LRRK2 Deficiency Protects Hearts from Myocardial Infarction Injury in Mice via the P53/HMGB1 Pathway

Yuan Liu

Zhengzhou University First Affiliated Hospital <https://orcid.org/0000-0003-3521-6410>

Changgui Chen

Wuhan NO.1 Hospital

Lu Chen

Zhengzhou University First Affiliated Hospital

Xiaoxin Pei

Zhengzhou University First Affiliated Hospital

Zekai Tao

Zhengzhou University First Affiliated Hospital

Yawei Xu

Zhengzhou University First Affiliated Hospital

Ran Li (✉ fcclir2@zzu.edu.cn)

Zhengzhou University First Affiliated Hospital <https://orcid.org/0000-0003-2877-2582>

Research Article

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Abstract

Purpose: LRRK2 is a Ser/Thr kinase with multiple functional domains. Current studies have shown that its mutations are closely related to hereditary Parkinson's disease. However, its role in cardiovascular disease, especially in myocardial infarction, is unclear. The aim of this study was to explore the functional role of LRRK2 in myocardial infarction.

Methods: Wild-type and LRRK2 knockout mice were subjected to coronary artery ligation (left anterior descent) to establish a myocardial infarction mouse model. Neonatal rat cardiomyocytes were subjected to hypoxia to induce hypoxia injury *in vitro*.

Results: We found increased LRRK2 expression levels in the infarct periphery of mouse hearts and hypoxic cardiomyocytes. LRRK2-deficient mice exhibited a decreased death rate and reduced infarction area compared to the wild-type controls 14 days after infarction. LRRK2-deficient mice showed reduced left ventricular fibrosis and inflammatory response, as well as improved cardiac function. In the *in vitro* study, LRRK2 silencing decreased the cleaved-caspase3 activity, reduced cardiomyocyte apoptosis, and diminished hypoxia-induced inflammation. However, LRRK2 overexpression enhanced the cleaved-caspase3 activity, increased the number of apoptotic cardiomyocytes, and caused remarkable hypoxia-induced inflammation. When exploring the related underlying mechanisms, we found that hypoxia induced an increase in HIF α expression, which enhanced LRRK2 expression. LRRK2 induced high expression of HMGB1 via P53. When blocking HMGB1 using the anti-HMGB1 antibody, the deteriorating effects caused by LRRK2 overexpression following hypoxia were inhibited in cardiomyocytes.

Conclusions: In summary, LRRK2 deficiency protects hearts from myocardial infarction injury. The mechanism underlying this phenomenon involves the P53-HMGB1 pathway.

Introduction

According to data reported by the World Health Organization in 2016, cardiovascular diseases (CVDs) accounted for 31% of all mortality in 2016, remaining the leading cause of death[1]. An important contributing factor is myocardial infarction (MI) and the heart failure induced by cardiac remodeling after acute MI (AMI)[2, 3]. When the main branch of the coronary artery is suddenly occluded, cardiomyocytes undergo ischemia or infarction. This process is called AMI, which eventually causes cardiomyocyte death and cardiac dysfunction[4]. With the development of revascularization techniques, the AMI survival rate keeps increasing each year, which causes an increasing prevalence of heart failure (HF)[5]. After AMI, numerous cardiomyocytes undergo necrosis and apoptosis, causing a diverse range of immune cells to be recruited into the infarcted heart, engulfing the dead cardiomyocytes and releasing various pro-inflammation and pro-fibrosis cytokines[6]. These cytokines promote an inflammatory cascade reaction and fibrotic proliferation, leading to cardiac systolic and diastolic dysfunction, and ultimately, HF[7]. Despite increased awareness of these processes and the development of various interventions, the

overall mortality rate due to HF induced by acute myocardial infarction remains high, and more effective treatment strategies are still needed[8].

