Photoactivation of Dok1/ERK/PPARγ signaling axis inhibits excessive lipolysis in insulin-resistant adipocytes

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Insulin resistance is a hallmark of the metabolic syndrome and type 2 diabetes. Increased plasma FFA level is an important cause of obesity–associated insulin resistance. Over-activated ERK is closely related with FFA release from adipose tissues in patients with type 2 diabetes. Nevertheless, there are no effective strategies to lower plasma FFA level. Low-power laser irradiation (LPLI) has been reported to regulate multiple biological processes. However, whether LPLI could ameliorate metabolic disorders and the molecular mechanisms involved remain unknown. In this study, we first demonstrated that LPLI suppresses excessive lipolysis of insulin-resistant adipocytes by activating tyrosine kinase-1 (Dok1)/ERK/PPARγ pathway. Our data showed that LPLI inhibits ERK phosphorylation through the activation of Dok1, resulting in decreased phospho-PPARγ level. Non-phosphorylated PPARγ maintains in nucleus to promote the expression of adipogenic genes, reversing excessive lipolysis in insulin-resistant adipocytes. In summary, the present research highlights the important roles of Dok1/ERK/PPARγ pathway in lowering FFA release from adipocytes, and our research extends the knowledge of the biological effects induced by LPLI.

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1. Introduction

Insulin resistance, a fundamental hallmark of type 2 diabetes, is closely associated with many biochemical and pathophysiologic sequelae, including atherosclerosis, hypertension, hyperglycemia and hypertriglyceridemia [1–3]. Obesity plays a key role in triggering insulin resistance and type 2 diabetes [4]. In obese individuals, excessive FFA release from adipose tissue is a major cause of insulin resistance. Accumulated evidences have shown that FFAs suppress insulin signaling pathway through phosphorylation of insulin receptor substrates (IRSs) [5–7]. Plasma FFAs discharging from adipose tissue are assembled as diacylglycerol in the muscle to inhibit the activity of IRS by activating members of the PKC family, or are incorporated into lipids in the liver and then result in nonalcoholic fatty liver disease (NAFLD) [8]. Moreover, excessive plasma FFAs also promote the synthesis and secretion of VLDL, eventually leading to atherosclerosis [8,9]. Thus, normalization of the excessive FFA release from adipose tissue could be one of the most important steps in treating obesity–associated insulin resistance.

PPARγ, a member of the nuclear receptor family of ligand-dependent transcription factors, is over-expressed in adipose tissue and is considered to be a master regulator of adipogenesis [10]. PPARγ promotes fatty acid storage in fat depots by regulating adipogenic gene expression. In insulin-resistant state, the transcriptional activity of PPARγ is significantly impaired. Over-activated ERK is an important reason for impaired transcriptional activity of PPARγ in type 2 diabetes patients’ adipocytes [11]. Phosphorylation of PPARγ by ERK promotes PPARγ translocation from nucleus to cytoplasm [12–14]. Downstream of tyrosine kinase 1 (Dok1), a major negative regulator of MEK/ERK, is highly expressed in adipose tissue [15,16]. Previous studies showed that Dok1 is a member of docking proteins for a wide range of receptor tyrosine kinases (RTKs) [15,17]. Additionally, Dok1 may act as a tumor suppressor due to its inhibitory effects on cell proliferation through blocking MEK/ERK [17,18]. Nevertheless, it is unclear whether Dok1 is involved in lipid metabolism through regulating MEK/ERK signaling axis in insulin-resistant adipocytes.

