

# Up-regulating Lipin1 Attenuates High Glucose-Induced Apoptosis in RSC96 cells Through Regulating Mitochondrial Dynamics

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## Research Article

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# Abstract

Diabetic peripheral neuropathy, one of the major complications resulting from diabetes, affects diabetic patients with high morbidity and mortality. It has emerged as a severe public health problem. However, its underlying pathogenesis and effective treatment strategies have not been fully studied. In the present study, we explored the role of lipin1 on mitochondrial function of Schwann cells under diabetic conditions. With high glucose stimulation or lipin1 down-regulation, the cell viability of Schwann cells was inhibited, accompanied with mitochondrial dysfunction and morphological abnormality. Besides, we found that high glucose stimulation or lipin1 silencing also disturbed the balance of mitochondrial dynamics, presented as increased levels of mitochondrial fission-related proteins (including DRP1 and FIS1) and decreased levels of mitochondrial fusion-related proteins (including MFN1 and OPA1). Furthermore, we demonstrated that up-regulating lipin1 ameliorated high glucose-induced disorder of mitochondrial dynamics and functions, and ultimately improved cell viability. Our results suggest that lipin1 may play a protective role on high glucose-stimulated Schwann cells through regulating the balance of mitochondrial dynamics.

## 1. Introduction

Diabetes mellitus is a complex metabolic disorder which is characterized by abnormal glucose and lipid metabolism (Huang et al., 2017). Diabetic peripheral neuropathy (DPN), one of the most common microvascular complications resulting from diabetes, affects more than 50% of diabetic patients over the course of the disease (Feldman et al., 2017). The clinical symptoms of DPN include numbness, burning, tingling sensation, and intractable pain, seriously affect the quality of life in patients (Sandireddy et al., 2014). Earlier works demonstrated that DPN was induced by multifactorial metabolic disorders, including nitrogen and oxidative stress, increased advanced glycation end-products, neuroinflammation, accumulation of sorbitol and so on (Juster-Switlyk and Smith, 2016). However, the underlying pathophysiologic mechanism of DPN remains incompletely understood and there is no effective therapy that could permanently reverse neuropathy.

Recently, increasing evidences suggest that mitochondrial disorder might be one of the key factors in the pathology of DPN. Positive relationship between mitochondrial disorder and diabetic neuropathy is found in diabetes animal models and in vitro cell model of diabetic neuropathy (Yerra et al., 2017; Yerra et al., 2018). However, the exact mechanism of mitochondrial dysfunction under high glucose condition in neurons remains elusive.

Mitochondria are semi-autonomous organelles which are characterized by double phospholipid membrane structure, including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and cardiolipin (CL). Recent studies demonstrate that mitochondria are highly dynamic organelles that continuously undergo fusion and fission to keep their normal function. Mitochondrial membrane lipid composition has close relationship with mitochondrial dynamics. For example, CL regulates mitochondrial fusion through modifying the morphology of mitochondria and

diacylglycerol (DAG) improves mitochondrial fission by recruiting fission-related proteins (Alshudukhi et al., 2017). As we know, blood glucose elevation could influence the lipid metabolism in cells. Previous studies demonstrated the alterations of lipid composition, including total-cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and fatty acid (FFA) levels in diabetic animal models (Jiang et al., 2019; Wang et al., 2020). Since lipid metabolism are associated with high glucose and mitochondrial dynamics, we hypothesis that high glucose stimulation may affect properties of mitochondrial membrane lipids and further disturb mitochondrial dynamics.

Lipin1 is the key phosphatidate (PAP) enzyme in lipid metabolism. It is well-known that DAG, function as a crucial second messenger in lipid metabolism, is the precursor for triacylglycerol (TAG), neutral phospholipid, phosphatidyllecithin, and cephalin. Lipin1 catalyzes the dephosphorylation of phosphatidic acid (PA) to DAG by its PAP enzyme activity, and further mediates the biogenesis of phospholipids and transduction of signaling pathway. Studies have shown that activity declines of PAP1 could control the pathway by which membrane lipids are synthesized, and influence the related cellular biological functions, including cell membrane stability, signal pathway transmission, adipocyte and nerve cell differentiation, and respiratory deficiency (Nadra et al., 2008; Csaki et al., 2013). Lipin1, serves as a key regulator in lipid metabolism, is crucial for myocyte injury in skeletal muscle though regulating mtDNA and total mitochondrial protein level (Schweitzer et al., 2019). Furthermore, previous study suggested that lipin1 may impact on mitochondrial function, presented as regulating mtDNA and total mitochondrial protein level in skeletal muscle (Schweitzer et al., 2019). Taken together, we propose that lipin1 modulate mitochondrial membrane lipid composition and thus influence mitochondrial dynamics and functions.

Until now, most investigations have tended to focus on the function of lipin1 in liver, adipose tissue, skeletal muscle and breast cancer (Arai et al., 2018; Rashid et al., 2019; Song et al., 2020), surprisingly little information is available concerning the functional status of lipin1 in peripheral nerves. Schwann cells are the most numerous glial cells of peripheral nerves and influence many aspects of nerve functions, such as neurotrophic factors secretion, nervous impulses along axons, extra cellular matrix production and repairment of injured nerves (Yan et al., 2018). Numerous researches demonstrated that Schwann cell dysfunctions and apoptosis caused by high glucose participate in the development of DPN (Yan et al., 2018; Dong et al., 2019; Liu et al., 2019; Cheng et al., 2020). Our previous research showed that the level of lipin1 protein in cultured Schwann cells was significantly decreased when exposed to high glucose, while up-regulating the expression of lipin1 attenuated neuronal damage in diabetic animal models (Xu et al., 2015). These results suggested that lipin1 may be related to the development of DPN.

Taken together, we intend to identify the relationship between lipin1 and DPN, and further to explore the underlying mechanisms. Our results showed that with exposure to high glucose or lipin1 silencing, the cell viability of Schwann cells was declined, concurrent with mitochondrial dysfunction and mitochondrial dynamics imbalance. However, over-expression of lipin1 could relieve the impairment of cell viability and mitochondrial functions in Schwann cells with high glucose stimulation. Our study

suggested that lipin1 could modulate the cell function of Schwann cells by affecting mitochondrial dynamics homeostasis, providing a novel approach for the prevention and treatment of DPN.