When the heart experiences ischemia injury, a ubiquitous protein high mobility group box 1 (HMGB1) could be released from necrotic cardiomyocytes and be actively secreted in stressed cells[9]. HMGB1 is a typical damage-associated molecular pattern (DAMP) that binds to various receptors and signaling molecules, such as rage and Toll-like receptor (TLR)2/4, and induces activation of NF- κ B and extracellular signal-regulated kinase (ERK) 1/2 signaling, triggering cells to produce pro-inflammatory cytokines [10, 11]. In many cardiovascular diseases, especially cardiac ischemia injury, HMGB1 was found to play an essential role in disease development[12, 13]. HMGB1 increases the expression of endothelial cell chemokine receptors and promotes the release of inflammatory factors, thereby inducing myocardial cell necrosis or apoptosis[14]. HMGB1 blockade can ameliorate cardiac fibrosis in experimental autoimmune myocarditis[15]. Therefore, HMGB1 is a therapeutic target for treating heart damage.

Leucine-rich repeat kinase 2 (LRRK2) is a Ser/Thr kinase with multiple functional domains, which is implicated in Parkinson's disease (PD) [16]. LRRK2 protein contains a Ras complex guanosine triphosphate hydrolase domain and a C-terminal Roc domain. Previous studies have focused on the functional role of LRRK2 in PD and other cerebrocortical diseases[16, 17]. LRRK2 was also reported to be involved in paraquat-induced inflammatory sickness and the stress phenotype[18]. Gu S et al. reported that downregulation of LRRK2 inhibited cholangiocarcinoma development[19] and promoted thyroid cancer cell apoptosis[20]. LRRK2 has also been reported to participate in mitochondrial Ca^{2+} efflux and function[21]. Despite these studies, the functional role of LRRK2 in cardiovascular disease is unclear. In this study, we established an MI model and used LRRK2 silencing to explore the functional role of LRRK2 in the cardiovascular system.

Materials And Methods

Animals

The animal protocols were approved by the Animal Care and Use Committee of Zhengzhou University and all the procedures were guided by the Guidelines for the Care and Use of Laboratory Animals, published by the United States National Institutes of Health (NIH Publication, revised 2011). The LRRK2 knockout (KO) mice were purchased from Jackson Laboratory (Jackson Laboratory, 016121). The wild-type littermates were used as a control. Male mice (8–10 weeks; 24–28 g) were subjected to left coronary artery ligation surgery. Fourteen days after surgery, the mice were sacrificed, and their hearts were removed.

Left coronary artery ligation surgery

Mice were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). After the pericardium was opened, a 7–0 silk suture was used to ligate the proximal left coronary descending artery (LAD) under the tip of the left atrial appendage. For the sham group, a 7–0 silk suture only encircled the LAD without ligation. After

closing the chest, 0.1 ml of 0.5% bupivacaine (Sigma-Aldrich) was injected into the mice to alleviate postoperative pain. The surgery was performed in a blinded manner.

Echocardiography and hemodynamics

The echocardiography and hemodynamic parameters were measured as our previous study described[22, 23]. Isoflurane (1.5%) was used to anaesthetize the mice. A MyLab 30CV ultrasound (Biosound Esaote) with a 10-MHz linear array ultrasound transducer was used to detect echocardiography parameters. For hemodynamic measurements, a microtip catheter transducer (SPR-839; Millar Instruments, Houston, TX, USA) was inserted into the right carotid artery and then advanced into the left ventricle (LV). A Millar Pressure-Volume System (MPVS-400; Millar Instruments) was used to record data. PVAN data analysis software was used for the analysis.

Histological analysis, immunohistochemistry, and TUNEL staining

The mouse heart sections were embedded in paraffin and cut into 4- to 5-mm thick sections. Hematoxylin and eosin (HE) and picric acid red (PSR) staining was performed as our previous study described[22, 23]. The infarct size was expressed as a percentage of the total LV area. A single myocyte was measured using a quantitative digital image analysis system (Image-Pro Plus, version 6.0). For immunohistochemistry, primary antibodies, i.e., anti-CD68 (ABD Serotec, MCA1957) and anti-CD45 antibodies (Abcam, ab10558) were used. A commercial kit was used to perform TUNEL staining and detect the apoptotic cells (Millipore, USA, #S7111). A fluorescence microscope (OLYMPUS DX51) was used to capture images.

Quantitative real-time polymerase chain reaction (RT-PCR) and western blot analysis

Total RNA was extracted from frozen mouse heart tissue and cardiomyocytes. RNA (2 µg per sample) was reverse transcribed into cDNA using the oligonucleotide (DT) primer and the transcript first strand cDNA synthesis kit (Roche). Then, we used a light Cyclor 480 instrument (software version 1.5, Roche) to perform the PCR analysis using the SYBR green PCR master mix (Roche). GAPDH was used as the internal reference gene.