Low-power laser irradiation (LPLI) is a non-thermal irradiation using light in visible to near infrared range and widely used to accelerate wound healing, reduce pain and inflammation in a variety of pathologies [19–21]. The effects of LPLI are verified by various animal models and clinical trials, whereas the mechanisms of how LPLI works remain obscure. A potential mechanism is that light energy is absorbed by intracellular chromophores, which accelerates electron transfer reactions, leading to the increase of reactive oxygen species (ROS) as versatile second messengers [22]. In addition, growing evidences have shown that LPLI activates RTKs and triggers its downstream effectors, such as

Abbreviations: Akt, protein kinase B; Dex, dexamethasone; Dok1, downstream of tyrosine kinases-1; ERK1/2, extracellular signal-regulated kinase 1 and 2; ap2, fatty acid binding protein 4; IBMX, isobutylmethylxanthine; IRS, insulin receptor substrate; InsR, insulin receptor; LPLI, low power laser irradiation; MEK, MAP kinase-ERK kinase; PI3K, phosphoinositide-3-kinase; RTKs, receptor tyrosine kinases; RXR, PPARγ receptor; ub, ubiquitin; WCL, whole cell lysate.

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Ras, PKC, and PI3-K/Akt [19,23]. Recent study has demonstrated that LPLI promotes glucose uptake through activation of PI-3K/Akt2/GLUT4 pathway in 3T3-L1 adipocytes [24]. The effects of LPLI on energy expenditure in adipocytes may provide potential for improving metabolic disorders in insulin-resistant condition. However, the underlying mechanisms are not fully elucidated.

In the present study, we investigated the effects of LPLI on lipid metabolism disorders using insulin-resistant 3T3-L1 adipocytes. Here we demonstrated that LPLI dramatically inhibited ERK activation through Dok1, resulting in the decrease of PPARγ phosphorylation. Non-phosphorylated PPARγ maintained in the nucleus to promote the expression of ap2, adiponectin, FAT, adipin and perilipin, reversing the excessive lipolysis in insulin-resistant adipocytes. Our data indicates that Dok1/ERK/PPAR signaling axis may be a potential target for ameliorating lipid metabolism disorders in insulin-resistant adipocytes, and our research extends the knowledge of the biological effects induced by LPLI.

2. Materials and methods

2.1. Reagents and plasmid

Dexamethasone (Dex), isobutylmethylxanthine (IBMX), Oil red O and PDB8059 were acquired from Sigma (St. Louis, MO). Insulin was obtained from Toscris Bioscience (Ellisville, MO). Lipid TOX™ Green Neutral Lipid Stains and Lipofectamine™ 2000 reagent were obtained from Invitrogen (Carlsbad, CA). Antibodies for western blot were as follows: anti-ERK, anti-phospho-Thr202/Tyr204-ERK, anti-PPARγ (Cell Signaling Technology, Danvers, MA); Anti-Dok1 (Bioworld Technology Inc., Minneapolis, MN); anti-Phospho-Tyr362-Dok1, anti-phospho-Ser112-PPARγ, anti-β-actin, anti-Na+/K+ ATPase (NKA) and anti-Histone H3 (Santa Cruz, Santa Cruz, CA). Dok1-GFP plasmid was a gift from Dr. Bakary S Sylla [25].

2.2. Cell culture and cell differentiation

3T3-L1 preadipocytes were cultured in Dulbecco’s Modified Eagle’s Medium/Ham’s F12 (1:1) medium (GIBCO, Grand Island, NY) with 10% bovine calf serum, maintained at 37 °C and 5% CO2. 3T3-L1 preadipocytes were differentiated as described previously [26]. Briefly, the preadipocytes were cultured in the 60-mm plates and supplemented with DMEM/F12 medium contained 10% fetal calf serum (FCS) (growth medium) to confluent (differentiated day 0) and were then induced to differentiate by incubating for 48 h in differentiation medium A (growth medium plus 0.5 mM 3-isobutyl-1-methylxanthine, 1 μM dexamethasone and 0.85 μM insulin). Thereafter, the medium was changed to differentiation medium B (growth medium supplemented with 0.85 μM insulin) and cells were incubated for a further 48 h. The medium was replaced with fresh growth medium every 2 days. The cells were maintained for up to differentiated day 8 and then confirmed by observing the accumulation of lipid droplets under the microscope via staining with Oil red O.