## 2. Methods

### 2.1 Cell Culture

RSC96 rat Schwann cells (RSC96 cells), a kind of myelin cells in rat peripheral nervous system, were obtained from Committee on Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). RSC96 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Hyclone, USA) containing 25-mM glucose, supplemented with 4-mM L-glutamine plus with 100 U/mL penicillin (Shandong Xinhua, China), 100- $\mu$ g/mL streptomycin (Sigma), and 10% fetal bovine serum (FBS, Biochrome, Germany), in a humidified 5% CO<sub>2</sub>, 95% air, 37°C incubator. The medium was changed every 2–3 days. Cells were trypsinized and sub-cultured in 96-well plates ( $1 \times 10^4$  cells/well) for subsequent experiments. After reaching ~70% confluence, RSC96 cells were then treated with normal concentration of glucose (25-mM glucose, NG) and high concentration of glucose (50, 75, and 100-mM glucose, HG) for 48 h.

### 2.2 Lentivirus transfection

Lipin1 silencing or over-expressing lentivirus transfection of Schwann cells were performed. Lentiviral vectors and the transfection enhancer were purchased from Genchem Biotechnology Company (Shanghai, China). Transduction RSC96 Schwann cells were seeded into 96-well plates at a density of  $4 \times 10^3$  cells for each well and incubated for the experiment with conditioned media. Cells were treated with lentiviral vectors and the transfection enhancer HitransG A according to the manufacture structures. In addition, empty lv-GFP lentivirus was also transfected and acts as a control. The cells were collected for quantitative PCR or western blotting assay to assess the transfection efficacy.

### 2.3 MTT assay

Cell proliferation was determined by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) colorimetric assay. Schwann cells were seeded in 96-well plates at a density of  $1 \times 10^4$  cells/well with various concentrations of glucose. Then the cells were incubated with 10  $\mu$ L of MTT (Invitrogen, USA) (5 mg/ml; pH 7.4) for 4 h at 37°C in a CO<sub>2</sub> incubator. After the supernatant was discarded, 150  $\mu$ L of dimethylsulfoxide (DMSO) (Invitrogen life technologies, USA) was added into each pore. The cell absorbance was detected at 570/630 nm wavelength using a microplate absorbance reader (RZ-9618; Ruize, Tianjin, China).

### 2.4 Flow cytometry

Cell apoptotic analysis was determined using propidium iodide (PI) and fluorescein isothiocyanate (FITC)-conjugated Annexin V staining. Briefly, Schwann cells were digested with trypsin and washed twice with cold phosphate-buffered saline (PBS). About  $1-5 \times 10^5$  cells were suspended in 500  $\mu$ L of 1X binding buffer containing 5  $\mu$ L Annexin V-FITC and 5  $\mu$ L 7- aminoactinomycin D (7-AAD) (Bioscience, USA). After

incubated for 30 min at room temperature in the dark, the cells were assessed immediately using a flow cytometer (FACSVerse, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) (excitation/emission (nm) <sup>488</sup>/<sub>530</sub>). Apoptotic cells were identified as Annexin V-FITC-positive cells.

## 2.5 ATP content

The ATP content levels of schwann cells in each group were measured using ATP bioluminescent assay kit (Solarbio, Beijing, China) by following the manufacturer's instructions. In brief, the supernatant was discarded and the cells were homogenized in hot double distilled water at a density of  $1 \times 10^6$  cells. Then the cells were mixed with detection reagent coming with the kit and centrifuged at 4000 rpm for 5 minutes. The optical density of the cells was assessed using a microplate luminometer (RZ-9618; Ruiz). The cellular ATP content was determined from the ATP standard curve, according to the manufacturer's instructions.

## 2.6 Mitochondrial membrane potential

Mitochondrial membrane potential (MMP) was assessed with JC-1 (5,50,6,60-tetrachloro-1,10,3,30-tetraethyl-benzimidazolyl-carbocyanine iodide) staining. Briefly, Schwann cells with different stimuli were rinsed with ice-cold PBS, and then stained with 2  $\mu$ L/mL JC-1 for 15 min at 37°C in the dark. After washing with PBS to remove the unbound dye, the results were examined using a flow cytometer equipped with an argon laser. The ratio of red and green fluorescence was measured as an indicator of membrane potential change. Fluorescence intensity was quantified using Image J software. The green fluorescence intensities from the JC-1 monomer leaked out to the cytoplasm and the red fluorescence intensities from the aggregated form of JC-1 within the mitochondria.

## 2.7 Western blot analyses

Schwann cells in different groups were collected and homogenized in ice-cold lysis buffer. Protein concentrations were determined using a bicinchoninic acid protein assay kit (Thermo Scientific, USA). Equal amount of protein was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto polyvinylidene difluoride (PVDF) membrane (Millipore, USA). Subsequently, the membranes were blocked with 5% fat-free milk (Solarbio, Beijing, China) and then incubated overnight at 4°C with primary antibodies: rabbit monoclonal anti-total-DRP1 (dynamic-related protein 1), rabbit monoclonal anti-MFN1 (mitofusin-1), rabbit monoclonal anti-OPA1 (optic atrophy-1) antibodies (Cell Signaling Technology, Boston, MA, USA) and rabbit polyclonal anti-FIS1 (mitochondrial fission protein-1) antibody (Abcam, Cambridge, UK). After being incubated with the appropriate horseradish peroxidase-conjugated secondary antibodies (1:5,000) in a shaker at 37°C for 1 h, the membrane was washed four times with PBS for 10 min. The protein complexes were visualized using an Enhanced Chemiluminescence kit and analyzed using the ImageJ software (National Institutes of Health, Bethesda, MD, USA). Finally, the relative protein expression levels were normalized to that of total actin (Bioss, Beijing, China).

## 2.8 Electron microscopy

The samples used for transmission electron microscopy were collected, fixed with 3% glutaraldehyde at 4°C for 2 h. After 3 times rinsed in phosphate buffer solution, the samples were dehydrated in a graded series of ethyl alcohol and embedded in epon. Then, the ultrathin sections were stained with uranyl acetate and lead citrate. Finally, the samples were examined under a transmission electron microscope (JEOL-1200EX, Osaka, Japan).

## 2.9 Statistical analysis

All data are expressed as mean  $\pm$  standard deviation values. The normality of the distribution of variables was assessed using the non-parametric Kolmogorov–Smirnov test. For data with a normal distribution, one-way ANOVA were applied, while for data that are not normally distributed, Kruskal-Wallis test were used. Post hoc comparisons were performed with a level of significance set at  $P \leq 0.05$ . Statistical analysis was performed using SPSS® version 17.0.

