC+oe-NC treatment, in the presence of oe-KDM6B, there showed decreased cell viability and SOD activity, increased cellular ROS and MDA content, and elevated apoptosis rate, and with miR-22-3p mimic, there exhibited elevated cell viability and SOD activity, reduced cellular ROS and MDA content, as well as decreased apoptosis rate; whereas, the simultaneous action of miR-22-3p mimic and oe-KDM6B reversed the functional indices of cells in the Exos+miR-22-3p mimic+oe-NC group (Fig. 5D–H).

In summary, we find that BMSC-Exos carrying miR-22-3p attenuates oxidative stress damage and apoptosis of neurons in AD by restraining KDM6B expression.

Discussion

AD is a multifactorial neurodegenerative disease and is chiefly featured with progressive impairment in emotion, cognition, language, as well as memory in the older population (Se Thoe et al. 2021). The impetus for comprehension of AD-pivoted mechanism and exploration of novel disease-modifying treatments, this work is started from the perspective of miR-22-3p released by BMSC-Exos and KDM6B.

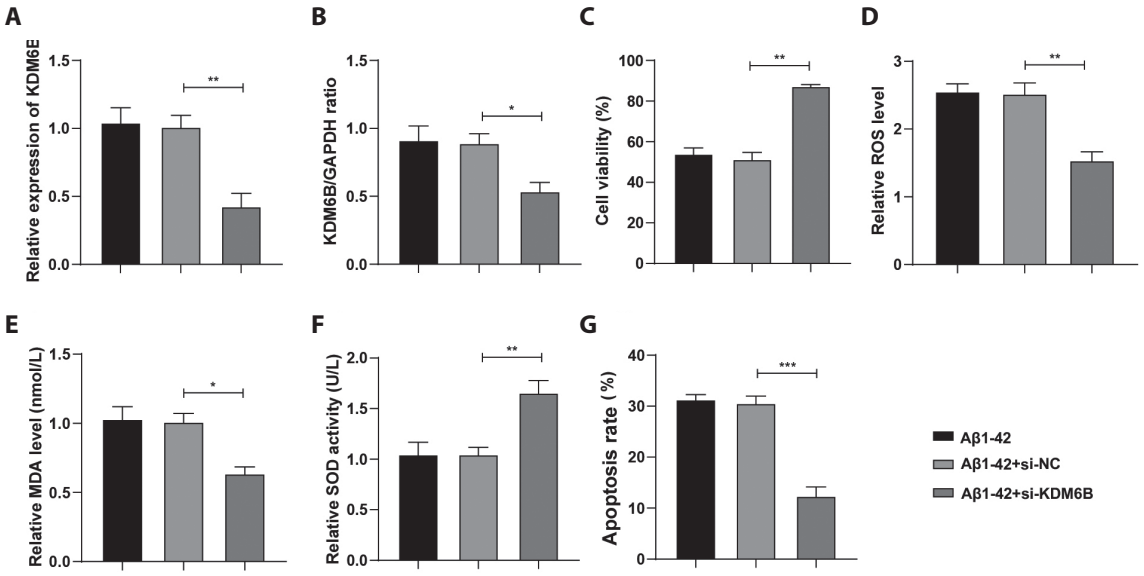

Figure 4. KDM6B promotes neuronal oxidative stress injury and apoptosis. KDM6B expression in HT22 cells after transfection with si-KDM6B was tested by RT-qPCR (**A**) and by Western blot (**B**). **C.** CCK-8 assay was utilized to measure the cell viability of HT22 cells. Detection of cellular ROS level (**D**), MDA level (**E**), and SOD activity (**F**) to assess oxidative stress. **G.** Flow cytometry was employed to evaluate the degree of apoptosis. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Thus, this study discussed the reciprocal between miR-22-3p and KDM6B, which indicated that BMSCs-derived exosomal miR-22-3p targeted KDM6B to regulate oxidative stress and neuronal apoptosis in AD.