2.3. Establishment of insulin-resistant cell model

Induction of insulin-resistant adipocytes was performed as described previously [27]. Briefly, the differentiated 3T3-L1 adipocytes were treated with Dex (20 nM) for 6–8 days. Medium with Dex was changed every 2 days during the induction period. For washout experiment, the adipocytes were cultured in induce medium (growth medium contained 20 nM Dex) and refreshed every 2 days. On the induced day 4, growth medium was changed and refreshed every 2 days.

2.4. LPLI treatment

Irradiation experiment was conducted as described in our previous work [28]. One day after Dex induction, the cells were irradiated with He–Ne laser (632.8 nm, HN–1000, Laser Technology Application Research Institute, Guangzhou, China) for 3.27, 6.54, 13.08 and 26.16 mW/cm² for 10 min in the dark with the corresponding doses of 2.5, 5, 10 and 20 J/cm², respectively. For 7 days continued LPLI treatment, the insulin-resistant adipocytes were irradiated with laser every day at 21:00.

2.5. FFAs and glycerol release, intracellular triglycerides assays

For FFA release detection, the culture supernatant was collected and then determined by FFA Detection kit (Nanjing Jiancheng Bioengineering Institute, Beijing). Briefly, FFAs in culture supernatant were coupled with cupric and the cuprate was soluble in chloroform. 2 min later, the mix was centrifuged at 10,000 g for 10 min at room temperature. The down organic phase reacted with color reagent and the absorbance at 540 nm was measured. For glycerol release detection, the culture supernatant was heated at 70 °C for 10 min to inactivate residue lipase activity in the samples. Glycerol content was determined by a Glycerol assay kit (Applygen Technologies Inc., Beijing) from the absorption of 490 nm [29]. For intracellular triglycerides analysis, cells were washed twice with 1 ml PBS, and then dissolved in lysis buffer (20 mM Tris, pH 7.4, 150 mM NaCl, 2 mM EDTA, 2 mM EGTA, 1 mM sodium orthovanadate, 50 mM sodium fluoride, 1% TritonX-100, 0.1% SDS). Whole-cell lysate was centrifuged at 10,000 g for 5 min at 4 °C. The TAG content of the supernatant was measured using a triglyceride assay kit (Applygen Technologies, Beijing) [30].

2.6. Western blot analysis

Cells in 60-mm plates were washed three times with PBS and then lysed with 150 μl lysis buffer added 100 μg/ml phenylmethylsulfonyl fluoride (PMSF). The lysates were collected and centrifuged at 10,000 g for 10 min at 4 °C. Protein concentrations were determined using the Bradford method. Cell lysates were mixed with sample buffer and boiled for 5 min. Proteins were separated in 10% SDS–polyacrylamide gels and then were transferred to PVDF membranes. The membranes with proteins were incubated with indicated primary antibodies and then were labeled with goat anti-rabbit conjugated to IRDye™ 800 secondary antibodies (Rockland Immunocchemicals, Gilbertsville, PA) or with goat anti-mouse conjugated to Alexa Fluor 680 secondary antibodies (Invitrogen, Carlsbad, CA). Detection was performed using the LI-COR Odyssey Scanning Infrared Fluorescence Imaging System (LI-COR, Inc., Lincoln, NE).

2.7. Cell-cycle analysis

After irradiated with He–Ne laser for continued 7 days, insulin-resistant adipocytes were fixed with 70% ethanol, and pretreated with 250 μg/ml RNase. Quantitation of proliferation by propidium iodide (PI, 50 μg/ml) staining was performed. Flow cytometry assay (FACS) was performed on a FACS canto flow cytometer (Becton Dickinson, Mountain View, CA) with excitation at 488 nm. Cell cycle profile was determined by using the program M software on the flow cytometer.