## 3. Results

### 3.1 Lipin1 affects cell viability in high-glucose-stimulated Schwann cells

To investigate appropriated experimental condition, RSC96 Schwann cells were incubated with 50–100 mM glucose to simulate an in vitro model of diabetic neuropathy, with 25 mM concentration of glucose serving as the normal concentration. The MTT assay was performed to assess the cell proliferation of Schwann cells in different groups. As shown in Fig. 1a, with the increase of glucose concentration, the cell viability was inhibited in a dose-dependent manner. Compared with the normal group, treatment of 50 mM glucose resulted in no obvious difference ( $P > 0.05$ ). However, stimulation with 75- and 100- mM glucose significantly decreased cell proliferation, with 52% inhibition at 100 mM glucose treatment. Hence, 100 mM was selected as the optimal stimulation concentration for the following experiments.

Earlier studies from our laboratory have provided the insights that protein level of lipin1 was reduced in RSC96 Schwann cells upon high glucose stimulation, accompanied with cell viability inhibition (Xu et al., 2014). Hence, to explore the relationship between lipin1 and cell viability in RSC96 Schwann cells, lipin1 silencing and overexpressing lentivirus transfection was constructed to further clarify this relationship between Lipin1 and cell viability. Cells overexpressing lipin1 (Lv-Lipin1) and the (Lv-Lipin1-Con) were subjected to HG conditions (HG + Lv-Lipin1 group and HG + Lv-Lipin1-Con group). Cells of lipin1 silencing (Lv-Lipin1 shRNA) and the corresponding controls (Lv-Lipin1 shRNA-Con) were cultured under normal glucose condition (NG + Lv-Lipin1 shRNA group and NG + Lv-Lipin1 shRNA-Con group). Successful genetic modification was visualized using fluorescence microscopy, with > 90% of these RSC96 Schwann cells were detected positive for green fluorescence (Fig. 1b).

The Schwann cells in all groups were tested by MTT assay and flow cytometry to assess the effect of Lipin1 on glucose - induced cell injury. As shown in Fig. 1c and 1d, compared with cells in the NG + LV-

Lipin1shRAN-Con group, the cell viability within both HG + LV-Lipin1-Con and NG + LV-Lipin1shRNA group were significantly decreased ( $P < 0.05$ ). And there is no significant difference between NG + LV-Lipin1shRAN and HG + LV-Lipin1-Con group. Moreover, the cell viability in HG + LV-Lipin1 group was significantly higher than that in HG + LV-Lipin1-Con group cells ( $P < 0.05$ ). Lipin1 overexpression ameliorated cell viability under high glucose condition. These results suggest that lipin1 may have relationship with the alteration in Schwann cells viability exposed to high glucose.

## **3.2 Lipin1 enhances mitochondrial function in Schwann cells exposed to high glucose concentration.**

To assess mitochondrial function, we measured mitochondrial membrane potential which is a vital parameter of mitochondrial metabolism (Alshudukhi et al., 2018) and ATP content in RSC96 Schwann cells under different conditions. Stimulation of Schwann cells with high glucose weakened mitochondrial function, reflected in decreased MMP (Fig. 2a, 2b) and ATP content (Fig. 2c) ( $P < 0.05$ ). Moreover, the effect of lipin-1 silencing on mitochondrial function was similar to those of high glucose exposure in Schwann cells. On the contrary, lipin1 overexpressing in Schwann cells ameliorated high glucose-induced mitochondrial dysfunction ( $P < 0.05$ ). These results indicated that high glucose-aroused impaired mitochondrial function and weakened mitochondrial metabolism was ameliorated upon lipin-1 overexpressing in Schwann cells.

## **3.3 Lipin1 protected against high glucose-induced mitochondrial morphological disorder in Schwann cells**

Using transmission electron microscopy (TEM), the mitochondria ultrastructure under different conditions were observed. As shown in Fig. 2d, mitochondria from NG + LV-Lipin1shRNA-Con group displayed intact morphology with parallel cristae. By contrast, mitochondria in the HG + Lv-Lipin1-Con group exhibited irregular, dense cristae and even leaving empty vesicle-like structure, which were also observed in the NG + LV-Lipin1shRNA group. After lipin1 up-regulating, disorganized cristae were ameliorated in HG + Lv-Lipin1 groups. These results demonstrate that Lipin1 reversed the mitochondrial morphological deficit observed in Schwann cells with high glucose stimulation.

## **3.4 Lipin1 ameliorates the imbalance of mitochondrial dynamics induced by high glucose stimulation**

To measure the effect of lipin1 on mitochondrial dynamics in Schwann cells exposed to hyperglycemia, western blot analysis was performed to assess the expression levels of mitochondrial fusion - and fission - related proteins. As shown in Fig. 3, the level of fission-related protein, such as total-DRP1, were elevated in the HG + LV-Lipin1-Con group and the NG + LV-Lipin1shRNA group, compared with those in NG + LV-Lipin1shRNA-Con group ( $P < 0.05$ ). However, lipin1 overexpression reduced the protein level of total-DRP1 exposed to high glucose ( $P < 0.05$ ). As that observed for total-DRP1, similar changes in FIS1 protein level (fission - related protein) were obtained in these conditions, but there was no significant statistical

difference. In contrast, lipin1 silencing inhibited the level of MFN1 under normal conditions, but there was no significant statistical difference. The expression level of MFN1 was suppressed after high glucose stimulation, while lipin1 overexpression reverse the inhibited MFN1 protein level exposed to high glucose ( $P < 0.05$ ). The level of OPA1—another mitochondrial fusion protein, was decreased in the HG + Lv-Lipin1-Con group and NG + Lv-Lipin1shRNA group, as compared with that observed in the NG + Lv-Lipin1shRNA-Con group. On the contrary, with lipin1 upregulation in the HG + Lv-Lipin1 group, the level of OPA1 was also increased, compared to the HG + Lv-Lipin1-Con group. However, there was no significant statistical difference, which might be related to the insufficient sample size. These results demonstrate that lipin1 could rectify high glucose - induced imbalance of mitochondrial dynamics in Schwann cells.

