PARK7 promotes repair in early steroid-induced osteonecrosis of the femoral head by enhancing resistance to stress-induced apoptosis in bone marrow mesenchymal stem cells via regulation of the Nrf2 signaling pathway

Fei Zhang1,2,3, Yanglin Yan1,2,3, Wuxun Peng1,2,3, Lei Wang1,2,4, Tao Wang1,2,4, Zhihong Xie2, Hong Luo2, Jian Zhang1,2 and Wentao Dong1,2

© The Author(s) 2021

INTRODUCTION
Steroid-induced osteonecrosis of the femoral head (SONFH), a joint dysfunction caused by long-term heavy use of glucocorticoids, results in a high rate of disability. Effective early-stage treatment strategies are urgently needed in the field of orthopedics [1, 2]. In recent years, cells with bone regeneration ability such as bone marrow mesenchymal stem cells (BMSCs) have been used to treat early-stage SONFH. However, the survival of the cells seeded into the osteonecrotic area is key to achieving successful transplantation [3, 4]. In our previous study, we used BMSCs to construct tissue-engineered bone for the repair of early-stage SONFH. However, transplantation efficacy was not satisfactory mainly because numerous BMSCs transplanted into the osteonecrotic area underwent stress-induced apoptosis [5].

Oxidative stress (OS) plays an important role in SONFH development and in osteonecrosis repair [6, 7]. OS results from increased levels of reactive oxygen species (ROS), decreased ROS-scavenging ability, or imbalance in oxidative and antioxidant defense system, leading to excessive ROS production in cells. These events can alter the cellular redox state; damage deoxyribonucleic acid (DNA), proteins, lipids, and other biological macromolecules; change cellular structure and function; and trigger a variety of apoptotic signaling pathways, such as those involving tumor protein 53 (p53) and p38/mitogen-activated protein kinase (p38/MAPK), to induce apoptosis [8–12]. An OS microenvironment exists in the osteonecrotic area of the femoral head; therefore, many BMSCs transplanted into this area undergo stress-induced apoptosis, which inhibits the reparative effectiveness of this therapy in...
early-stage SONFH [13, 14]. Therefore, enhancing resistance to OS in BMSCs will likely improve their therapeutic efficacy in early-stage SONFH.

The highly conserved Parkinson disease protein 7 (PARK7) exists as a homologous dimer composed of 189 amino acids. PARK7 is widely expressed in various cells and tissues, including BMSCs. As an active oxygen scavenger and antioxidant, PARK7 has an important role in cell survival and resistance to OS [15–18]. The results obtained in our previous study confirmed that PARK7 enhances OS resistance in BMSCs, thereby enabling them to resist stress-induced apoptosis [19]. However, the molecular mechanism of PARK7-mediated resistance to OS, and whether PARK7-mediated resistance to stress-induced apoptosis improves the efficacy of BMSC transplantation in early-stage SONFH, remain unclear.

In this study, we further explored the molecular mechanism underlying PARK7-mediated resistance to OS and evaluated the role of PARK7 in BMSC transplantation for the repair of early-stage SONFH. Our study will provide new methods for promoting resistance to stress-induced apoptosis in BMSCs and improving their therapeutic effectiveness in early-stage SONFH.

RESULTS

PARK7 enhances BMSC resistance to OS
First, we explored the role of PARK7 in BMSC response to OS. For this, we inserted the coding sequence region of the PARK7 gene into an overexpressed lentiviral vector (OE-PARK7). We then designed a short-hairpin ribonucleic acid-targeting PARK7 and inserted it into an interfering lentiviral vector (Sh-PARK7). After we successfully isolated and cultured BMSCs (Fig. S1), these lentiviruses were consequently transfected into BMSCs. At 5 days after transfection, we observed that green fluorescent protein was successfully expressed in BMSCs, and the transfection efficiency was >90% (Fig. 1A, B). The results of real-time quantitative polymerase chain reaction (qPCR) and immunoblotting also confirmed that PARK7 expression was successfully up or downregulated in BMSCs (Fig. 1C, D). Finally, we treated BMSCs with a high concentration of hydrogen peroxide (H2O2; 1000 μM) for 24 h to simulate conditions of OS. Our results indicate that after prolonged exposure to OS, BMSCs showed downregulated expression of antioxidant enzymes including manganese superoxide dismutase (MnSOD), catalase (CAT), and glutathione peroxidase (GPx) (Fig. 1E–H); increased contents of malondialdehyde (MDA) (Fig. 1I); decreased mitochondrial membrane potential (MMP) (Fig. 1M, N); and increased rate of apoptosis (Fig. 1O, P). However, these results were reversed in PARK7-overexpressing BMSCs, which showed upregulated expression of MnSOD, CAT, and GPx (Fig. 1E–H); decreased intracellular ROS (Fig. 1K, L) and MDA levels (Fig. 1I); increased MMP expression (Fig. 1M, N) and cell activity (Fig. 1J); and decreased rate of apoptosis (Fig. 1O, P). Conversely, when PARK7 expression was downregulated in BMSCs, the results obtained using the abovementioned indicators were the opposite of those obtained using PARK7 overexpression (Fig. 1E–P). These findings suggest that PARK7 enhanced the ability of BMSCs to resist OS and promoted BMSC survival under conditions of OS.