Heart tissues and cardiomyocytes were lysed in RIPA Lysis Buffer. Protein samples (50 µg) were separated by SDS-PAGE and transferred to a PVDF membrane (Millipore, Beijing, China), which was then incubated with different primary antibodies including LRRK2, phosphorylated (P-)-P53, HMGB1 (purchased from Abcam, 1:1000 dilution), and GAPDH (purchased from Cell Signaling Technology, 1:1000 dilution). The blots were developed with enhanced chemiluminescence (ECL) reagents (Bio-Rad, Hercules, CA, USA) and captured by a ChemiDoc MP Imaging System (Bio-Rad). GAPDH (Santa Cruz) acted as an internal reference protein.

Cell culture

Neonatal rat cardiomyocytes (NRCMs) were isolated as described previously[22]. Sprague-Dawley rats (1 to 3-day-old)-derived NRCMs were seeded (1×10^6 cells/well) into six-well plates with DMEM/F12 with 10% fetal bovine serum (FBS). 5-bromo-2-deoxyuridine (0.1 mM; BrdU, Sigma, B5002) was used for suppressing fibroblast growth. Then, cells were serum-starved for 8 h, followed by transfection with si-LRRK2 (Santa Cruz) or the negative control (ScRNA), and then subjected to hypoxia for 24 h. The hypoxia model was induced as described previously [24]. The cells were transfected with adenovirus (Ad-)LRRK2 (Vigene, Shangdong, China) for 8 h to overexpress LRRK2. They were then treated with HMGB1-specific neutralizing antibody (50 µg/ml, ST326052233; Shino-Test, Tokyo, Japan) for 8 h to block HMGB1. Finally, the cells were treated with KC7F2 (20 µM, MedChemExpress) for 24 h to inhibit HIF-1α.

ELISA analysis and C-caspase-3 activity

ELISA kits (BioLegend) were used to test the release of tumor necrosis factor (TNF)-α, interleukin (IL)-1, and IL-6 according to a previous study[25]. An ELISA plate reader (Synergy HT, BioTek, VT, USA) was used to measure the optical density of the samples at 450 nm. The HMGB1 ELISA kit (Cloud Clone, Wuhan, China) was used to detect HMGB1 concentration in heart tissues and cardiomyocytes.

C-caspase-3 activity (Beyotime Biotech, China) was detected by a commercial assay according to the manufacturer's instructions.

Statistical analysis

Data are expressed as the mean ± SD. The unpaired Student's *t*-test was used to compare differences between two groups. One-way ANOVA was used to compare differences between groups. P values <0.05 were considered statistically significant .

Results

Expression level of LRRK2 in ischemic hearts and cardiomyocytes

In order to explore whether LRRK2 participates in the pathology of MI, we detected the transcription and protein levels of LRRK2 at 3, 7, and 14 days after MI in the mice. As a result, we observed an increasing trend in LRRK2 after MI in heart tissue, which tended to increase at 3, 7, and 14 days after MI (Figure 1 A-C) at both the transcription and protein level. We then detected the expression pattern of LRRK2 in cardiomyocytes. As shown in Figure 1 D-F, the transcription and protein levels of LRRK2 were sharply upregulated in hypoxic cardiomyocytes (24 h). This result indicates that LRRK2 may play a key role in the progression of cardiac MI to HF.

LRRK2 silencing relieves cardiomyocyte hypoxia injury

We then isolated NRCMs to perform the *in vitro* study. NRCMs were transfected with LRRK2 siRNA and the negative control ScRNA for LRRK2 knockdown (Figure 2A). An MTT assay was used to detect cell viability after 24 h of hypoxia. NRCMs in the hypoxia group revealed a reduced cell viability and increased

number of TUNEL-positive cells. NRCMs transfected with LRRK2 siRNA revealed higher cell viability and reduced TUNEL-positive cell numbers when compared with cells transfected with ScRNA in hypoxia conditions (Figure 2B, C). We then detected the activity of cleaved-caspase3 (c-caspase3), which is an apoptotic actuator. As shown in Figure 2D, hypoxia induced a sharply augmented c-caspase3 expression and activity, compared to the case in cells under conditions of normoxia. LRRK2 knockdown diminished this augmentation of c-caspase3 activation (Figure 2D). As inflammation is a key feature in cardiomyocyte hypoxia injury, we detected the release of pro-inflammatory cytokines. As shown in Figure 2E, the increased release of TNF α , IL-1, and IL-6 was downregulated by LRRK2 silencing. These data suggest that LRRK2 knockdown could protect cardiomyocytes from hypoxia injury.