## 4. Discussion

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder that is characterized by abnormal glucose and lipid metabolism. Hence, exploring the mechanisms of lipid metabolism is vital and may offer clues to the discovery of new strategies for diabetes mellitus. Lipin1, acts as the phosphatidate phosphates, play a key role in the regulation of lipid metabolism at multiple nodal points. Lipin1 usually locates in the cytosol and translocate to the endoplasmic reticulum (ER) to catalyze the conversion of PA to DAG, which is a key substrate for the synthesis of TAG, PE and PC. Lipin1 is highly expressed in liver, adipose, skeletal muscle, and is also present in endoneurium of peripheral nerve of adult mice (Nadra et al., 2008). Earlier studies from our laboratory have found that the expression of lipin1 in sciatic nerve and hippocampus were significantly decreased in diabetic neuropathy rat models (Xu et al, 2015(Shang et al., 2020)). Meanwhile, over-expression of lipin1 alleviated HG-induced peripheral neuropathy, emerged as enhanced motor nerve conduction velocity, improved nerve pathological morphology, and increased nerve fiber diameter and nerve fiber density (Xu et al, 2015). These observations were consistent with previous studies showing that lipin1 deficiency animals develop peripheral neuropathy, characterized by myelin degradation, decreased motor nerve conduction velocity and compound muscle action potentials (Nadra et al., 2008). Taken together, these observations demonstrated that lipin1 may have a relationship with the development of diabetic peripheral neuropathy.

Peripheral nerves are composed of three distinct tissue compartments: the epineurium, perineurium, and endoneurium. The outermost epineurium surrounds perineurium, which in turn surrounds the fascicle of axons, Schwann cells, and fibroblasts. Schwann cells, derived from the neural crest, are the most abundant cells of the endoneurium. In the peripheral neural system, the myelin sheath allows for efficient action potential transmission and provides trophic support for related axons (Nadra et al., 2008). Nerve myelin sheath is the extended plasma membrane of the Schwann cell, most of the myelin lipid is provided from Schwann cell. Therefore, Schwann cells viability could play a crucial role in the development of peripheral nerve growth. Since diabetic peripheral neuropathy is characterized by nerve demyelination and degeneration of nerve fibers in dorsal root ganglion and peripheral nerves (Hong et al., 2008), the viability of Schwann cells is closely associated with diabetic peripheral neuropathy.

In our study, the cell viability of Schwann cells was significantly weakened with high glucose treatment or with lipin1 silencing. In contrast, over-expression of lipin1 ameliorated cell viability with HG stimulation. It suggested that the loss of lipin1 suppressed cell viability of Schwann cells, while lipin1 treatment could alleviate HG-aroused cell apoptosis. Our gain-of-function and loss-of-function studies uniformly revealed that lipin1 alleviated HG-aroused decline in cell viability of Schwann cells. However, the underlying molecular mechanisms remain elusive.

Mitochondria are semi-autonomous organelles that maintain the bilateral structure of the membranes. Mitochondria, served as bioenergetic powerhouses, provide the cell with energy in the form of ATP generated by oxidative phosphorylation. Hence, mitochondria are the powerhouses of the cell (Spinelli and Haigis, 2018) and are critical for cells survival. Previous studies demonstrated that mitochondrial disorder have close relationship with DPN, presented as hyperglycemia decreased mitochondrial respiration and ATP production in cultured dorsal root ganglion neurons (DRGs) (Rumora et al., 2019). Peripheral nerves have long axons that are wrapped with myelin produced by Schwann cells. Because of its morphological complexity and rapid changes in metabolic requirements, neurons are critically dependent on energy metabolism, predominantly supplied with mitochondrial oxidative phosphorylation (Pellerin and Magistretti, 2003). Besides, due to the limited capacity for self-renewal, the nervous system has the lowest capacity to maintain healthy after impairment of mitochondrial bioenergetics (Sajic, 2014). Therefore, peripheral nerves are particularly dependent on effective mitochondrial function and distribution.

In our study, we tested the mitochondrial membrane potential and ATP content in Schwann cells to assess mitochondrial metabolism. In our experiments, mitochondrial dysfunction occurs in Schwann cells insulted to high glucose and lipin1 silencing under normal glucose, presented as decreased levels of MMP and ATP content. By contrast, over-expression of lipin1 alleviate high glucose aroused mitochondrial dysfunction. In addition, in order to see if metabolic disorder induced by hyperglycemia are also reflected in the morphological changes, mitochondrial structures were also observed using electron microscopic. Mitochondrial after high glucose stimulation and lipin1 silencing exhibited morphological disorder, manifested as displayed irregularly and dense cristae, whereas lipin1 up-regulation reversed high glucose-aroused mitochondrial swelling and chromatin condensation. Our observations are consistent with previous reports that EDL muscle of lipin-1 deficiency *fld* mice presented impaired mitochondrial clearance and loss of membrane potential mitochondrial (Alshudukhi et al., 2018). Glycolysis muscle fibers in patients with heritable Lipin1-null mutations displayed mitochondrial aggregates and declined mitochondrial cytochrome *c* oxidase activity (Alshudukhi et al., 2018). Overall, these observations demonstrate that lipin1 reversed high glucose-induced mitochondrial dysfunction and morphological disorder. Lipin1 overexpression might improve cell viability through regulating mitochondrial functions in Schwann cells.

Mitochondria are highly dynamic organelle that constantly undergo fusion and fission, which are pivotal for the distribution of mtDNA and the maintenance of mitochondrial function (Friedman and Nunnari, 2014). Mitochondrial fusion is a process in which two separate mitochondria are fused into one (Sajic, 2014). Mitochondrial fusion mediates material mixing between damaged mitochondria and healthy

mitochondria to ensure the integrity of the entire mitochondrial network. Mitochondrial fission is the division of one mitochondrial divide into two, which facilitates segregation of damaged mitochondria and enhance the number of mitochondria (Wu et al., 2016). It has previously been observed that the alterations of mitochondrial membrane lipid composition, such as CL and DAG, could change the balance of mitochondrial dynamics (Alshudukhi et al., 2017). Furthermore, mitochondrial dynamin-related family of large GTPases are all located at mitochondrial membranes, which are composed of proteins and lipids. Therefore, we speculate that lipin1, the key regulator of membrane lipid composition, maybe associated with mitochondrial dynamics by regulating phospholipid synthesis of the mitochondrial membrane (He et al., 2017).

The fusion of mitochondria is regulated by the proteins belonging to the dynamin-related family of large GTPases. MFN1 located in the outer membrane (OMM) and OPA1 located in the inner membrane (IMM). MFN1 serves as a tether between fusing mitochondria and contributed to artificial membrane clustering (Giacomello et al., 2020), OPA1 function as coordinate OMM and IMM, and remodel mitochondrial cristae (Giacomello et al., 2020). The fission of mitochondria is primarily regulated by DRP1, which translocate from the cytosol onto mitochondria outer membrane, more precisely onto ER-mitochondria contact sites (Lee and Yoon, 2016). This can be mediated by the OMM anchoring adaptors, including FIS1, MFF, Mid49 (Sabouny and Shutt, 2020). Although FIS1 is not essential for mitochondrial division, it could inhibit the mitochondrial fusion machinery and its over-expression leads to mitochondrial fragmentation (Giacomello et al., 2020). Studies have demonstrated that lipin1 catalytic domain co-localized with DRP1 at constricted regions of the tubules. Through the same localized domain, lipin1 could participate in the fission process and promotes fission in a PA-independent manner (Huang et al., 2011).

