

ORIGINAL RESEARCH ARTICLE

Lipopolysaccharide-induced proliferation and glycolysis in airway smooth muscle cells via activation of Drp1

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Abstract

Abnormal airway smooth muscle cells (ASMCs) proliferation is an important pathological process in airway remodeling contributes to increased mortality in asthma. Mitochondrial dynamics and metabolism have a central role in the maintenance of the cell function. In this study, lipopolysaccharide (LPS)-induced ASMCs proliferative model was used to investigate the effect of mitochondria on the proliferation of ASMCs and the possible mechanism. We used cell and molecular biology to determine the effect of dynamin-related protein 1 (Drp1) on LPS-mediated ASMCs cell cycle progression and glycolysis. The major findings of the current study are as follows: LPS promoted an increased mitochondrial fission and phosphorylation of Drp1 at Ser616 (p-Drp1 Ser616). LPS-induced ASMCs proliferation and cell cycle progression, which was significantly inhibited application of Drp1 RNA interfering. Glycolysis inhibitor 2-deoxyglucose (2-DG) depressed ASMCs proliferative process induced by LPS stimulation. LPS caused mitochondrial metabolism disorders and aerobic glycolysis in a dependent on Drp1 activation. These results indicated that Drp1 may function as a key factor in asthma airway remodeling by mediating ASMC proliferation and cell cycle acceleration through an effect on mitochondrial metabolic disturbance.

KEYWORDS

DRP1, glycolysis, LPS, mitochondria, proliferation

*Lixin Zhang and Cui Ma have contributed equally to this work.

1 | INTRODUCTION

Airway smooth muscle cells (ASMCs) are the main orchestrators of the lung inflammatory responses and remodeling processes associated to the respiratory physiology (Choi et al., 2018; D. Liu, Pan, Zhao, & Liu, 2018; Prabhala, Bunge, Ge, & Ammit, 2016). Changes in ASMCs function are well-established contributors to airway hyperresponsiveness and airway remodeling in asthma (M. Chen, Huang, et al., 2016; Li, Lu, Wu, Zhang, & Yao, 2015). However, the mechanisms by which exposure to inflammation influence ASMCs proliferation and migration contribute to bronchial airway structure and function disorder have not been firmly established.

Lipopolysaccharide (LPS), the major component of the outer membranes of Gram-negative bacteria which is recognized as an important virulence to aggravate progression of numbers of inflammatory disorders (Z. W. Dong & Yuan, 2018; L. Liu et al., 2012). LPS liberates and activates the ubiquitous nuclear transcription factor NF- κ B to regulate transcription of diverse genes coding for cytokines including tumor necrosis factor- α (TNF- α), Interleukin-6 (IL-6), IL-8, and IL-1 β which together contribute to the upregulation of inflammatory responses (De Bosscher, Vanden Berghe, & Haegeman, 2003; J. Dong et al., 2018). Since ASMCs proliferation is one of the leading factors in the progression of airway remodeling in asthma, inhibition of ASMCs proliferation and migration represents an important therapeutic strategy for the prevention of asthma (Sung et al., 2017; Wang, Shang, Cai, & Liu, 2018). In the current study, an LPS-induced ASMCs proliferative model was used to investigate the mechanisms in subacute airway remodeling. The mechanisms by which distinct receptors are controlled by LPS, the physiological factors and functional proteins in LPS-induced ASMCs proliferation are not well understood.

Mitochondria exist in a dynamic network comprised of individual organelles that continuously undergo fusion and fission to adapt to changes in the cellular environment (Rambold & Pearce, 2018; Youle & van der Blik, 2012). Mitochondrial fission is regulated by the phosphorylation and recruitment of cytoplasmic GTPase, dynamin-related protein 1 (Drp1) which localized on the outer mitochondrial membrane to mitochondrial fission sites (Cho et al., 2009; Schmitt et al., 2018). Growing evidence suggests that Drp1 is critical for determination of not only cellular function but also cell fate through the regulation of mitochondrial metabolism activity (H. Zhang et al., 2017). Drp1-dependent mitochondrial fission regulating various cellular dynamics, including proliferation, autophagy, apoptosis, innate immunity, acute injury, and other processes required for development and maintenance of organ systems (Hu, Huang, & Li, 2017). There is currently a little information focus on mitochondrial fission-fusion dynamics in LPS-induced ASMCs proliferation or the importance of Drp1 in asthma.