LRRK2 overexpression deteriorates cardiomyocyte hypoxia injury

To explore whether LRRK2 overexpression would cause deteriorating effects, NRCMs were transfected with Ad-LRRK2 to overexpress LRRK2 (Figure 3A). Contrary to the results shown in LRRK2 siRNA experiments, LRRK2 overexpression caused a decreased cell viability and increased TUNEL-positive cell number during hypoxia conditions when compared to cells transfected with Ad-NC, the negative control (Figure 3B, C). The C-caspase3 staining and activity results were also consistent with the results above. LRRK2 overexpression increased C-caspase3 expression and activation in hypoxia conditions compared to the Ad-NC group (Figure 3D). The release of TNF α , IL-1, and IL-6 in the Ad-LRRK2 group was also greater than that in the Ad-NC group under hypoxia conditions (Figure 3E). Collectively, all the above data suggest that LRRK2 has a pro-cardiomyocyte injury effect in response to hypoxia.

LRRK2 deficiency in mice ameliorates heart MI injury and improves survival rate

Does LRRK2 play a role in the process of cardiac remodeling after MI leading to HF? LRRK2-null mice (Figure 4A) were subjected to LAD surgery to establish the MI model. Fourteen days after MI, the hearts of the mice were removed. We observed a reduction in the death rate of the LRRK2-null mice during the 14 days of observation when compared to the WT mice (Figure 4B). The myocardial infarction area was detected via HE staining, and we found that LRRK2 deficiency diminished the infarction area, compared to the WT control (Figure 4C). PSR staining was used to detect LV fibrosis. As shown in Figure 4D, the LV collagen volume in LRRK2-null mouse hearts was less than that in the WT mouse hearts after 14 days of MI. As increased inflammatory cell infiltration and pro-inflammatory cytokines release leads to serious LV fibrosis and cardiac dysfunction, we explored the inflammatory response after MI. As shown in Figure 4E and F, the number of CD68-positive macrophages and CD45-positive leucocytes was obviously reduced in LRRK2-deficient mice, and the concentration of pro-inflammatory cytokines in heart tissue was remarkably abated when compared with that in WT mice. These data clearly imply that LRRK2 exerts a pro-cardiac remodeling effect from MI to HF.

LRRK2 deficiency in mice improves cardiac function

Cardiac function was also detected via echocardiography and hemodynamic measurement. As shown in Figure 5A, B, the LV end diastolic diameter (LVEDd) was increased and LV ejection fraction (LVEF) and LV

fractional shortening (LVFS) were reduced in WT mice after 14 days of MI compared with sham mice, while these parameters were improved in LRRK2-null mice after MI. End diastolic left ventricular posterior wall thickness (LVPWd) was increased in both WT and KO mice after MI and revealed no significant difference. The cardiac output was decreased in WT-MI mice but increased in KO-MI mice. The heart rate was not significantly different among the four groups (Figure 5C). The heart mass and lung mass were increased in the MI group as compared with the sham group, as evidenced by the heart weight or lung weight to body weight ratios of the mice. These parameters were reduced in KO-MI mice when compared with WT-MI mice (Figure 5D). These data imply that LRRK2 deficiency in mice improves cardiac dysfunction after MI.