In our experiments, the levels of mitochondrial fission related proteins, including DRP1 and FIS1 were increased, whereas fusion related protein, such as MFN1 and OPA1 were decreased under high glucose conditions. The role of lipin1 silencing is similar to that under high glucose conditions. In contrast, lipin1 treatment inhibited DRP1 and FIS1 protein expression and promoted MFN1 and OPA1 protein expression. These findings suggested that lipin1 may have an antagonist effect to high glucose inducement on mitochondrial dynamic equilibrium of Schwann cells. Lipin1 treatment may protect against cells from mitochondrial dynamics imbalance induced by high glucose, and then keep mitochondria healthy and functional.

Our results are all based on in vitro experiments, which will be further verified by improving in vivo experiments in future studies. In addition, we only detected the influence of lipin1 on the expression of mitochondrial dynamic - related proteins, and lacked the analysis of changes in the lipid composition of mitochondrial membrane, which will be improved in the subsequent experiments.

Taken together, our study demonstrates for the first time that lipin1 could ameliorate cell viability of Schwann cells by affecting mitochondrial dynamics and functions, providing a novel approach for the prevention and treatment of DPN.

# Declarations

## Ethics approval and consent to participate

RSC96 rat Schwann cells were obtained from Committee on Type Culture Collection of Chinese Academy of Sciences (Shanghai, China).

## Consent for publication

Informed consent was obtained from all authors.

## Availability for data and materials

All data generated or analyzed during this study are included in this published article

## Competing of interests

The authors declare that they have no conflict of interest.

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## Author Contributions

All authors contributed to the study conception and design. Conceptualization, Chen S.H.; Methodology: Xu M., Yu S.Y.; Software: Li H.Z.; Validation: Sun W.J., Zhuang X.H.; Investigation: Zhuang X.H.; Data Curation: Liu W.; Writing-Original Draft Preparation: Sun W.J.; Writing-Review and Editing: Zhuang X.H.; Supervision: Xie G.A. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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# References

1. Abdullah Ali Alshudukhi. Exploring the role of lipin1 in mitophagy process using lipin1 deficient – EGFP tagged IC3 transgenic mice. [dissertation]. [Ohio]: Wright State University
2. Alshudukhi AA, Zhu J, Huang D, Jama A, Smith JD, Wang QJ (2018) Lipin-1 regulates Bnip3-mediated mitophagy in glycolytic muscle. FASEB J fj201800374. <https://doi.org/10.1096/fj.201800374>

3. Arai T, Tanaka M, Goda N (2018) HIF-1-dependent lipin1 induction prevents excessive lipid accumulation in choline-deficient diet-induced fatty liver. *Sci Rep* 8: 14230. <https://doi.org/10.1038/s41598-018-32586-w>
4. Cheng YC, Chu LW, Chen JY, Hsieh SL, Chang YC, Dai ZK (2020) Loganin Attenuates High Glucose-Induced Schwann Cells Pyroptosis by Inhibiting ROS Generation and NLRP3 Inflammasome Activation. *Cells* 9:1948. <https://doi.org/10.3390/cells9091948>
5. Csaki LS, Dwyer JR, Fong LG, Tontonoz P, Young SG, Reue K (2013) Lipins, lipinopathies, and the modulation of cellular lipid storage and signaling. *Prog Lipid Res* 52:305–316. <https://doi.org/10.1016/j.plipres.2013.04.001>
6. Dong J, Li H, Bai Y, Wu C (2019) Muscone ameliorates diabetic peripheral neuropathy through activating AKT/mTOR signalling pathway. *J Pharm Pharmacol* 71:1706–1713. <https://doi.org/10.1111/jphp.13157>
7. Feldman EL, Nave KA, Jensen TS, Bennett DLH (2017) New Horizons in Diabetic Neuropathy: Mechanisms, Bioenergetics, and Pain. *Neuron* 93:1296–1313. <https://doi.org/10.1016/j.neuron.2017.02.005>
8. Friedman JR, Nunnari J (2014) Mitochondrial form and function. *Nature* 505: 335–343. <https://doi.org/10.1038/nature12985>
9. Giacomello M, Pyakurel A, Glytsou C, Scorrano L (2020) The cell biology of mitochondrial membrane dynamics. *Nat Rev Mol Cell Biol* 21:204–224. <https://doi.org/10.1038/s41580-020-0210-7>
10. He J, Zhang F, Tay LWR, Boroda S, Nian W, Levental KR (2017) Lipin-1 regulation of phospholipid synthesis maintains endoplasmic reticulum homeostasis and is critical for triple-negative breast cancer cell survival. *FASEB J* 31:2893–2904. <https://doi.org/10.1096/fj.201601353R>
11. Hong S, Agresta L, Guo C, Wiley JW (2008) The TRPV1 receptor is associated with preferential stress in large dorsal root ganglion neurons in early diabetic sensory neuropathy. *J Neurochem* 105:1212–1222. <https://doi.org/10.1111/j.1471-4159.2008.05220.x>
12. Huang H, Gao Q, Peng X, Choi SY, Sarma K, Ren H (2011) piRNA-associated germline nuage formation and spermatogenesis require MitoPLD profusogenic mitochondrial-surface lipid signaling. *Dev Cell* 20:376–387. <https://doi.org/10.1016/j.devcel.2011.01.004>
13. Huang S, Huang S, Wang X, Zhang Q, Liu J, Leng Y (2017) Downregulation of lipin-1 induces insulin resistance by increasing intracellular ceramide accumulation in C2C12 myotubes. *Int J Biol Sci* 13:1–12. <https://doi.org/10.7150/ijbs.17149>
14. Jiang X, Yu J, Wang X, Ge J, Li N (2019) Quercetin improves lipid metabolism via SCAP-SREBP2-LDLr signaling pathway in early stage diabetic nephropathy. *Diabetes Metab Syndr Obes* 12:827–839. <https://doi.org/10.2147/DMSO.S195456>
15. Juster-Switlyk K, Smith AG (2016) Updates in diabetic peripheral neuropathy. *F1000Res* 5. <https://doi.org/10.12688/f1000research.7898.1>
16. Lee H, Yoon Y (2016) Mitochondrial fission and fusion. *Biochem Soc Trans* 44:1725–1735. <https://doi.org/10.1042/BST20160129>