The Nrf2 signaling pathway participates in reversal of OS in BMSCs
The antioxidant enzymes MnSOD, CAT, and GPx, and downstream effector proteins of the nuclear factor (erythroid-derived 2)–like 2 (Nrf2) signaling pathway, are crucial for the elimination of intracellular ROS from cells [20, 21]. Therefore, we further studied the role of the Nrf2 signaling pathway in reducing OS in BMSCs. We transfected BMSCs with an Nrf2-overexpressing lentivirus (OE-Nrf2) or interference lentivirus (Sh-Nrf2) to upregulate or downregulate the expression of Nrf2 in BMSCs. The results of qPCR and immunoblotting confirmed that Nrf2 expression was successfully upregulated or downregulated in BMSCs (Fig. 2A–C). We then treated BMSCs with 1000 μM H2O2 for 24 h. Our results show that after prolonged exposure to OS, the expression of MnSOD, CAT, and GPx were downregulated (Fig. 2F–I); the levels of MDA (Fig. 2J) and ROS (Fig. 2K, L) were increased, and the apoptotic rate was significantly increased (Fig. 2M, N) in BMSCs. However, upregulating the expression of Nrf2 in BMSCs significantly increased the content of free Nrf2 in the nucleus (Fig. 2D, E); increased cellular expression of MnSOD, CAT, and GPx (Fig. 2F–I); decreased the levels of MDA and ROS (Fig. 2J–L); and significantly decreased the apoptotic rate (Fig. 2M, N) compared with the values obtained in the H2O2/NC group. Downregulation of Nrf2 expression yielded results that were opposite of those obtained using upregulation of Nrf2 expression (Fig. 2D–N). These findings suggest that the Nrf2 signaling pathway enhanced resistance to OS in BMSCs by regulating the expression of antioxidant enzymes, such as MnSOD, CAT, and GPx, which are crucial for maintaining cellular redox homeostasis.

PARK7 protects BMSCs from OS by regulating the Nrf2 signaling pathway
Our results suggest that by regulating the expression of antioxidant enzymes, such as MnSOD, CAT, and GPx, the Nrf2 signaling pathway can enhance resistance to OS in BMSCs (Fig. 2). Functional experiments involving PARK7 show that PARK7 can also promote the expression of these antioxidant enzymes and enhance the ability to resist OS in BMSCs (Fig. 1). Therefore, we hypothesized that PARK7 can protect BMSCs from OS by regulating the Nrf2 signaling pathway. To verify this hypothesis, we first transfected BMSCs with PARK7-overexpressing lentivirus to upregulate the expression of PARK7 in BMSCs (Fig. 3A, B); then, we treated these BMSCs with 1000 μM H2O2 for 24 h. Our results show that BMSCs subjected to extended exposure to OS downregulated their expression of MnSOD, CAT, and GPx (Fig. 3E–H) and increased their levels of MDA and ROS (Fig. 3D, I, J); in addition, numerous cells underwent stress-induced apoptosis (Fig. 3K, L). However, in BMSCs-overexpressing PARK7 and subjected to extended exposure to OS, the expression of MnSOD, CAT, and GPx was upregulated (Fig. 3E–H), whereas MDA and ROS levels were decreased (Fig. 3D, I, J), which can effectively inhibit OS-induced apoptosis (Fig. 3K, L). Based on the results obtained using upregulation of PARK7 expression, we then downregulated the expression of Nrf2 in BMSCs (Fig. 3A, C) and treated these BMSCs with 1000 μM H2O2 for 24 h. In these cells, the expression of MnSOD, CAT, and GPx was downregulated (Fig. 3E–H), whereas MDA and ROS levels, and rate of apoptosis, were increased (Fig. 3D, I–L). These findings show that downregulation of Nrf2 expression weakened PARK7-mediated protection against OS in BMSCs. Taken together, these results confirm that PARK7 protected BMSCs from OS-induced stress by regulating the Nrf2-signaling pathway.