miRNAs have been demonstrated to be involved in multiple physiological processes together with pathological pathways, consisting of embryonic development, tumorigenesis, as well as cardiac disease (Lozano-Velasco et al. 2022). Many miRNAs have been suggested as down-regulated and participate in senile plaques along with neurofibrillary tangles (Takousis et al. 2019). This paper revealed lower miR-22-3p and higher KDM6B in the hippocampal CA3 area in AD rats. Next, restored miR-22-3p or depleted KDM6B were transfected into cells *in vitro* and it was manifested that restored miR-22-3p or depleted KDM6B enhanced cell viability and inhibited oxidative stress and apoptosis of neurons in AD. As previously reported, miR-22-3p is down-regulated in the serum of AD patients (Guo et al. 2017). Furthermore, another article has shown that miR-22-3p regulates the Aβ deposition in AD models *via* mitogen-activated protein kinase 14 (Ji et al. 2019), yet miR-22 modulates the AD silence disease neuroinflammation (Han et al. 2020). To further reveal the downstream regulatory targets of miR-22-3p, we further searched the TargetScan database to forecast the downstream genes of miR-22-3p, and identified a site where miR-22-3p and KDM6B bind to each other. As to KDM6B expression, it is reflected that overexpression of KDM6B is manifested in neurons in oxygen-glucose deprivation injury (Zhang et al. 2018). KDM6B, serving as an epigenetic regulator, modulates transcriptional activation in the process of hematopoietic stem and progenitor cell differentiation, along with immune responses (Wei et al. 2018). KDM6B depletion has been demonstrated to alleviate neurological deficits and decrease infarct volume post-ischemic injury (Zhang et al. 2018).

BMSCs are typical stem cells, which were differentiated into varying cells under changed physiological conditions (Li et al. 2002). Some studies have investigated the biological capabilities of BMSCs and exosomes from BMSCs, which highlights their beneficial influences against diverse diseases (Yu et al. 2015; Jin et al. 2019). As reported, exosomes derived from BMSCs are able to reduce neuroinflammation together with neuronal apoptosis in primary neurons of transgenic AD mice (Lee et al. 2018). Liu et al. have stated that BMSCs-Exos reduce inflammation and protect from abnormal neurogenesis together with memory dysfunction post-status epilepticus (Liu S et al. 2022). In this research, we also observed that BMSC-Exos enhances antioxidant capacity and reduces neuronal apoptosis in the hippocampal CA3 area in AD rats. Moreover, we found that BMSC-Exos up-regulate miR-22-3p and down-regulate KDM6B to modulate antioxidant capacity and neuronal apoptosis in AD. Similar to our findings, exosomal miR-22-3p could mitigate brain ischemic injury via impeding KDM6B-modulated influences on the BMP2/BMF axis (Zhang et al. 2021).

A study has reported a novel mechanism by which advanced glycation end products influence the osteogenic process and antioxidant capacity of periodontal ligament