17. Liu Y, Chen X, Yao J, Kang J (2019) Circular RNA ACR relieves high glucose-aroused RSC96 cell apoptosis and autophagy via declining microRNA-145-3p. *J Cell Biochem*. <https://doi.org/10.1002/jcb.29568>
18. Nadra K, de Preux Charles AS, Medard JJ, Hendriks WT, Han GS, Gres S (2008) Phosphatidic acid mediates demyelination in *Lpin1* mutant mice. *Genes Dev* 22:1647–1661. <https://doi.org/10.1101/gad.1638008>
19. Pellerin L, Magistretti PJ (2003) How to balance the brain energy budget while spending glucose differently. *J Physiol* 546:325. <https://doi.org/10.1113/jphysiol.2002.035105>
20. Rashid T, Nemazanyy I, Paolini C, Tatsuta T, Crespín P, de Villeneuve D (2019) *Lipin1* deficiency causes sarcoplasmic reticulum stress and chaperone-responsive myopathy. *EMBO J* 38(1). <https://doi.org/10.15252/embj.201899576>
21. Rumora AE, Savelieff MG, Sakowski SA, Feldman EL (2019) Disorders of mitochondrial dynamics in peripheral neuropathy: Clues from hereditary neuropathy and diabetes. *Int Rev Neurobiol* 145::127–176. <https://doi.org/10.1016/bs.irn.2019.05.002>
22. Sabouny R, Shutt TE (2020) Reciprocal Regulation of Mitochondrial Fission and Fusion. *Trends Biochem Sci* 45:564–577. <https://doi.org/10.1016/j.tibs.2020.03.009>
23. Sajic M (2014) Mitochondrial dynamics in peripheral neuropathies. *Antioxid Redox Signal* 21:601–620. <https://doi.org/10.1089/ars.2013.5822>
24. Sandireddy R, Yerra VG, Areti A, Komirishetty P, Kumar A (2014) Neuroinflammation and oxidative stress in diabetic neuropathy: futuristic strategies based on these targets. *Int J Endocrinol* 2014: 674987. <https://doi.org/10.1155/2014/674987>
25. Schweitzer GG, Collier SL, Chen Z, McCommis KS, Pittman SK, Yoshino J (2019) Loss of *lipin 1*-mediated phosphatidic acid phosphohydrolase activity in muscle leads to skeletal myopathy in mice. *FASEB J* 33:652–667. <https://doi.org/10.1096/fj.201800361R>
26. Shang P, Zheng F, Han F, Song Y, Pan Z, Yu S et al (2020) *Lipin1* mediates cognitive impairment in fld mice via PKD-ERK pathway. *Biochem Biophys Res Commun* 525(2):286–291. [doi:10.1016/j.bbrc.2020.02.070](https://doi.org/10.1016/j.bbrc.2020.02.070)
27. Song L, Liu Z, Hu HH, Yang Y, Li TY, Lin ZZ (2020) Proto-oncogene *Src* links lipogenesis via *lipin-1* to breast cancer malignancy. *Nat Commun* 11:5842. <https://doi.org/10.1038/s41467-020-19694-w>
28. Spinelli JB, Haigis MC (2018) The multifaceted contributions of mitochondria to cellular metabolism. *Nat Cell Biol* 20:745–754. <https://doi.org/10.1038/s41556-018-0124-1>
29. Wang Z, Zhu Y, Zhang Y, Zhang J, Ji T, Li W (2020) Protective effects of AS-IV on diabetic cardiomyopathy by improving myocardial lipid metabolism in rat models of T2DM. *Biomed Pharmacother* 127:110081. <https://doi.org/10.1016/j.biopha.2020.110081>
30. Wu H, Wei H, Sehgal SA, Liu L, Chen Q (2016) Mitophagy receptors sense stress signals and couple mitochondrial dynamic machinery for mitochondrial quality control. *Free Radic Biol Med* 100:199–209. <https://doi.org/10.1016/j.freeradbiomed.2016.03.030>

31. Xu M, Zhuang XH, Sun AL, Ni YH, Sun FD, Chen sh. High glucose induced RSC96 Schwann cells injury in vitro. *Journal of Shandong University (Health Sciences)* 5: 44–48
32. Xu Y, Zhuang XH, Liu YT, Sun AL, Li XB, Jiang DQ (2015) Over expression of lipin1 attenuates diabetic peripheral neuropathy in rats. *Journal of Shandong University (Health Sciences)* 9:53–57
33. Yan L, Xie M, Lu H, Zhang H, Shi M, Zhang Y (2018) Anti-Apoptotic Effect of IGF1 on Schwann Exposed to Hyperglycemia is Mediated by Neurtin, a Novel Neurotrophic Factor. *Mol Neurobiol* 55:495–505. <https://doi.org/10.1007/s12035-016-0331-3>
34. Yerra VG, Areti A, Kumar A (2017) Adenosine Monophosphate-Activated Protein Kinase Abates Hyperglycaemia-Induced Neuronal Injury in Experimental Models of Diabetic Neuropathy: Effects on Mitochondrial Biogenesis, Autophagy and. *Mol Neurobiol* 54: 2301–2312. <https://doi.org/10.1007/s12035-016-9824-3>
35. Yerra VG, Kalvala AK, Sherkhane B, Areti A, Kumar A (2018) Adenosine monophosphate-activated protein kinase modulation by berberine attenuates mitochondrial deficits and redox imbalance in experimental diabetic neuropathy. *Neuropharmacology* 131:256–270. <https://doi.org/10.1016/j.neuropharm.2017.12.029>