PARK7 regulates the Nrf2 signaling pathway by promoting dissociation of the Keap1–Nrf2 complex
Under conditions of OS, the Nrf2 transcription factor binds to Kelch-like echinacoside (ECH)–associated protein 1 (Keap1) in the cytoplasm to form the Keap1–Nrf2 complex [22]. When stimulated by OS, Nrf2 dissociates from Keap1. Then, the free Nrf2 translocates to the nucleus and binds to antioxidant-response elements with the assistance of the musculoaponeurotic fibrosarcoma oncogene homolog protein, thereby initiating the transcription and expression of downstream genes encoding antioxidant enzymes [23, 24]. To investigate the mechanism underlying PARK7-mediated regulation of the Nrf2 signaling pathway, we first treated PARK7-overexpressing BMSCs with H2O2 to simulate conditions of OS. Then, we used immunoprecipitation with an
antibody specific for Nrf2 to analyze the effect of PARK7 expression on the dissociation of the Keap1–Nrf2 complex. Our results show that under conditions of OS, PARK7 did not regulate the content of total Nrf2 and Keap1 in BMSCs (Fig. 4A, D, E), but did affect the interaction between Nrf2 and Keap1 (Fig. 4A–C). Partial dissociation occurred in both H₂O₂/BMSC and H₂O₂/BMSC/NC groups (Fig. 4A–C). In contrast, the dissociation of Keap1–Nrf2 complex increased in the group overexpressing PARK7 (Fig. 4A–C). Having established that PARK7 promoted the dissociation of the Keap1–Nrf2 complex, we next investigated whether PARK7 could
Fig. 1 PARK7 enhances the ability of BMSCs to resist oxidative stress. After BMSCs were transfected with the PARK7 overexpression or PARK7-interfering lentivirus: A Expression levels of the reporter gene GFP were observed using an inverted fluorescence microscope (n = 6). PARK7 Parkinson disease protein 7, BMSCs bone marrow mesenchymal stem cells, GFP green fluorescent protein, NC negative control, Sh-PARK7 short-hairpin ribonuclease acid of PARK7, OE-PARK7 overexpression of PARK7. B GFP expression levels were quantified in BMSCs as shown in A (n = 6). C RT-qPCR analysis of PARK7 mRNA expression in BMSCs (n = 3). RT-qPCR, real-time quantitative polymerase chain reaction. D Immunoblot analysis of PARK7 expression levels in BMSCs (n = 3). After PARK7 expression was upregulated or downregulated in BMSCs, BMSCs were treated with H2O2 to simulate oxidative stress. E Immunoblot analysis of CAT, GPx, and MnSOD expression in BMSCs (n = 3). MnSOD manganese superoxide dismutase, CAT catalase, GPx glutathione peroxidase, H2O2 hydrogen peroxide. F Quantification of CAT expression is shown in E (n = 3). G Quantification of GPx expression is shown in E (n = 3). H Quantification of MnSOD expression is shown in E (n = 3). I Detection of MDA levels using thiobarbituric acid assay (n = 6). MDA malondialdehyde. J Detection of cell viability using Cell Counting Kit-8 (n = 6). K Detection of ROS levels using DHE (n = 6). ROS reactive oxygen species, DHE dihydroethidium. L Quantification of ROS levels is shown in K (n = 6). M Detection of MMP using a JC-1 assay (n = 5). MMP mitochondrial membrane potential, DAPI 4',6-diamidino-2-phenylindole; JC-1, 5,5′,6′,6′-tetrachloro-1,1′,3′,3′-tetraethyl-imidacarbocyanine. N Quantification of MMP is shown in M (n = 5). O Flow cytometry analysis of apoptosis (n = 5). PI propidium iodide, FITC fluorescein isothiocyanate. P Quantification of apoptosis levels is shown in O (n = 6). All data are represented as mean ± standard deviation (SD). *P < 0.05. Differences were tested using one-way analysis of variance (ANOVA) with Tukey’s post hoc test (B–D, F–J, L, N, P).
the necrotic area of the femoral head, which greatly limits transplantation efficacy [5, 27, 28]. Inhibiting stress-induced apoptosis of BMSCs is key to resolving this issue. In this study, we investigated the role of PARK7 in promoting resistance to stress-induced apoptosis in BMSCs. Our results show that PARK7 promoted disintegration of the Keap1–Nrf2 complex, which led to activation of Nrf2. The activated Nrf2 entered the nucleus and initiated the expression of antioxidant enzymes such as MnSOD, CAT, and GPx, which eliminate excessive cellular ROS and protect cells from OS-induced injury and stress-induced apoptosis. We then investigated whether PARK7-mediated resistance to stress-induced apoptosis could improve the transplantation efficacy of
BMSCs in early-stage SONFH. For this, we transfected BMSCs-overexpressing PARK7 into rats with induced early-stage SONFH. Our results indicate that PARK7 overexpression effectively imparted resistance to stress-induced apoptosis in BMSCs transplanted into the osteonecrotic area, thereby improving the transplantation efficacy of BMSCs in early-stage SONFH.