LRRK2 affects the P53-HMGB1 pathway

We screened the potential target and the upstream molecules of LRRK2. As a result, we found that the increase in LRRK2 expression in hypoxic cardiomyocytes was induced by HIF-1 α . Cells were treated with the HIF-1 α inhibitor KC7F2 (20 μ M) for 24 h. As shown in Figure 6A, the expression of HIF-1 α was upregulated in NRCMs after hypoxia, but decreased to the baseline level after treatment with KC7F2. The expression trends of LRRK2 were the same as those of HIF-1 α . When treating cells with KC7F2, the hypoxia-induced increase in the expression level of LRRK2 was inhibited (Figure 6A). These results indicate that when cells suffer from hypoxia, high levels of HIF-1 α trigger the expression of LRRK2, which participates in the process of cardiac remodeling after MI. Next, we screened the potential targets of LRRK2. Studies have reported the role of LRRK2 on phosphorylation of P53, which has a role in the release of HMGB1[26]. We detected the protein level of P-P53 and found that P-P53 was upregulated in NRCMs after hypoxia and downregulated by LRRK2 knockdown (Figure 6B). Then, we detected the expression of HMGB1, the strong pro-inflammation and pro-apoptosis DAMP. As a result, an increased HMGB1 level was observed in hypoxic NRCMs, while the HMGB1 expression was downregulated in LRRK2-silenced cells (Figure 6B). We further detected the concentration of HMGB1 in heart tissue after MI and in NRCMs after hypoxia. As shown in Figure 6C-E, the concentration of HMGB1 was augmented in both heart tissues after MI and cardiomyocytes after hypoxia, but LRRK2 deficiency decreased the level of HMGB1 in both ischemic heart tissue and hypoxic cardiomyocytes, whereas LRRK2 overexpression increased the level of HMGB1 in hypoxic cardiomyocytes. These data indicate that LRRK2 may exert its function via the P53-HMGB1 pathway.

HMGB1 antibodies counter negative effects of LRRK2

We then used the HMGB1 antibody to block the extracellular HMGB1 to counteract the ligand effect of HMGB1. NRCMs were also transfected with Ad-LRRK2 for LRRK2 overexpression. As shown in Figure 7A, B, the cell viability was increased, and the number of TUNEL-positive cells was reduced in the anti-HMGB1 group compared to the hypoxia group. The c-caspase3 expression and activity, and pro-inflammatory cytokine concentrations were also diminished in the anti-HMGB1 group compared to the hypoxia group (Figure 7C, D). The pro-apoptosis and pro-inflammatory effects of LRRK2 overexpression were counter by anti-HMGB1 antibody treatment, as shown by the diminished number of TUNEL-positive cells, reduced c-

caspase3 activation and pro-inflammatory cytokine concentration, and increased cell viability in the Ad-LRRK2+anti-HMGB1 group (Figure 7 A-D). Taken together, these data suggest that hypoxia-induced increase in HIF-1 α expression causes upregulation of LRRK2, which exerts a deteriorating effect during the cardiac remodeling process after MI via regulating P53-mediated HMGB1 expression and release.

Discussion

Myocardial infarction occurs when the coronary atherosclerotic plaque ruptures and the coronary arteries are completely occluded, causing ischemia in the blood-supplying myocardium[8]. After myocardial infarction, a series of molecular, cellular, extracellular, and tissue changes occur in the left ventricle of surviving patients, who show clinical changes in heart size, structure, and function, ultimately leading to maladaptation (HF)[27]. The role of LRRK2, previously studied as a PD-associated protein, in cardiovascular disease was first examined in our study. We found that LRRK2 was upregulated in the process of cardiac remodeling after MI and exerts deteriorating effects in this process. During cardiac ischemia, cardiomyocytes show an increase in the HIF-1 α expression [28]. As a transcriptional factor, HIF-1 α regulates the expression of several molecules to compensate for the oxygen and nutrition deprivation[28]. In our study, we observed that HIF-1 α increased the expression of LRRK2 during hypoxia, as HIF-1 α inhibitor inhibited the hypoxia-induced upregulation of LRRK2.