Glycolysis is a central element of the primary metabolic activity and generates biomass precursors and energy from sugars which is a key element in the metabolism of mammalian cells (Rehberg, Ritter, & Reichl, 2014). Antiapoptotic and highly proliferative cells exhibit a

shift from glucose oxidation to lactate synthesis and aerobic glycolysis, accompanied by upregulation of cytoplasmic glycolysis (Caruso et al., 2017; Sun et al., 2010). This phenomenon, known as the "Warburg effect," was observed in colon and pancreatic tumors and patients with pulmonary arterial hypertension (de Alteriis, Carteni, Parascandola, Serpa, & Mazzoleni, 2018; Sithara, Arun, Syama, Reshmitha, & Nisha, 2017; W. H. Zhang et al., 2014). Accumulating evidence suggests that mitochondrial abnormality related to oxidative metabolism, cellular stress, and energy transduction resulting in different pathophysiological responses of neurons, cardiomyocytes, pulmonary, and immune cells (Ma et al., 2017; VanLinden et al., 2015). However, the precise effects of Drp1 on the mitochondrial metabolism in ASMCs are largely unknown and its mechanisms still remain to be explained.

In the current study, LPS-induced ASMCs proliferation and Drp1 expression were found, as evidenced by mitochondrial imaging, cell viability, and cell-cycle progression analysis. The glycolysis is also activated during an LPS-challenged inflammatory condition. After specifically silenced Drp1 expression, the elevated expression of proliferating cell nuclear antigen (PCNA) and cyclin A/E in ASMCs during LPS treatment could be reversed. Our results showed that Drp1 could mediate the LPS-induced mitochondrial fission and excessive proliferation of ASMCs, associated with the mitochondrial metabolism regulation.

2 | MATERIAL AND METHODS

2.1 | Materials

Antibodies against Cyclin E, Hexokinase II (HK II), Pyruvate Kinase Muscle Isozyme Isoform M2 (PKM2), Pyruvate Dehydrogenase (PDH), Phosphorylation of Drp1 at Ser616 (p-Drp1 Ser616) were obtained from Cell Signaling Technology (Danvers, MA). Antibody against Drp1 and Tom20 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies against cyclin A, β -actin, and PCNA were obtained from Boster Biological Technology (Wuhan, China). The Cell Counting Kit-8 Kit, Cell Cycle and Apoptosis Analysis Kit, and MitoTracker Green were provided by Beyotime Institute of Biotechnology (Wuhan, China). Lipopolysaccharide and 2-deoxyglucose (2-DG) from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO). All the drugs were used from the XF Cell Mito Stress Test Kit and XF Cell Glycolysis Stress Test Kit (Seahorse Biosciences, Billerica, MA). All other reagents were from common commercial sources.

2.2 | Animals use

Adult male Wistar rats (180–200 g) were from the Experimental Animal Center of Harbin Medical University, which is accredited by the Institutional Animal Care and Use Committee (IACUC). All animal experiments complied with the guidelines of the National Institutes of Health (NIH) for the Care and Use of Laboratory Animals.

2.3 | Cell culture

Airway smooth muscle cells (ASMCs) were collected according to the previously published protocol (Hirst, 1996; Ning et al., 2011). Briefly, primary cultured ASMCs were prepared from pulmonary tracheas isolated from rat lung; the isolated pulmonary tracheas were digested with 0.2% collagenase and 0.2% bovine serum albumin in phosphate-buffered saline (PBS) solution for 3 hr at 37°C. The digested ASMCs were cultured in Dulbecco's modified Eagle's (DMEM) medium supplemented with 20% fetal bovine serum (FBS) for 3 days in a humidified incubator with 5% CO₂ at 37°C. Before each experiment, the cells were incubated in DMEM without serum for 24 hr.

2.4 | Small interfering RNA (siRNA) design and transfections

To silence the expression of DRP1 protein, ASMCs were transfected with the siRNA, which was designed and synthesized by GenePharma (Shanghai, China). Nontargeted control siRNA (siNC) was used as the negative control. The sense sequence of siRNA against DRP1 and nontargeted control sequence was listed below: DRP1: (NM_053655.3)-5'-GCAGAACUCUAGCUGUAAUTT-3'; negative control (NC): 5'-UUCUCCGAACGUGUCCGUTT-3'. Briefly, the ASMCs at 60–80% confluence were used, and then 1.5 µg siRNA and 7.5 µl Lipofectamine siRNA Transfection Reagent were diluted in the serum-free 100 µl Opti-MEM-1 medium (Gibco, Grand Island, NY), and after 5 min we mixed them together. Then we incubated the mixture (siRNA/Transfection Reagent) at room temperature for 18 min and added onto cells. After transfection, cells were quiescent for 24 hr and used as required.

2.5 | 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay

ASMCs were cultured at a density of 5,000 cells/well in a 96-well culture plate, and cells were treated with different groups in DMEM. Then treated with LPS in DMEM with 5% FBS. After 24 hr of the incubation at 37°C, the cells were incubated for 4 hr in a medium containing 0.5% MTT. The reaction was terminated by adding 150 µl dimethyl sulfoxide (DMSO) to the medium followed by incubation for 10 min at 37°C. Absorbance at 540 nm was recorded by an ELISA plate reader.