OS is an important pathophysiological mechanism in SONFH [6, 7]. Dysfunction of the cellular mitochondrial aerobic respiratory chain results in the production of excessive ROS due to hypoxia in the femoral head necrotic area [29]. After the femoral head becomes necrotic, numerous inflammatory cells infiltrate, and these inflammatory cells will also produce excessive ROS in BMSCs [30]. Excessive ROS can change the redox state of transplanted BMSCs and damage mitochondria, DNA, proteins, lipids, and other biomacromolecules, resulting in stress-induced BMSC apoptosis [31, 32]. Our results indicate that under conditions of OS, BMSCs decreased their expression of MMPs and increased those of ROS and MDA; consequently, numerous BMSCs underwent stress-induced apoptosis. Therefore, enhancing the ability of BMSCs to resist OS is conducive to their survival in the OS microenvironment, and will improve the transplantation efficacy of BMSCs in early-stage SONFH.

PARK7 is an antioxidant protein that can enhance cellular resistance against OS and stress-induced apoptosis [33, 34]. In our present study, the results of our functional experiments with PARK7 showed that PARK7 overexpression reduced ROS levels in BMSCs; increased the expression of MnSOD, CAT, and GPx; protected the mitochondria; and enhanced BMSC resistance to OS and stress-induced apoptosis. Conversely, downregulation of PARK7 expression led to a decrease in BMSC resistance to OS, and numerous BMSCs underwent stress-induced apoptosis under conditions of OS. This study demonstrates how PARK7 can protect the mitochondria and maintain mitochondrial function via auto-oxidation of cysteine residues at sequences 46, 53, and 106. PARK7 can also auto-oxidize to monitor OS and can activate extracellular signal-regulated kinase 1/2 (ERK1/2), apoptotic signal-regulating kinase 1 (ASK1), and phosphatidylinositol 3-kinases (PI3K)/Akt signaling pathways, thereby enhancing resistance to OS and inhibiting stress-induced apoptosis [35–40]. In this study, we investigated the role of the Nrf2 signaling pathway in PARK7-mediated resistance to stress-induced BMSC apoptosis. Our results indicate that PARK7 promotes the dissociation of Keap1–Nrf2 complex, thereby activating Nrf2. The activated Nrf2 then entered the nucleus to initiate the expression of MnSOD, CAT, GPx, and other genes encoding antioxidant enzymes. This cascade resulted in the removal of excessive cellular ROS and protected the cells from OS-induced injury and stress-induced apoptosis. To evaluate the effect of PARK7 in vivo, we transfected BMSCs-overexpressing PARK7 into rats with early-stage SONFH. Our results show that PARK7 imparted resistance to stress-induced apoptosis in BMSCs transplanted into the osteonecrotic area, thereby improving the therapeutic efficacy of transplantation.

In conclusion, our study shows that PARK7 enhanced resistance to OS and inhibited stress-induced apoptosis in BMSCs by regulating the Nrf2 signaling pathway. This enhanced protection against OS improved the therapeutic efficacy of BMSCs used to repair early-stage SONFH. Our study provides a new approach for preventing stress-induced BMSC apoptosis and improves their therapeutic efficacy in early-stage SONFH.

MATERIALS AND METHODS

Animals

All animal studies were approved by the Experimental Animal Bioethics Committee of the Guizhou Medical University (GUMU), Guiyang, China. All procedures involving animals conformed to the Guide for the Care and Use of Laboratory Animals in accordance with Directive 2010/63/EU of the European Parliament. BMSCs were extracted from young male Sprague Dawley (SD) rats (20–30 g, 60 rats). The model of SONFH was established in adult male SD rats (400–500 g, 150 rats). All SD rats were provided by the Laboratory Animal Center of GUMU (no. 1800815).

BMSC isolation and culture

Bilateral femurs and tibias were harvested under aseptic conditions from young male SD rats weighing 20–30 g. The medullary cavity was flushed with l-glutamine Dulbecco’s modified Eagle medium (l-DMEM; Gibco (Thermo Fisher Scientific, Waltham, MA, USA)). Bone marrow tissue was centrifuged at 1000 rpm for 5 min to remove suspended adipose tissue. Bone marrow precipitates were then resuspended in complete l-DMEM (10% fetal bovine serum (FBS) and 1% double-antibody (aB); Gibco) and cultured at 37°C and 5% CO2. When primary BMSCs reached 90% confluence were differentiated using osteogenic- or chondrogenic-differentiation medium (Cyagen Biosciences, Shanghai, China). After osteogenic differentiation, they were digested using 0.25% trypsin–0.02% ethylenediaminetetraacetic acid (EDTA; Gibco) and passaged at a ratio of 1:3. Third-generation BMSCs were used for subsequent experiments.