## Figures

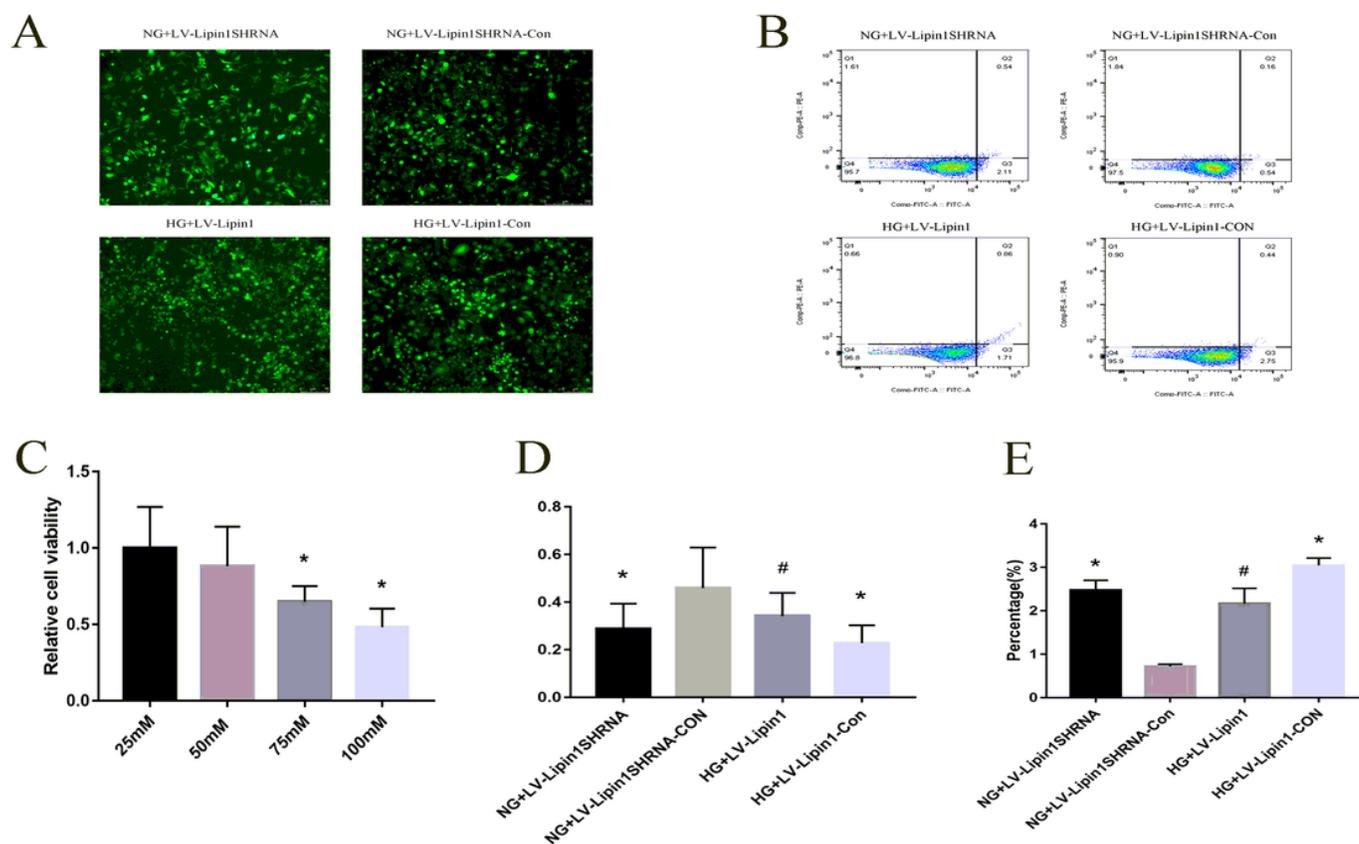
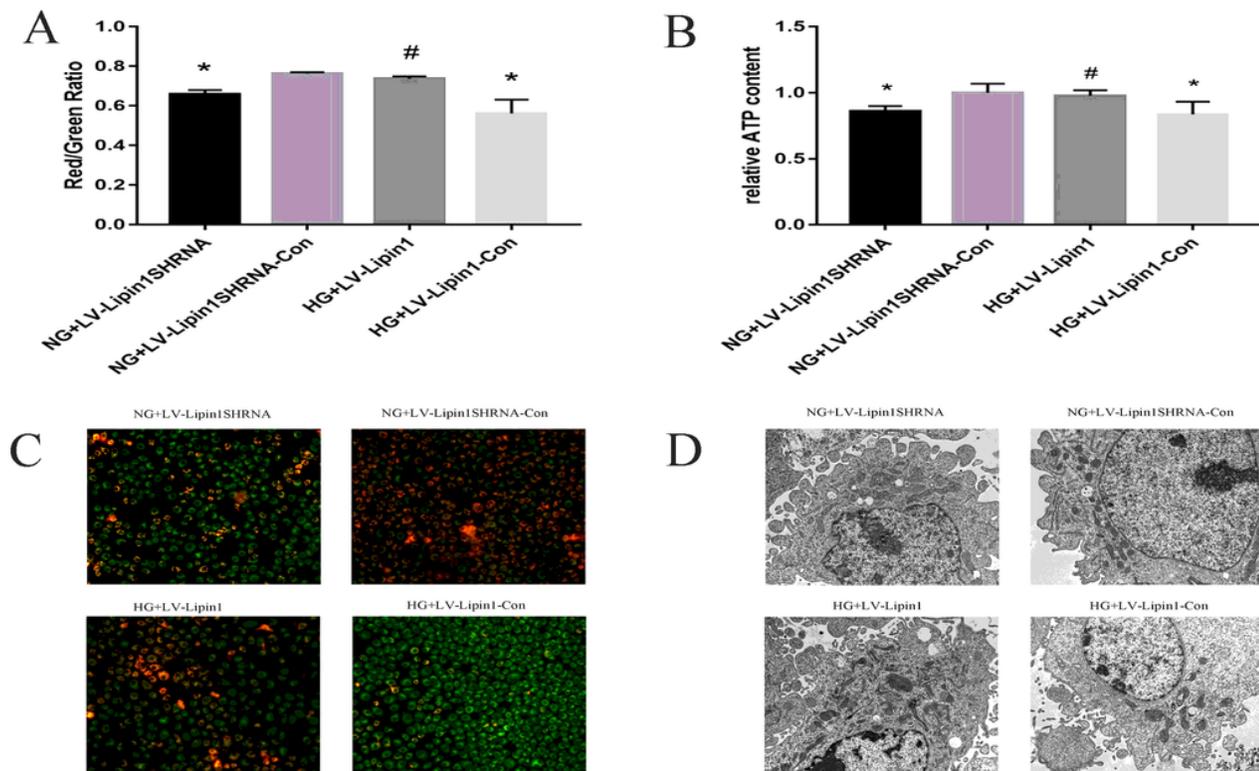