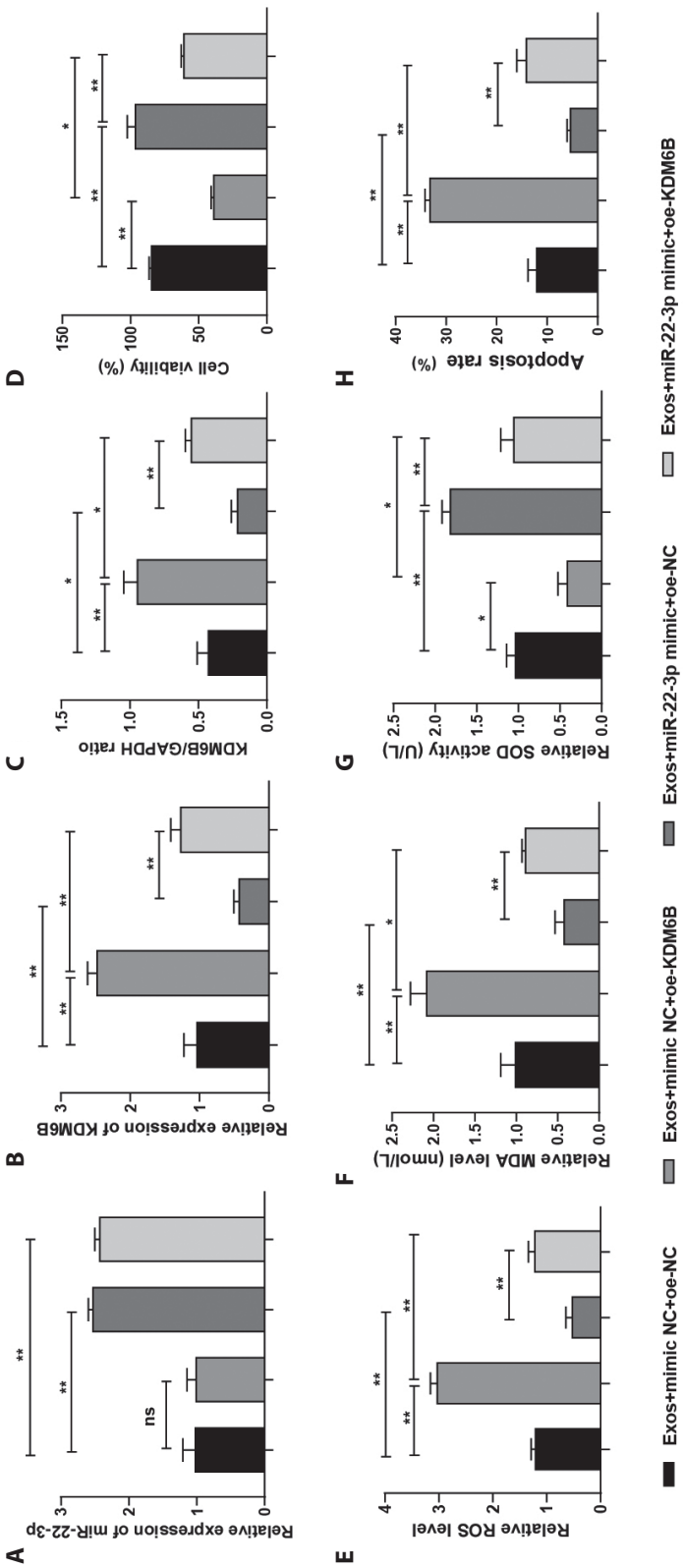

Figure 5. BMSC-Exo attenuates oxidative stress damage and apoptosis in neurons through the miR-22-3p/KDM6B axis. miR-22-3p expression (**A**) and KDM6B mRNA expression (**B**) in HT22 cells after co-treatment with Aβ1-42, exosomes, miR-22-3p mimic, or KDM6B overexpressing lentivirus was determined by RT-qPCR. **C.** KDM6B protein expression in HT22 cells after co-treatment with Aβ1-42, exosomes, miR-22-3p mimic, or KDM6B overexpressing lentivirus was assessed by Western blot. **D.** CCK-8 assay for the detection of cell viability in HT22 cells after co-treatment. Detection of ROS level (**E**), MDA level (**F**), and SOD activity (**G**) to assess oxidative stress in cells after co-treatment. **H.** Flow cytometry for the determination of the degree of apoptosis in cells after co-treatment. * $p < 0.05$, ** $p < 0.01$.

stem cells through a KDM6B/Wnt self-reinforcing loop under orthodontic force (Ying et al. 2024). Shuangyu Han et al. indicated that KDM6B inhibition can reduce the expression of NOX4, thereby decreasing oxidative stress (Han et al. 2025). Furthermore, data suggests that silencing KDM6B can inhibit neurological deficits, decrease cerebral infarction volume, alleviate neuronal apoptosis, and ameliorate inflammation. Additionally, KDM6B-mediated demethylation of IRF4 leads to aggravated ischemic brain injury through SOX9 activation (Chang et al. 2022). As revealed by the results of this study, KDM6B promotes neuronal oxidative stress damage and apoptosis in AD. As a histone demethylase, KDM6B may affect cellular antioxidant capacity by regulating the expression of genes related to antioxidant defense. When KDM6B is inhibited, the expression and activity of certain antioxidant enzymes (such as SOD) may increase, thereby more effectively scavenging ROS produced within cells. Moreover, oxidative stress is closely related to inflammatory responses. Inhibition of KDM6B may reduce the production and release of inflammatory cytokines, mitigating the damage to neurons caused by inflammatory responses and indirectly reducing oxidative stress. On the other hand, inhibition of KDM6B may regulate apoptosis by affecting the expression and activity of apoptosis-related genes (such as members of the Bcl-2 family). Simultaneously, inhibition of KDM6B may also affect apoptosis by regulating intracellular signaling pathways (such as PI3K/Akt, MAPK, etc.). In other words, in AD, inhibiting KDM6B can enhance neuronal antioxidant capacity and anti-apoptotic ability, thereby protecting neurons from AD-related damage. Additionally, the regulation of KDM6B may also involve the modulation of neuroinflammation. In AD, neuroinflammation is an important pathological process. By inhibiting KDM6B, the production and release of inflammatory cytokines may be reduced, thereby alleviating the damage to neurons caused by neuroinflammation. However, further mechanistic studies are needed to elucidate how KDM6B inhibition specifically leads to the reduction of oxidative stress and apoptosis.

To conclude, this study highlights that exosomal miR-22-3p enhanced cell viability and inhibited oxidative stress and apoptosis of neurons in AD. This paper helps the development of a new strategy of neuroprotection in AD. As a key mediator in this study, miR-22-3p exerts protective effects by targeting and regulating KDM6B. In addition to miR-22-3p, there may be other microRNAs involved in this process, which may synergistically protect neurons from oxidative stress and apoptosis by regulating different targets. However, this study did not delve deeper into other microRNAs and potential mediators that may be involved, nor did it explore in detail the mechanism by which KDM6B inhibition specifically reduces oxidative stress and apoptosis. Future research should aim to further unravel the regulatory mechanisms of KDM6B, as well as the broad impacts of other microRNAs and potential mediators in AD, with the goal of providing more effective strategies and methods for the treatment of AD.

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