Identification of BMSC surface antigens using flow cytometry

First, 5 μL of mouse anti-CD90-PeCy7TM7, mouse anti-CD106-PE, mouse anti-CD11b-V450, and mouse anti-CD45-fluorescein isothiocyanate (FITC) (BD Biosciences, Franklin Lakes, NJ, USA) was added into an FCM tube, followed by the addition of 50 μL cell suspension (2 × 10^7/mL). The mixture was incubated at room temperature (RT) in the dark for 30 min, and the contents of each tube were then washed twice using a standing buffer. Then, 500 μL staining buffer was added to resuspend the cells, and FCM (Beckman Coulter Life Sciences, Brea, CA, USA) was used to detect expression levels of CD11b, CD45, CD90, and CD106.

BMSC identification using osteogenic, cartilage, and adipogenic differentiation

Third-generation BMSCs at 60–70% confluence were differentiated using osteogenic- or chondrogenic-differentiation medium (Cyagen Biosciences, Santa Clara, CA, USA). After 2 weeks of osteogenic induction, 0.1% Alizarin

Fig. 2 Nrf2 signaling pathway participates in reversing oxidative stress in BMSCs. After BMSCs were transfected with the Nrf2 overexpression or Nrf2 interfering lentivirus. A Immunoblot analysis of Nrf2 protein expression in BMSCs (n = 3). Nrf2 nuclear factor (erythroid-derived 2)-like 2, Sh-Nrf2 short-hairpin ribonucleic acid of Nrf2, OE-Nrf2 overexpression of Nrf2. B Quantification of Nrf2 expression is shown in A (n = 3). C RT-qPCR analysis of Nrf2 mRNA expression in BMSCs (n = 3). After Nrf2 was upregulated or downregulated in BMSCs, BMSCs were treated with H2O2 to simulate oxidative stress. D Immunoblot analysis of Nrf2 protein expression in BMSC nuclei (n = 3). H3, histone 3. E Quantification of Nrf2 expression is shown in F (n = 3). F Immunoblot analysis of MnSOD, CAT, and GPx protein expression in BMSCs (n = 3). G Quantification of MnSOD expression is shown in F (n = 3). H Quantification of CAT expression is shown in F (n = 3). I Quantification of GPx expression is shown in F (n = 3). J Detection of MDA levels using thiobarbituric acid assay (n = 6). K Detection of ROS levels using DCFH-DA (n = 5). DCFH-DA dichlorodihydrofluorescein diacetate. L Quantification of ROS levels is shown in K (n = 5). M Analysis of apoptosis activity using TUNEL/DAPI staining (n = 5). TUNEL terminal deoxynucleotidyl transferase deoxyuridine-5′-triphosphate nick end labeling. N Quantification of TUNEL-positive signal in BMSCs is shown in M (n = 5). All data are represented as mean ± SD. *P < 0.05. Differences were evaluated using one-way ANOVA with Tukey’s post hoc test (B–C, E, G–J, L, N).
Red stain (Cyagen) was used to identify calcium nodules, and a modified Gomori calcium cobalt stain (Cyagen) was used to detect ALP activity. After 4 weeks of induction, an Alisin Blue stain (Cyagen) was used to identify acid mucopolysaccharides in cartilage. When the degree of cellular fusion of third-generation BMSCs reached 100%, an adipogenic-induction medium (Cyagen) was used to induce BMSC differentiation. After 3 weeks of induction, Oil Red O stain (Cyagen) was used to identify lipid droplets in the cells.

**BMSC model of OS**

Third-generation BMSCs were treated using 1000 μM H₂O₂ for 24 h to mimic conditions of OS as described previously [5, 41].
Fig. 3  PARK7 protects BMSCs from oxidative stress by regulating the Nrf2 signaling pathway. After BMSCs were transfected with the PARK7 overexpression or Nrf2 interfering lentivirus. A Immunoblot analysis of PARK7 and Nrf2 protein expression in BMSCs (n = 5). B Quantification of PARK7 expression is shown in A (n = 5). C Quantification of Nrf2 expression is shown in A (n = 5). After PARK7 or Nrf2 expression was successfully up or downregulated in BMSCs, BMSCs were treated with H2O2 to simulate oxidative stress. D Detection of MDA levels using thiobarbituric acid (n = 6). E Immunoblot analysis of MnSOD, CAT, and GPx protein expression in BMSCs (n = 3). F Quantification of MnSOD expression is shown in E (n = 3). G Quantification of CAT expression is shown in E (n = 3). H Quantification of GPx expression is shown in E (n = 3). I Detection of ROS levels using DHE (n = 5). J Quantification of ROS levels is shown in I (n = 5). K Analysis of apoptosis levels using TUNEL/DAPI staining (n = 5). L Quantification of TUNEL-positive signal in BMSCs is shown in K (n = 5). All data are represented as mean ± SD. *P < 0.05. Differences were tested using one-way ANOVA with Tukey’s post hoc test (B–D, F–H, J, L).