2.6 | Western blot analysis

When cells reached 70–80% confluence, they were placed in the DMEM medium without serum for 24 hr. Cells were treated with the different experiment group. Protein was harvested by washing cells in cold PBS three times and then cells were scraped into 200 µl protein loading buffer, containing protease inhibitors on ice. The lysates were sonicated for 1 min and then centrifuged at 13,500 rpm for 18 min at 4°C. The protein concentrations in the supernatant were confirmed using the Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Inc., Berkeley, CA). Samples containing 20 µg total

proteins were heated with SDS-PAGE sample buffer for 5 min at 100°C and separated by 10 sodium dodecyl sulfate polyacrylamide gelelectrophoresis (SDS-PAGE) gels. Protein was then transferred to nitrocellulose membrane and incubated with Tris-buffered saline (Tris 20 mM, NaCl 150 mM, pH = 7.6, Tween 20 0.1%) containing 5% nonfat dry milk for 1 hr and incubated with DRP1 (1:500), p-DRP1 (1:500), PCNA (1:1,000), cyclin A (1:500), cyclin E (1:500), HK II (1:500), PDH (1:500), PKM2 (1:500), β-actin (1:5,000) and secondary antibodies as described β-actin as an internal control. These proteins were visualized with enhanced chemiluminescence reagents.

2.7 | Cell cycle and DNA analysis

ASMCs were treated in groups as indicated, harvested and fixed using 70% ethanol. The ethanol was removed, and the cells were incubated in 200 µl PBS. DNA fluorescence and flow cytometry were performed using a BD FACSCalibur Flow Cytometer (BD Biosciences, Bedford, MA). The cells were stained according to the CycleTEST PLUS DNA Reagent Kit protocol. For each sample, 2 × 10⁵ events were accumulated in a histogram. The proportions of cells in the different phases of the cell cycle were calculated from each histogram.

2.8 | Image analysis of mitochondrial morphology

To quantify mitochondrial fragmentation count, ASMCs were transfected with mitochondrial-tracker, photoactivatable green fluorescent tracker. Briefly, images of mitochondrial matrix-targeted green fluorescent protein-transfected ASMCs were obtained, thresholded, and binarized to identify mitochondrial segments using Image J (Rawak Software Inc., Stuttgart, Germany). The mitochondrial fragmentation count was calculated by normalizing the number of individual mitochondria to the entire mitochondrial pixel count.

2.9 | Cell Counting Kit-8 (CCK8) assay

ASMCs were separately seeded into four 96-well plates at a density of 1 × 10⁴/well, and then cells were treated with different agents according to the different experimental groups. After 24 hr of the incubation at 37°C, the CCK8 kit (10 µl) was added into each well. After 2 hr of incubation at 37°C, the optical density (OD) was detected by a spectrophotometer at the wavelength of 450 nm.

2.10 | Mitochondrial glycolysis assays

Mitochondrial glycolysis was analyzed using a Seahorse XF24 Extracellular Flux Analyzer (Seahorse Biosciences, North Billerica, MA) by real-time measurements of extracellular acidification rate (ECAR, indicative of glycolysis). To estimate changes in cellular glycolytic rates we measured changes in the ECAR. ASMCs were glucose-starved in XF assay medium in a CO₂-free XF prep station at 37°C for 60 min and then sequentially injected with glucose

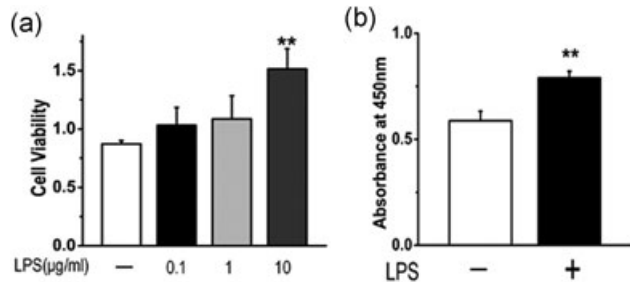


FIGURE 1 Scheme of LPS-induced ASMCs proliferative model. (a) Cells were growth-arrested for 24 hr and then treated with LPS (0.1–10 µg/ml) for 48 hr, cell viability was determined by MTT assay in vitro (** $p < 0.01$; $n = 5$). (b) CCK8 assay showed that the cell proliferation increased with 10 µg/ml LPS treatment (** $p < 0.01$; $n = 5$). All values are denoted as means $\pm$ SEM. ASMCs: abnormal airway smooth muscle cells; CCK8: Cell Counting Kit-8; LPS: lipopolysaccharide; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide

(2 mg/ml), oligomycin (1 µM) and 2-deoxy-D-glucose (100 mM). Differences in basal-, maximal-, and spare glycolytic capacity were then determined and the data represented as milli-pH per minute.