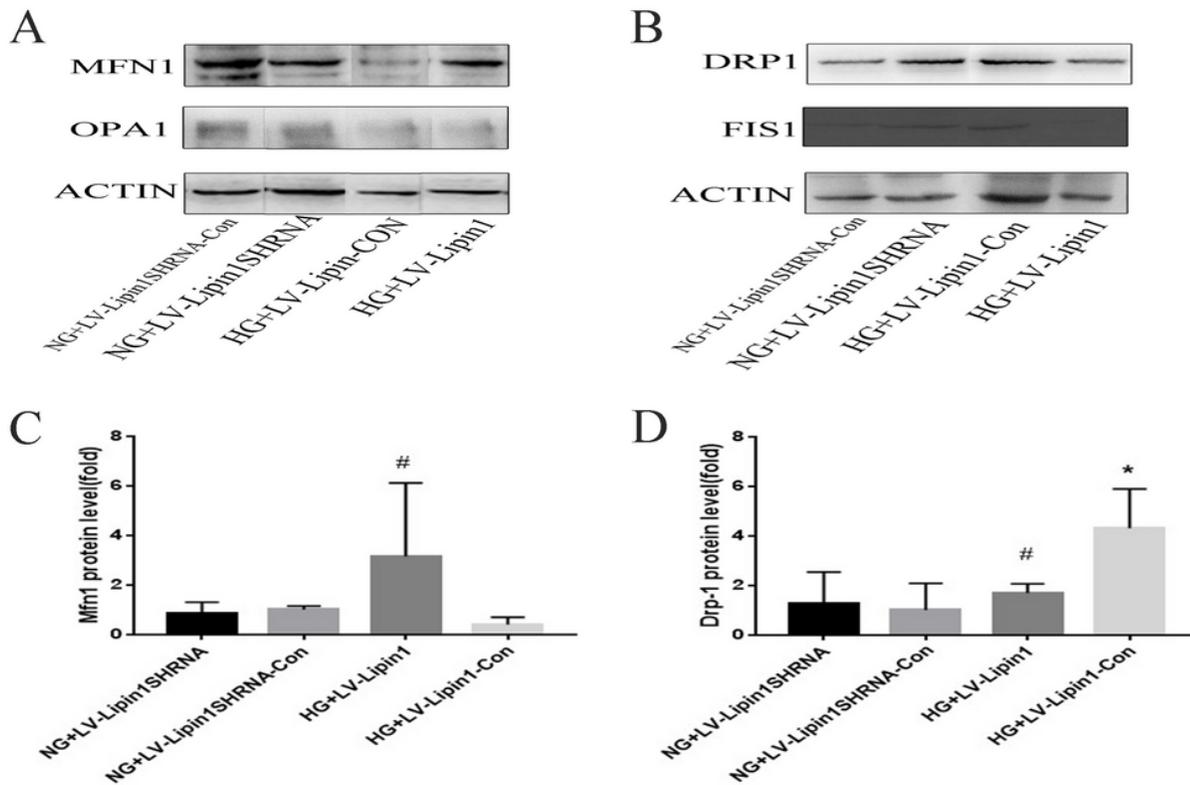
Figure 1

Lipin1 protected against high glucose - induced injury in Schwann cells (A) Schwann cells infected with Lv-Lipin1shRNA, Lv-Lipin1 shRNA-Con, Lv-Lipin1 and Lv-Lipin1-Con. Infection efficiencies were assessed using fluorescence microscopy. (B, E) Apoptotic rate of Schwann cells in different groups. Percentage of apoptotic Schwann cell analyzed by flow cytometry. Apoptotic rate with different concentrations was standardized to that of the control group (NG+LV-Lipin1 shRNA-Con group). (C) Schwann cells were treated with 25(the control group), 50, 75, 100 mM high glucose for 24 h. Cell viability with various concentrations was standardized to that of the control group. (D) Assessment of viability in cells transfected with different viruses under different treatment conditions. Data are expressed as means  $\pm$  SEMs. Values are representative of three independent experiments. \*P<0.05, vs. NG+LV-Lipin1 shRNA-Con group; #P<0.05, VS. HG+LV-Lipin1-Con group.



**Figure 2**

lipin1 attenuated mitochondrial dysfunction and morphological disorder of Schwann cells exposed to high glucose (A, C) Fluorescent microscopic images showing the mitochondrial membrane potential (JC-1 fluorescence) in Schwann cells under different experimental conditions. (B) The ATP content with different stimulation was standardized to that of NG+LV-Lipin1 shRNA-Con group. (D) Transmission electron microscopy revealed that lipin1 attenuated high glucose-induced mitochondrial pathological changes. Data are expressed as means  $\pm$  SD. \*P<0.05, vs. NG+LV-Lipin1 shRNA-Con group; #P<0.05, VS. HG+LV-Lipin1-Con group. The results are representative of three independent experiments.



**Figure 3**

Effect of lipin1 on mitochondrial fusion and fission related proteins expression changes in Schwann cells (A) Representative images of western blot show expression changes of MFN1 and OPA1 in Schwann cells under different conditions. The differences of MFN1 and OPA1 between HG+LV-Lipin1-Con and HG+LV-Lipin1 group, NG+LV-Lipin1 shRNA-Con and NG+LV-Lipin1 shRNA group were obvious. There is no difference of MFN1 and OPA1 protein levels between HG+LV-Lipin1-Con and NG+LV-Lipin1 shRNA group. (B) Representative images of western blot show expression changes of DRP1 and OPA in Schwann cells under different conditions. The differences of FIS1 and DRPI between HG+LV-Lipin1-Con and HG+LV-Lipin1 group, NG+LV-Lipin1 shRNA-Con and NG+LV-Lipin1 shRNA group were obvious. There is no difference of FIS1 and DRP1 protein levels between HG+LV-Lipin1-Con and NG+LV-Lipin1 shRNA group. (C) Quantification analysis of MFN1 protein expression levels. The difference of DRP1 between HG+LV-Lipin1-Con and HG+LV-Lipin1 group was significant. Actin was used as the loading control. (D) Quantification analysis of DRP1 protein expression levels. The differences of DRP1 between HG+LV-Lipin1-Con and HG+LV-Lipin1 group, HG+LV-Lipin1-Con and NG+LV-Lipin1 shRNA-Con group were significant. Actin was used as the loading control. Data are expressed as means  $\pm$  SD (n=3). \*P<0.05, vs. NG+LV-Lipin1 shRNA-Con group; #P<0.05, VS. HG+LV-Lipin1-Con group. MFN1: mitofusin-1; OPA1: optic atrophy-1; DRP1: dynamic-related protein 1; FIS1: mitochondrial fission protein-1