Lentiviral transfection and screening of mixed clones

PARK7 and Nrf2 overexpression and interference lentiviruses were purchased from China Shanghai Genechem Co., Ltd. (Shanghai, China). Based on pre-transfection experiments conducted to determine the optimal multiplicity of infection (100) and transfection conditions (HitransG P; China Shanghai Genechem), BMSCs were infected with the lentiviruses; NC and blank controls were processed concurrently using identical transfection conditions (HitransG P; China Shanghai Genechem). BMSCs were infected with the lentiviruses; optimal multiplicity of infection (100) and transfection conditions (HitransG P; China Shanghai Genechem) were used to screen the cells successfully transfected by lentivirus. After all the blank control cells died, the concentration of purinomycin was reduced to 1 μg/mL to maintain screening in order to obtain mixed clone cell lines.

Real-time qPCR

RNA was extracted from BMSCs using column affinity purification (QIAGEN, Hilden, Germany). Complementary DNA was synthesized using M-MuLV RT Master Mix with Oligo(dT) (Sangon Biotech, Shanghai, China). RT-qPCR was performed on a StepOnePlus system (Applied Biosystems, Foster City, CA, USA) in 96-well plates using specific primers and SYBR Green Mix (Sangon Biotech). Rat primer (Sangon Biotech) sequences were as follows: PARK7-F: AGGCGAGCTGGGATTAAGGT; PARK7-R: GAGCAGGACTGATTACATACGGCC; Nrf2-F: CCTGATGACATGAGTCGC; Nrf2-R: AACATGGATATGCTGCTTA; β-actin (ACTB)-F:CACCCGGGAGTACCACTTCT; ACTB-R: CCATACCCACATCACACCC. Fold change values in RNA expression over that of control were calculated using the ΔΔCt method.
Immunoblotting

Cells were lysed using radioimmunoprecipitation assay cell lysate buffer (Beyotime Institute of Biotechnology, Shanghai, China). The lysates were centrifuged at 13,000 × g/min for 10 min, and protein concentration in the supernatants was determined using a bicinchoninic acid protein concentration detection kit (Solarbio, Beijing, China). Proteins were separated using sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to a polyvinylidene fluoride membrane (MilliporeSigma, Burlington, MA, USA). The membrane was first blocked in 5% bovine serum albumin at RT for 2 h and then incubated overnight at 4 °C with the following primary antibodies: rabbit anti-rat PARK7 (1:5000; ab76008) and mouse anti-rat Nrf2 (1 µg/ml; ab89443; Abcam).
Fig. 5 PARK7 promotes BMSC survival in the oxidative stress microenvironment of the femoral head necrotic area. At 6 weeks after the establishment of the early-SONFH model using treatment with methylprednisolone. A MRI assessment of osteonecrosis (n = 6). B Micro-CT assessment of osteonecrosis (n = 6). C HE staining of osteonecrotic areas (n = 6). D Expression level of PARK7 in the necrotic area of femoral head was detected by immunofluorescence labeling (n = 6). E Quantification of PARK7 expression levels is shown in D (n = 6). F The level of ROS in the necrotic area of femoral head was detected by DHE staining (n = 6). G Quantification of ROS levels is shown in F (n = 6). At 48 h after BMSC transplantation, H Expression level of PARK7 in the transplanted area was detected using immunofluorescence labeling (n = 6). L Detection of ROS was detected using immunofluorescence labeling (n = 6). I Quantification of PARK7 expression levels is shown in H (n = 6). J Expression level of MnSOD in the transplanted area was detected using immunofluorescence labeling (n = 6). K Quantification of MnSOD expression levels is shown in J (n = 6). L Level of ROS in the transplanted area was detected using DHE staining (n = 6). M Quantification of ROS levels is shown in L (n = 6). N Live-animal imaging shows DiR fluorescence intensity in the transplanted area (n = 6). O Quantification of DiR fluorescence intensity is shown in N (n = 6). P Detection of BMSC apoptosis in the transplanted area using TUNEL staining (n = 6). Q Quantification of TUNEL-positive signal in BMSCs is shown in P (n = 6). R Immunoblot analysis of Bax and Bcl-2 protein expression in the transplanted area (n = 6). S Quantification of Bax expression is shown in Q (n = 6). T Quantification of Bcl-2 expression is shown in R (n = 6). All data are represented as mean ± SD. *P < 0.05. A two-tailed unpaired Student’s t test was used for comparative analyses involving two groups (E, G). One-way ANOVA with Tukey’s post hoc test was used for analyses involving more than two groups (I, K, M, O, Q, S, T).
hydrate (10%, 3 mL/kg), placed on the imaging platform, and imaged using a small-animal imaging system (PerkinElmer, MA, USA). Fluorescence intensity in the femoral head necrotic area was then calculated using Living Image software (PerkinElmer).