2.11 | Statistical analysis

The composite data were expressed as means $\pm$ SEM. Statistical analysis was performed with χ^2 test, Student's t test or one-way

ANOVA followed by Dunnett's test where appropriate. $p < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | LPS treatment induces ASMCs proliferation

Proliferative responses of isolated ASMCs to LPS stimulation were investigated by cell viability and proliferation assays. As illustrated in Figure 1a, 48 hr treatment with LPS resulted in a significant and concentration-dependent increase in cell viability, reaching a maximum at a concentration of 10 µg/ml. LPS (0.1 µg/ml) had no additional effect on cell viability when compared to none treatments. Similarly, LPS-induced an increase in cell proliferation as assessed by Cell Counting Kit-8 (CCK8) assay (Figure 1b).

3.2 | LPS increases mitochondrial fission and activates mitochondrial Drp1

To determine whether mitochondrial dysfunction is involved in LPS-mediated ASMCs proliferation, the mitochondrial morphology was first analyzed by MitoTracker Fluorescent Probe. We found that after stimulation with LPS, mitochondrial morphology from a mixture of elongated tubules and short fragments to less elongated and more fragmented form (Figure 2a). To examine whether LPS-affected

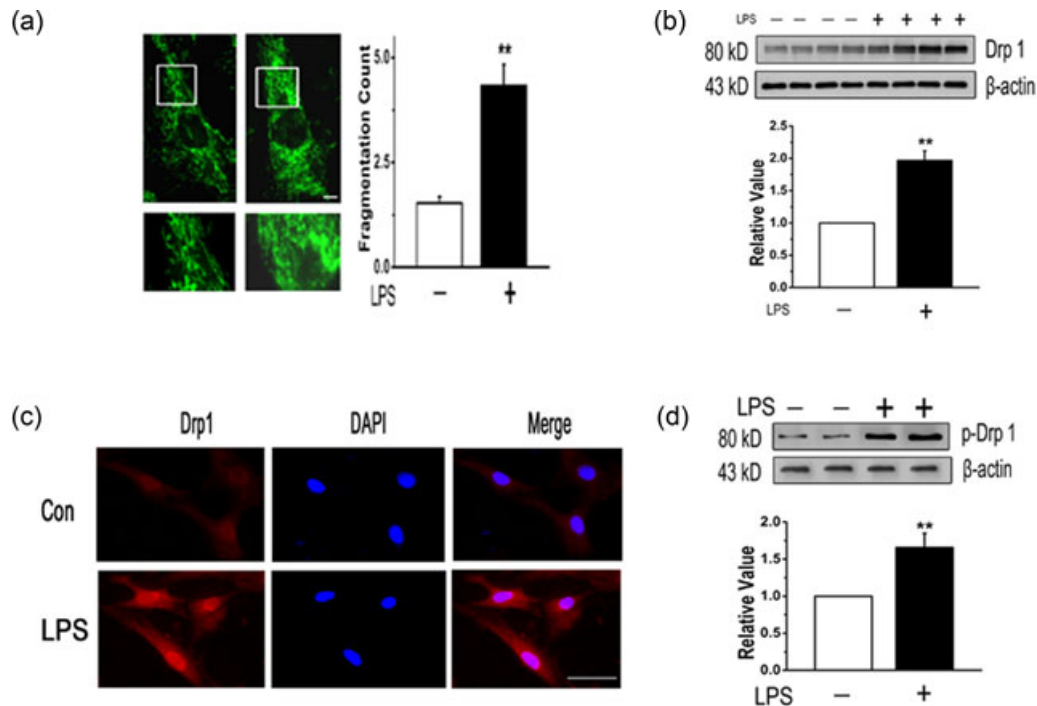


FIGURE 2 DRP1 expression was increased after LPS treatment. (a) Representative images from Mito-Tracker Green-labeled mitochondria which showed that LPS induced morphological alteration of mitochondria. Scale bars = 50 µM (** $p < 0.01$; $n = 6$). (b) LPS significantly increased DRP1 protein expression (** $p < 0.01$; $n = 4$). (c) Immunofluorescence evaluation of the expression of DRP1 in ASMCs of rats under LPS treatment conditions. Scale bars = 25 µM. (d) Immunoblotting of activated Drp1 (p-Drp1 Ser616) in LPS stimulated ASMCs (** $p < 0.01$; $n = 4$). All values are denoted as means $\pm$ SEM. ASMCs: abnormal airway smooth muscle cells; Drp1: dynamin-related protein 1; LPS: lipopolysaccharide; p-Drp1 Ser616: phosphorylation of Drp1 at Ser616 [Color figure can be viewed at wileyonlinelibrary.com]