MI is often fatal, and patients that survive acute myocardial infarction undergo an adaptive response to compensate for the disordered hemodynamics and maintain cardiac function[27]. After the ischemic attack, loss of myocardial cells causes the overloading of the ventricular wall, leading to dilatation of the ventricular chamber[7]. During this phase, the LVEF decreases abruptly, drops the cardiac output, and increases the LVEDV[7]. The body activates the sympathetic nervous system (SNS) and the renin-angiotensin-aldosterone system (RAAS) to compensate for the prolonged reduction in cardiac output[7]. In our study, we found that LRRK2 deficiency reduced the LVEDd and increased the LVEF, LVFS, and cardiac output after 14 days of MI. This implies that LRRK2 deficiency may be beneficial to the adaption of the heart to MI injury. After myocardial ischemia leads to increased myocardial cell necrosis, a variety of immune cells including neutrophils and monocytes infiltrate into the myocardial tissue to phagocytize dead cells[6]. Subsequently, the inflammatory phase subsides, the heart transitions to the repair and proliferative phase, and the factors secreted by the inflammatory cells promote the proliferation of myofibroblasts and collagen deposition, leading to scar formation. Finally, a maturation process follows, with the quiescence of myofibroblasts. A balance of the inflammation phase, proliferative phase, and maturation process is essential for the maintenance of cardiac function[6, 27]. However, after 14 days of MI, we found that cardiac dysfunction in wild-type mice is accompanied by persistent inflammatory infiltration, inflammatory factor secretion, and fibrotic proliferation. LRRK2 deficiency attenuated the increased inflammatory response, reduced the inflammatory factor concentration, and decreased the collagen volume. LRRK2 deficiency also decreased the infarction area and improved the survival rate. In the *in vitro* study, LRRK2 overexpression could enhance cell apoptosis and inflammatory response in response to hypoxia. This indicates that LRRK2 serves as a detrimental factor in cardiomyocytes under hypoxia conditions.

Cardiomyocyte death results in a massive release of DAMP-related proteins, binding to pattern recognition receptors (PRRS), or binding to TLRs, activating inflammatory signaling pathways[1]. HMGB1 is one of the most important DAMPs [9]. Under ischemia or other stress, HMGB1 migrates from the nucleus to the cytoplasm through the Golgi/endoplasmic reticulum pathway, moves to the cell membrane, and is finally secreted into the extracellular matrix [10]. Extracellular HMGB1 plays an important role in inflammation, apoptosis, necrosis, and autophagy[29]. HMGB1 binds to cell membrane receptors such as RAGE and TLR2/4 and induces activation of NF-κB and ERK1/2 signaling, triggering cells to produce pro-inflammatory cytokines[30]. In this study, we found that LRRK2 increased HMGB1 release in cardiomyocytes under hypoxia. LRRK2 deficiency dropped HMGB1 release in the ischemic heart and cardiomyocytes. How did LRRK2 regulate HMGB1 release or expression? Transcription, translation, mRNA, and protein stability of HMGB1 are regulated by many molecules. Studies have proven that P53, CCAAT-binding transcription factor 2 (CTF2), and JAK/STAT can regulate HMGB1 expression at the transcription level [31, 32]. We found that LRRK2 silence decreased the HMGB1 protein level. Ho DH et al. reported that LRRK2 could phosphorylate P53, leading to P53 activation in differentiated SH-SY5Y cells and microglia model BV2 cells[26, 33]. We detected the activation (phosphorylation) of P53 and found that LRRK2 deficiency reduced the level of P-P53 in cardiomyocytes. Thus, LRRK2 may regulate HMGB1 transcription via P53 activation.

In summary, our study demonstrated that during the ischemia condition, high levels of HIF-1α stimulate LRRK2 expression, which promotes P53 phosphorylation and activation, causing the transcription of HMGB1. The increased release of HMGB1 accelerates the inflammation and apoptosis of the ischemic heart, which leads to HF. Thus, targeting LRRK2 may become a new therapeutic method for hindering the progression of cardiac remodeling after MI.

Declarations

Conflict of interests

The authors declare no conflicts of interest.

Ethics approval

The Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and the ARRIVE guidelines and the Animal Care and Use Committee of Zhengzhou University guided our study.

Availability of data and material

The original data will be available when requiring to the corresponding author.

Authors' contribution

Liu Yuan and Li Ran contributed to the conception and design of the experiments; Chen Chang-gui and Chen Lu carried out the experiments; Pei Xiao-xin, Tao Ze-kai and Xu Ya-wei analysed the experimental results and revised the manuscript; Liu Yuan and Li Ran wrote and revised the manuscript.