**Micro-CT**
At 12 weeks after BMSC transplantation, the femoral head tissues were harvested and fixed in paraformaldehyde (4%, 2 days). The tissues were then scanned using micro-CT with a high-resolution system to evaluate bone repair. In brief, the tissue samples were scanned continuously at a resolution of 6.5 µm per voxel. The defect area of the femoral head is the region of interest (ROI). Tb.N, Tb.Th, BV, BV fraction, and the size of the defect area were then calculated for each ROI. NRecon software (Micro Photonics Inc., Allentown, PA, USA) was used for 3D image reconstruction, and CTAn software (Bruker) was used for 3D analysis.

**HE and Masson trichrome staining**
At 12 weeks after BMSC transplantation, the bone tissue was decalcified in 10% EDTA solution at 37 °C for 2 months, dehydrated, embedded in paraffin, and sectioned at the thickness of 3 µm. The sections were deparaffinized, rehydrated, and stained using an HE staining kit (Solarbio, China) or a Masson trichrome staining kit (Solarbio, China) per instructions of the respective manufacturer. The sections were then dehydrated, cleared in xylene, sealed with neutral balsam, and examined under a biological microscope (OLYMPUS BX53, Tokyo, Japan).

**Statistical analysis**
All statistical data were analyzed and graphed using GraphPad Prism software version 6 (GraphPad Software, San Diego, CA, USA). The Kolmogorov–Smirnov test was used to analyze normally distributed data. A two-tailed unpaired Student’s t test was used for comparative analyses.
involving two groups. One-way analysis of variance with Tukey’s post hoc test was used for analyses involving more than two groups. All error bars are expressed as mean ± SD. P < 0.05 was considered statistically significant.

DATA AVAILABILITY
Additional data or reagents are available from the corresponding author upon reasonable request.