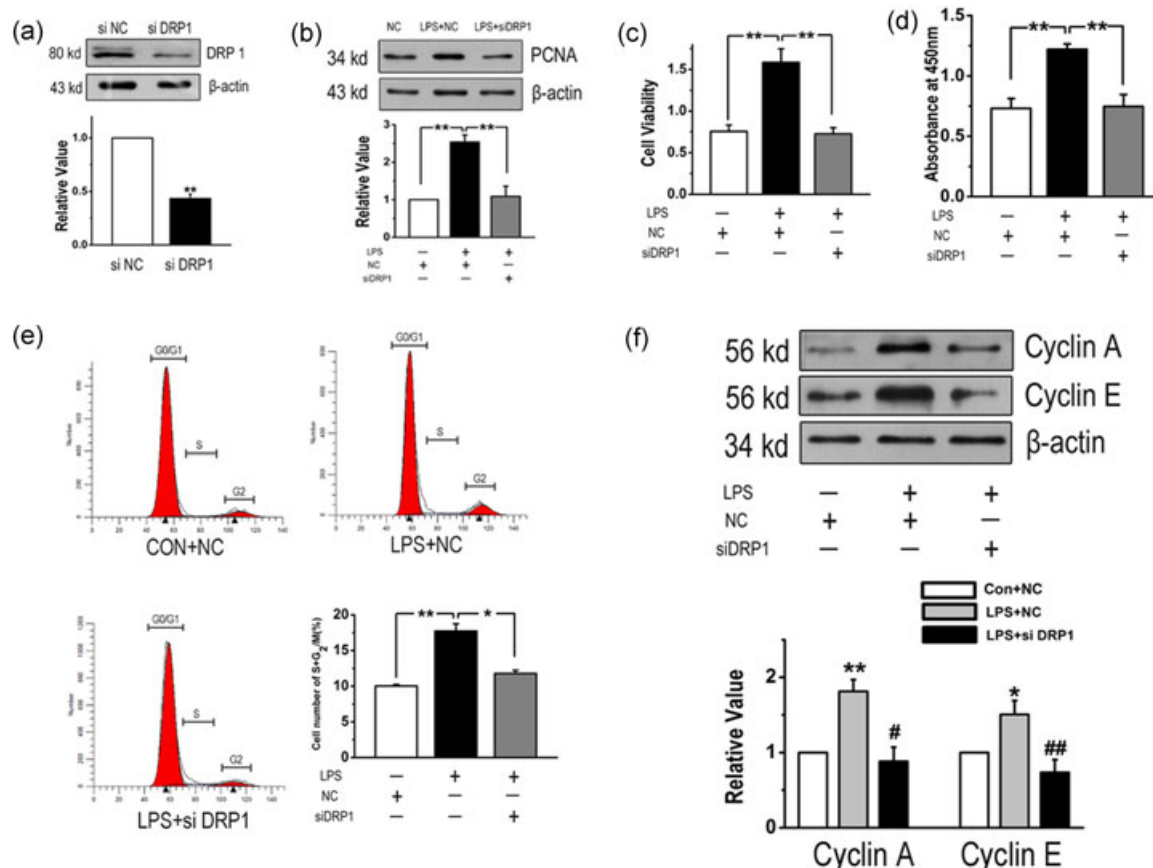


FIGURE 3 LPS induced pathological process of ASMCs through Drp1 activation. (a) Protein expression of DRP1 was measured to assess the efficiency and specificity of siRNAs targeting DRP1 knockdown (** $p < 0.01$; $n = 5$). (b) LPS mediated increased expression of PCNA was reversed by siDRP1 (** $p < 0.01$; $n = 5$). Cell viability (c) and proliferation (d) were measured under LPS treatment with or without DRP1 RNA interference (** $p < 0.01$; $n = 6$). (e) LPS increased the cell number in the S and G₂/M phases compared with control. This effect was blocked by siDRP1 (* $p < 0.05$, ** $p < 0.01$; $n = 6$). (f) The protein expression of cyclin A and cyclin E were determined after knocking down DRP1 (* $p < 0.05$ and ** $p < 0.01$ vs. control; # $p < 0.05$ and ## $p < 0.01$ vs. LPS; $n = 6$). All values are denoted as means $\pm$ SEM. ASMCs: abnormal airway smooth muscle cells; CON: control; LPS: lipopolysaccharide; NC: negative control; PCNA: proliferating cell nuclear antigen; siDRP1: siRNA targeting DRP1 [Color figure can be viewed at wileyonlinelibrary.com]

mitochondrial fission by regulating cytoplasmic GTPase, Drp1, we performed western blot analysis and immunofluorescent assay. As shown in Figure 2b,c, LPS administration significantly increased the expression of Drp1. Furthermore, LPS activated p-Drp1 Ser616 in ASMCs (Figure 2d).