2.8. Cell fractionation

To prepare cytoplasm and membrane fractions, cells were homogenized in buffer 1 (10 mM Tris–HCl, 2 mM MgCl2, 0.2 M sucrose, 0.5 mM EDTA, pH 7.5), and centrifuged at 1000 g for 10 min at 4 °C. The supernatant was pooled and centrifuged at 100,000 g for 40 min to get the cytoplasm fraction. The remaining pellet was homogenized in buffer 2 (6 mM Tris–HCl, 1 mM EDTA, pH 8.0), and centrifuged another 40 min at 100,000 g. The pellet was then resuspended in buffer 3 (20 mM Tris–HCl, 1 mM EDTA, pH 7.5) and centrifuged at 100,000 g for 40 min to get the membrane fraction [31]. Cytoplasmic and nuclear fractions were obtained as described previously [23]. Briefly, cells were washed
with ice-cold PBS, then scraped and homogenized with ice-cold hypotonic buffer (10 mM HEPES pH 7.4; 10 mM KCl; 1.5 mM MgCl2; 1 mM EDTA; 1 mM DTT) containing PMSF (100 μg/ml). Then the cellular fractions were analyzed by western blot.

2.9. Co-immunoprecipitation (Co-IP)

For co-immunoprecipitation, the indicated antibodies were added into cell lysates for 2 h at 4 °C. The immunocomplexes were captured by the addition of protein A-sepharose beads (50% slurry) (Roche Applied Sciences, Indianapolis, IN) mixed overnight at 4 °C. The beads were resuspended with SDS sample buffer, boiled for 5 min to remove sepharose beads and analyzed by western blot [23].

2.10. Real-time quantitative PCR

Total RNA was extracted from cells using the RNAiso Plus (Tokyo, Japan). PrimeScript II 1st strand cDNA synthesis kit (TaKaRa Bio, Tokyo, Japan) was used to synthesize cDNA. The following primers were used: Dok1, GA-GGCTTCTGAACGCTGCGGCTTGC and AGAACATTACCTGACACACCAGCTTA; PPARγ, GCTGTGCAGGAGATCACAGA and GGGCTCCATAAAGTACCAA; ap2, TACCAGAAAGCTTGTAGATGAAT and ACGATTCCACCACGGTTTTATCA; FAT, ATGGGCTGTGATCGGAACTG and AGCCAGGACTGCACCAATAAC; adiponectin, TCTGTGGCAATGGCAAAAAG and CGATCCACATCCGGTAGGAT; and perilipin, CTCTGGGAAGCATCGAGAAG and GCATGGTGTGTCGAGAAAGA. The cDNA was loaded into capillary tubes with SBRYII and then incubated in the LightCycler for an initial denaturation at 95 °C for 10 s, followed by 40 cycles, each cycle consisting of 95 °C for 5 s, 60 °C for 10 s, and 72 °C for 10 s. The expression of GAPDH was detected to normalize gene expression.

2.11. RNAi-mediated gene silencing

The following specific siRNA was used: Dok1, 5′-GGUCAUGUUCUUUCGAGTT-3′; ERK, 5′-GCAUGACCAUAUCGCUATT-3′. siRNAs were transfected into 3T3-L1 adipocytes with Lipofectamine™ 2000 reagent at the induced day 4. After 72 h, cells were lysed and analyzed by western blot.

2.12. Statistics analysis

All assays were performed at least three times. All the error bars represented as the mean ± SEM (n ≥ 3). For statistical evaluation Student’s paired t-test was used and significance was defined as P < 0.05.