REFERENCES
1. Chang C, Greenspan A, Gershwin ME. The pathogenesis, diagnosis and clinical manifestations of steroid-induced osteonecrosis. J Autoimmun. 2020;110:102460.
2. Xu H, Wang C, Liu C, Peng Z, Li J, Jin Y, et al. Cotransplantation of mesenchymal stem cells and endothelial progenitor cells for treating steroid-induced osteonecrosis of the femoral head. Stem Cells Transl Med. 2021;10:781–96.
3. Xiong M, Zhang X, Wang E, Gao L, Chang J. Bone tissue engineering strategy based on the synergistic effects of silicon and strontium ions. Acta Biomater. 2018;72:381–95.
4. Wang T, Teng S, Zhang Y, Wang F, Ding H, Guo L. Role of mesenchymal stem cells on differentiation in steroid-induced avascular necrosis of the femoral head. Exp Ther Med. 2017;13:669–75.
5. Zhang F, Peng W, Zhang J, Dong W, Wu J, Wang T, et al. P53 and Parkin co-regulate mitophagy in bone marrow mesenchymal stem cells to promote the repair of early steroid-induced osteonecrosis of the femoral head. Cell Death Dis. 2020;11:142.
6. Chen K, Liu Y, He J, Pavlos N, Wang C, Kenny J, et al. Steroid-induced osteonecrosis of the femoral head reveals enhanced reactive oxygen species and hyperactive osteoclasts. Int J Biol Sci. 2020;16:1888–1900.
7. Deng G, Niu K, Zhou F, Li B, Kang Y, Liu X, et al. Treatment of steroid-induced osteonecrosis of the femoral head using porous Se@SiO2 nanocomposites to suppress reactive oxygen species. Sci Rep. 2017;7:43914.
8. Gu Y, Han J, Jiang C, Zhang Y, Biomarkers, oxidative stress and autophagy in skin aging. Ageing Res Rev. 2020;59:101036.
9. Ping Z, Peng Y, Lang H, Xinyong C, Zhiyong Z, Xiaocheng W, et al. Oxidative stress in radiation-induced cardiotoxicity. Oxid Med Cell Longev. 2020;2020:3579143.
10. Di Meo F, Cucinelli R, Marzucchi S, Bergamo P, Petillo O, Peluso G, et al. Ginkgo biloba prevents oxidative stress-induced apoptosis blocking p53 activation in neuroblastoma cells. Antioxid (Basel). 2020;9:279.
11. Hu Q, Wang H, He C, Jin Y, Fu Z. Polyacrylate nanoparticles trigger the activation of p38 MAPK and apoptosis via inducing oxidative stress in zebrafish and macrophage cells. Environ Pollut. 2021;269:116075.
12. Dong Y, Zhao J, Zhu Q, Liu H, Wang J, Lu W. Melatonin inhibits the apoptosis of rooster Leydig cells by suppressing oxidative stress via AKT-Nrf2 pathway activation. Free Radic Biol Med. 2020;160:1–12.
13. Li R, Lin QX, Liang XZ, Liu GB, Tang H, Wang Y, et al. Stem cell therapy for treating steroid-induced osteonecrosis of the femoral head: from clinical applications to related basic research. J Cell Mol Res Ther. 2018;9:291.
14. Wauquier F, Leotoing L, Coxam V, Guichoux E, Wittman Y. Oxidative stress in bone remodelling and disease. Trends Mol Med. 2009;15:468–77.
15. Guzman JN, Sanchez-Padilla J, Wokosin D, Kondapalli J, Ilijic E, Schumacker PT, et al. Oxidant stress evoked by pacemaking in dopaminergic neurons is attenuated by DJ-1. Nature 2010;468:696–700.
16. Zhou J, Wang M, Zhou L, Feng X, Yu L, et al. CPX targeting DJ-1 triggers ROS-induced cell death and protective autophagy in colorectal cancer. Theranostics 2019;9:5577–94.
17. Amratullah H, Maron-Gutierrez T, Shen Y, Gupta S, Tsiporidis JN, Varoukhi AK, et al. Protective function of DJ-1/PARK7 in lipopolysaccharide and ventilator-induced acute lung injury. Redox Biol. 2021;38:101796.
18. Upadhya M, Milliner C, Bell BA, Bonilha VL. Oxidative stress in the retina and retinal pigment epithelium (RPE): Role of aging, and DJ-1. Exp Eye Res. 2019;189:107830.
19. Zeng J, Zhao H, Chen B. The role of DJ-1/PARK7 inhibits high glucose-induced oxidative stress to prevent retinal pericyte apoptosis via the PI3K/AKT/mTOR signaling pathway. Exp Eye Res. 2019;189:107830.
20. Zhang XL, Yuan YH, Shao QH, Wang ZZ, Zhu CG, Shi JG, et al. DJ-1 regulating PI3K-NFκB signaling plays a significant role in bibenzyl compound 20C-mediated neuroprotection against rotenone-induced oxidative insult. Toxicol Lett. 2017;271:74–83.
21. Zhang F, Peng W, Zhang J, Dong W, Yuan D, Zheng Y, et al. New strategy of bone marrow mesenchymal stem cells against oxidative stress injury via Nrf2 pathway: oxidative stress preconditioning. J Cell Biochem. 2019;120:19902–14.

ACKNOWLEDGEMENTS
This study was completed in the Clinical Medicine Research Center of the Affiliated Hospital of GMU. We would like to thank the teachers at this center for their guidance. We would like to thank LetPub (http://www.letpub.com.cn) for providing English language editing. This work was supported by the National Natural Science Foundation of China (grant nos. 81902226, 82060397, 81860387), the Guizhou Provincial Natural Science Foundation (grant no. Qiankehejuchu[2020]1Y311), the Postgraduate Research Foundation of the Guizhou Provincial Department of Education (grant no. Qianjiaohe YJSCXJH[2020]141), and the Science and Technology Foundation of Guizhou Provincial Health Committee (grant no. gzwj2021–232, gjwzkj2020–1–130 and gjwzkj2021–234).

AUTHOR CONTRIBUTIONS
F.Z. designed the study, performed experiments, analyzed data, and wrote the manuscript. Y.L.Y. performed experiments as well as collected and analyzed the data. W.X.P. designed the study and revised the manuscript. L.W. and T.W. performed experiments and revised the manuscript. Z.H.X. and H.L. performed experiments and collected the data. J.Z. and W.T.D. performed experiments, provided technical assistance, and helped with language editing.
ETHICS STATEMENT
This study was approved by the Experimental Animal Bioethics Committee of the GMU (no. 1800815), Guiyang, China.

COMPETING INTERESTS
The authors declare that there are no conflicts of interest.

ADDITIONAL INFORMATION
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41419-021-04226-1.

Correspondence and requests for materials should be addressed to Wuxun Peng.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2021