3.3 | Drp1 inhibition by RNA interference reduces ASMCs cell proliferation

RNA interference transfection of rats ASMCs was used to significantly downregulate Drp1 expression (Figure 3a). As shown in Figure 3b-d, the exposure of LPS led to the increase of PCNA protein expression, cell viability, and cell proliferation in ASMCs. Moreover, the advanced effects of LPS on cell proliferation were attenuated by siRNA targeting DRP1 (siDRP1). LPS-induced cell proliferation is also demonstrated by an increased percentage of cells entering S-phase and G₂/M-phase. And cells treated with siRNA targeted at Drp1 reversed cell-cycle transition from G₀/G₁ phase to S and G₂/M phase induced by LPS (Figure 3e). Since cyclin A and cyclin E play important roles in both the S and G₂/M

phases of the cell cycle, we analyzed the posttranslational levels of cyclin A and cyclin E in ASMCs. A significant reduction in the expression of the cell cycle regulated proteins was observed after knocking down Drp1 (Figure 3f). These results suggest that Drp1 is involved in the ASMCs cell cycle control of LPS.

3.4 | Inhibition of mitochondrial aerobic glycolytic pathway prevents ASMCs hyperproliferation

After confirming the pathological influence of LPS in ASMCs, we explored the mechanisms for the occurrence of proliferation. Previous research indicated that continuously growing mammalian cells often exhibit a disturbed glucose metabolism (Rehberg et al., 2014). To further explore the influence of the Warburg effect on induction of ASMCs proliferative process, we treated cells with glycolysis inhibitor 2-DG. Cell viability and proliferation assays demonstrated that 2-DG abrogated the increasing proliferation of ASMCs induced by LPS (Figure 4a,b). We evaluated two key regulatory molecules in the cell cycle, cyclin A and cyclin E;

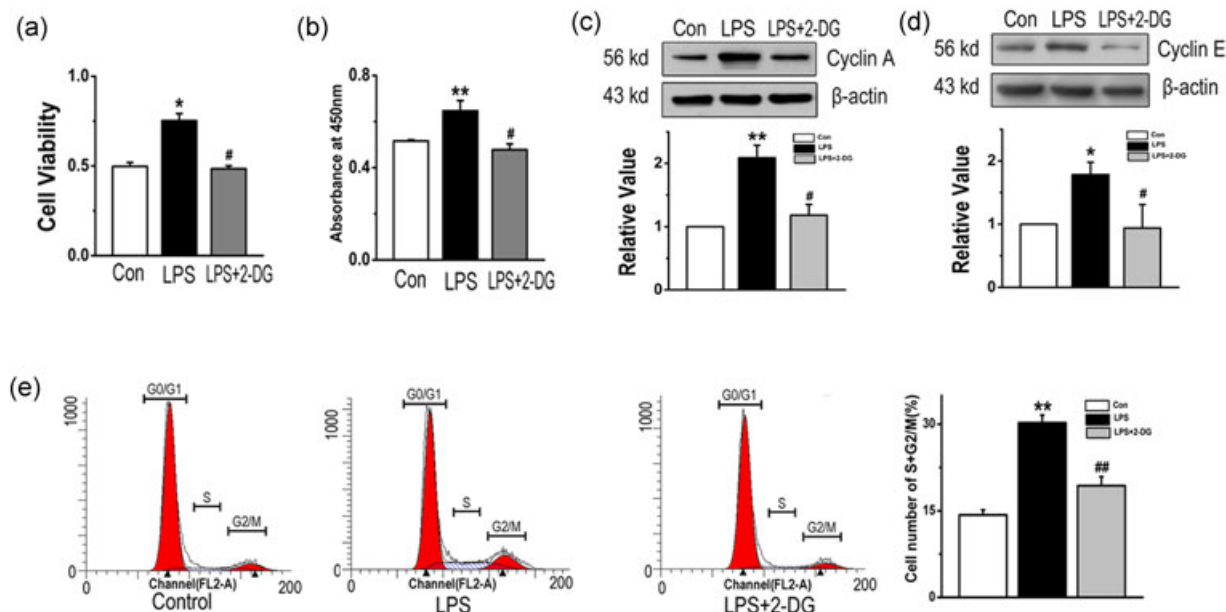


FIGURE 4 Impact of hyperactive glycolysis on ASMCs proliferation. (a, b) The survival rate of ASMCs following treatment of LPS was studied by MTT and CCK8 assay. Cells were growth-arrested for 24 hr and then stimulated with glycolysis inhibitor 2-DG ($*p < 0.05$ and $**p < 0.01$ vs. control; $#p < 0.05$ vs. LPS; $n = 6$). (c, d) Effects of 2-DG on the expression of cyclin A and cyclin E. (e) Cell-cycle analysis indicated that LPS stimulated cell progression into G₂/M + S-phase, and this effect was inhibited by 2-DG ($*p < 0.05$ and $**p < 0.01$ vs. control; $##p < 0.01$ vs. LPS; $n = 6$). All values are denoted as means $\pm$ SEM. ASMCs: abnormal airway smooth muscle cells; CCK8: Cell Counting Kit-8; 2-DG: 2-deoxyglucose; LPS: lipopolysaccharide; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide [Color figure can be viewed at wileyonlinelibrary.com]

decreased levels of cyclins were observed in ASMCs treated with 2-DG (Figure 4c,d). The LPS induced increase of cells entering S-phase and G₂/M-phase was inhibited by glycolysis inhibitor 2-DG (Figure 4e). The above results indicated that LPS affected ASMCs proliferation and cell cycle progression partly through changing the glycolytic metabolic behavior.