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Figures

Figure 1

Figure 1

Expression level of LRRK2 in ischemic hearts and cardiomyocytes A and B. Protein level of LRRK2 in hearts after MI (n = 6). C. mRNA level of LRRK2 in hearts after MI (n = 6). C and D. Protein level of LRRK2 in NRCMs after 24 h of hypoxia (n = 6). F. mRNA level of LRRK2 in NRCMs. aP < 0.05 vs. CON/Normoxia group.

Figure 2

LRRK2 silence relieves cardiomyocyte hypoxia injury A. Protein expression of LRRK2 in NRCMs after transfection with LRRK2 siRNA (n = 6). B. Cell viability after hypoxia for 24 h (n = 6). C. TUNEL staining and quantified result (n = 5). D. C-caspase3 staining and activity (n = 6). F. ELISA detection of pro-inflammatory cytokine concentrations (n = 6). aP < 0.05 vs. Normoxia-ScRNA group, bP < 0.05 vs. hypoxia-ScRNA group.

Figure 3

Figure 3

LRRK2 overexpression deteriorates cardiomyocyte hypoxia injury A. Protein level of LRRK2 in NRCMs after transfection with Ad-LRRK2 (n = 6). B. Cell viability after hypoxia for 24 h (n = 6). C. TUNEL staining and quantification of apoptotic cells (n = 5). D. c-caspase3 staining and activity (n = 6). F. ELISA for the detection of pro-inflammatory cytokine concentrations (n = 6). aP < 0.05 vs. Normoxia-Ad-NC group, bP < 0.05 vs. hypoxia-Ad-NC group.

Figure 4

LRRK2 deficiency in mice ameliorates heart MI injury and improves survival rate A. Protein expression of LRRK2 in WT and KO mice (n = 6). B. Survival rate 14 days after MI (n = 25 per group). C. H&E staining and quantification of LV fraction area (n = 6). D. PSR staining and quantification of LV collagen volume (n = 6). E. CD45 and CD68 staining and quantification of CD45- and CD68-positive cell numbers (n = 6). F. ELISA for the detection of pro-inflammatory cytokine concentrations in heart tissue (n = 6). aP < 0.05 vs. WT-sham group, bP < 0.05 vs. WT-MI group.

Figure 5

Figure 5

LRRK2 deficiency in mice improves cardiac function A and B. Echocardiography measurement of LVEDd, LVPWd, LVEF, and LVFS (n = 10). C. Hemodynamic measurement of cardiac output and heart rate (n = 10). D. Heart weight to body weight ratio, lung weight to body weight ratio (n = 10). aP < 0.05 vs. WT-sham group, bP < 0.05 vs. WT-MI group.

Figure 6

Figure 6

LRRK2 affects the P53-HMGB1 pathway A. NRCMs were treated with KC7F2 and exposed to hypoxia for 24 h. Protein level of HIF-1α and LRRK2 (n = 6). aP < 0.05 vs. CON group, bP < 0.05 vs. hypoxia group. B. P53 and HMGB1 protein level in NRCMs transfected with LRRK2 siRNA (n = 6). aP < 0.05 vs. Normoxia-ScRNA group, bP < 0.05 vs. hypoxia-ScRNA group. C-E. ELISA for the detection of HMGB1 in NRCMs transfected with LRRK2 siRNA(n = 6, C, aP < 0.05 vs. Normoxia-ScRNA group, bP < 0.05 vs. hypoxia-ScRNA group); in NRCMs transfected with Ad-LRRK2(n = 6, D, aP < 0.05 vs. Normoxia-Ad-NC group, bP <

0.05 vs. hypoxia-Ad-NC group.); and in heart tissue (n = 6, E aP < 0.05 vs. WT-sham group, bP < 0.05 vs. WT-MI group).

Figure 7

Figure 7

HMGB1 antibody counters LRRK2’s negative effects A-D. NRCMs were transfected with Ad-LRRK2 and treated with anti-HMGB1 antibodies. A. Cell viability after hypoxia for 24 h (n = 6). B. TUNEL staining and quantification of apoptotic cells (n = 5). C. C-caspase3 staining and activity (n = 6). D. ELISA for the detection of pro-inflammatory cytokine concentrations (n = 6). aP < 0.05 vs. normoxia group, bP < 0.05 vs. hypoxia group, cP < 0.05 vs. Ad-LRRK2-hypoxia group.