3.5 | LPS regulates mitochondrial metabolism disorders in a Drp1-dependent manner

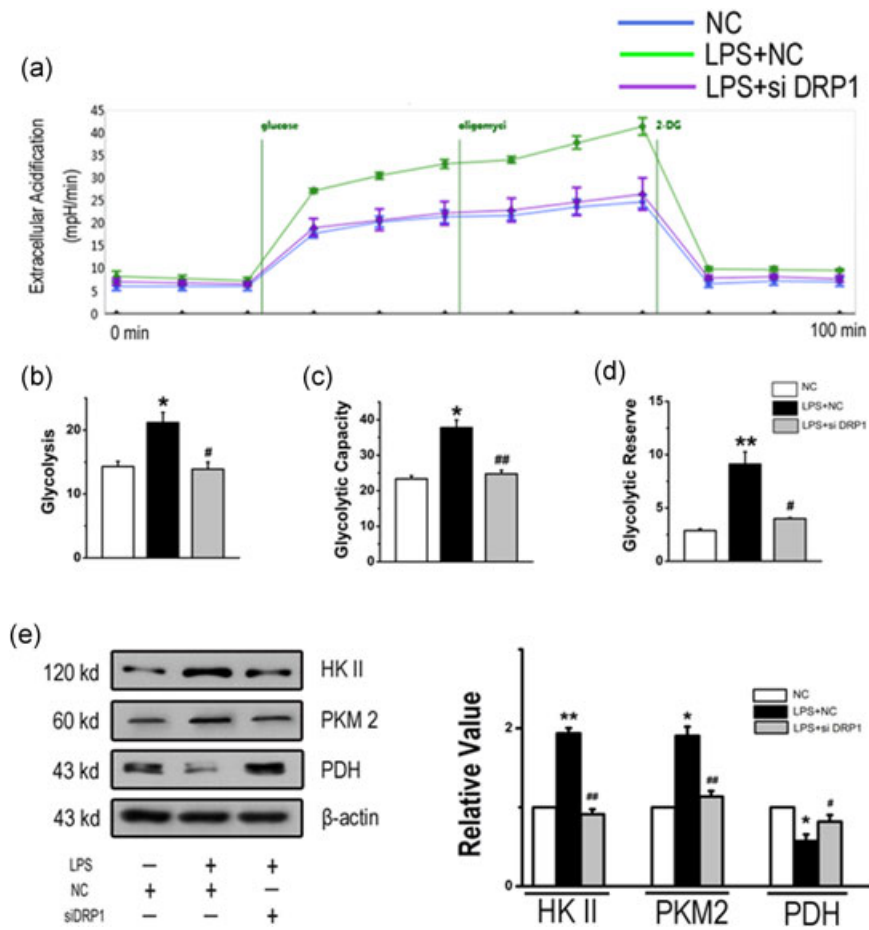
The mechanism of action of 2-DG suggests that ASMCs displaying higher levels of aerobic glycolysis may be responsible for hyperproliferation to LPS, and Drp1 is not only essential for ASMCs proliferation, but that ectopic expression of Drp1 also increases the mitochondrial metabolism disorders. This hypothesis is supported by experiments in a Seahorse XFe24 Extracellular Flux Analyzer to monitor the metabolic patterns of mitochondria in ASMCs. The ECAR was monitored in real time to estimate glycolysis. The results showed that glycolytic stress activation stimulated by LPS was blocked with siDrp1 (Figure 5a-d). Furthermore, the expression of glycolytic key enzymes including HK II, pyruvate kinase (PKM), and PDH was analyzed in vitro. Increased glycolysis in LPS stimulation was confirmed by the increased expression of HK II and PKM2 and downregulated expression of PDH, which could be reversed by silencing Drp1 (Figure 5e). The energy phenotype test and metabolic enzyme immunoblotting both indicate that the LPS-induced glycolysis in ASMCs was mediated by Drp1.

4 | DISCUSSION

In this study, we demonstrated that LPS induce a profound and concentration-dependent increase in cell number of cultured ASM cells. The LPS-induced cell proliferation is dependent on phosphorylation of DRP1 at Ser616. Moreover, blocking the progress of glycolysis by 2-DG has been proved to reduce ASMCs proliferation stimulated by LPS. In addition, we demonstrated that LPS treatment changed mitochondrial biogenesis, the high glycolytic activity of ASMCs after LPS stimulation, which is also associated with increased expression of DRP1. These data confirmed our hypothesis that DRP1-mediated mitochondrial metabolic dysfunction contributed to LPS-induced ASMC proliferation, which plays an important role in the LPS-induced airway remodeling.

Prior studies have utilized LPS administration to model asthma and chronic obstructive pulmonary disease (COPD), and showed that LPS-induced ASMCs proliferation was associated with the activation of the Toll-like receptor 4 (TLR4) signaling as well as transient receptor potential channel-3 (TRPC3) mediated Ca²⁺ influx resulting in inflammation and airway remodeling (X. X. Chen, Zhang, et al., 2016; Toward & Broadley, 2002; Vernooij, Dentener, van Suylen, Buurman, & Wouters, 2002). Furthermore, we and other researchers have reported that mitochondrial fragmentation and networked morphology damages after the hypoxia and cigarette smoke extract (CSE) stimulation via Drp1 and reactive oxygen species (ROS) pathways influence cell proliferation and apoptosis relevant to vascular and airway diseases (Aravamudan et al., 2018; Zhang

FIGURE 5 Higher glycolytic stress activation stimulated by LPS was relieved by siDRP1. (a) Extracellular acidification rates (ECAR) of cultured ASMCs measured by Seahorse XFe24 ($n = 5$). (b, c, d), Glycolysis and glycolytic reserve following 10 mM glucose and 1 μ M oligomycin were measured, and nonglycolytic acidification after treatment with 100 mM 2-DG was detected (* $p < 0.05$ and ** $p < 0.01$ vs. control, # $p < 0.05$ and ## $p < 0.01$ vs. LPS; $n = 6$). (e) The protein expression of HK II, PKM2, and PDH in anticipated groups showed the effects of LPS on ASMCs glycolysis were inhibited by siDRP1 (* $p < 0.05$ and ** $p < 0.01$ vs. control, # $p < 0.05$ and ## $p < 0.01$ vs. LPS; $n = 5$). All values are denoted as means $\pm$ SEM. ASMCs: abnormal airway smooth muscle cells; 2-DG: 2-deoxyglucose; HK II: hexokinase II; LPS: lipopolysaccharide; NC: negative control; PDH: pyruvate dehydrogenase; PKM2: pyruvate kinase muscle isozyme isoform M2; siDRP1: siRNA targeting DRP1 [Color figure can be viewed at wileyonlinelibrary.com]



et al., 2016). Consistent with the work, we provide evidence here that a direct effect of LPS on ASM cell proliferation may contribute to airway remodeling. In the current study, we found that DRP1 mediated LPS-induced proliferation of ASMCs, which is key process underlying the formation of airway remodeling in asthma.

Impaired mitochondrial function and stalling glycolysis with reduced availability of pyruvate are essential regulators of mitochondrial-dependent apoptosis and necrosis respond to stimuli (Handy & Loscalzo, 2012). However, the effects of mitochondrial metabolism on LPS-induced bronchial airways inflammation contributes to wheezing are less understood. The glucose analogue 2-DG is typically used to inhibit the glycolytic metabolism which can be actively taken up by the cells via glucose transporters and phosphorylated (2-DG6-phosphate) by hexokinase, but not metabolized further by phosphohexose isomerase resulting in its trapping causing the metabolic block (Cheng et al., 2012; Maschek et al., 2004). The important role of aerobic glycolysis in mediating ASMCs changes evoked by LPS was verified by application of 2-DG in combination with LPS. In the current study, we found that 2-DG treatment cells had decreased levels of cyclin A/E and cell cycle arrest (Figure 4), which was in accordance with other studies showing downregulation Warburg effect contributing to the antitumor effect (Gu et al., 2017; Singh et al., 2015). Moreover, the detailed in vitro characterization of key glycolytic enzymes, such as HK, PKM, and PDH were further

explored in the study. We observed that the LPS-induced shift of metabolic pathway to hyperactive glycolysis in ASMCs as showed by the increased expression of HK, PKM, and PDH are relieved by siDRP1. Although this protection is somewhat incomplete, it plays a crucial role in maintaining mitochondrial metabolic behavior and benefit to maintain normal growth status of ASMCs. Studies revealed that mitochondrial defects and mitochondrial oxidative stress may play a large role in the development of allergic asthma (Reddy, 2011).

Here, DRP1 is highlighted in the influence on mitochondrial metabolism in which DRP1 mediated LPS-induced proliferation of ASMCs for the first time. Consequently, these results suggest that mitochondrial metabolism may represent a new potential therapeutic target in airway remodeling in asthma.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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