3. Results

3.1. Excessive lipolysis in Dex-induced insulin-resistant adipocytes

To establish insulin-resistant cell model, 3T3-L1 preadipocytes were differentiated and then stained by Oil red O. As shown in Fig. 1A, large fat content was accumulated in differentiated cells, which were considered as mature adipocytes [26]. Subsequently, adipocytes were exposed to 20 nM Dex for 8 days to set insulin-resistant cell model as reported previously [27] and the characteristics of the insulin-resistant state were detected. The results consistent with the previous study that the insulin-stimulated glucose uptake and the insulin-stimulated
phospho-Akt level were significantly reduced after chronic Dex incubation (data not showed), confirming that the 3T3-L1 adipocytes treated with chronic Dex were under the insulin-resistant state.

We next examined the effects of Dex on lipid metabolism. Firstly, the intracellular triglyceride level and the neutral lipid drops were assessed. As shown in Fig. 1B and C, the intracellular triglyceride level was much lower after Dex treatment, suggesting that the fat storage capacity of adipocytes was impaired under Dex treatment. The following experiments showed that the release of FFAs (Fig. 1D) and glycerol (Fig. 1E) was significantly increased in the Dex treatment group. Taken together, these findings demonstrate that the chronic Dex exposure leads to insulin resistance, accompanied with excessive lipolysis in 3T3-L1 adipocytes.

3.2. LPLI suppresses excessive lipolysis dependent on inhibition of ERK

In order to determine the effects of LPLI treatment on lipid metabolism disorders in insulin-resistant 3T3-L1 adipocytes, we co-treated the adipocytes with 20 nM Dex and LPLI at different doses ranging from 2.5 to 20 J/cm². 7 days later, the release of FFAs and glycerol was measured.

The results showed that LPLI partially restored the FFA and glycerol release in insulin-resistant 3T3-L1 adipocytes, and a significant decrease was observed at the dose of 10 J/cm² (Fig. 2A and B).

ERK is an important component in obesity-associated metabolic disorders [32,33]. Based on the increased phosphorylation of ERK in adipose tissues of patients with type 2 diabetes, we next investigated whether activated ERK is involved in Dex-induced insulin-resistant. We found that the level of phospho-ERK was remarkably increased with Dex treatment (Fig. 2C). However, LPLI treatment significantly reduced the phospho-ERK level in insulin-resistant 3T3-L1 adipocytes, and LPLI at 10 J/cm² was the most effective dose according to the results (Fig. 2D), suggesting that down-regulation of ERK might be responsible for the anti-lipolytic effects of LPLI.

10 J/cm² LPLI did not affect the proliferation of insulin-resistant 3T3-L1 adipocytes (Fig. 2E). Therefore, 10 J/cm² was selected as the optimum irradiation dose in our following studies. To further confirm the role of ERK in the inhibitory effect of LPLI on FFAs eruption, the ERK specific siRNA was used to knockdown ERK in insulin-resistant adipocytes (Fig. 2E). We found that the release of FFAs (Fig. 2F) and glycerol...
(Fig. 2G) was decreased both in the LPLI treatment group and the ERK knockdown group. Additionally, the FFA release was positively correlated with the activity of ERK using Pearson product moment correlation test ($r = 0.864, P < 0.01$) (Fig. 2H). Those results suggest that LPLI suppresses lipolysis in insulin-resistant adipocytes partially through inhibition of ERK.

### 3.3. Dok1 is essential for LPLI-induced ERK inhibition

Dok1 has been reported to act as a tumor suppressor through inhibiting MEK/ERK [15,16]. The following experiments were conducted to evaluate whether the negative regulation of MEK/ERK by Dok1 could suppress lipolysis in the insulin-resistant adipocytes. Firstly, we found
that Dok1 protein level (Fig. 3A) and mRNA level (Fig. 3B) were folds increased in differentiated 3T3-L1 adipocytes. Membrane recruitment of Dok1 is essential for its negative effect on MEK/ERK signaling [31]. To determine whether LPLI can activate Dok1, 3T3-L1 adipocytes were transfected with GFP-Dok1 or GFP vector plasmid and treated with LPLI. As shown in Fig. 3C, GFP-Dok1 was predominantly localized in the cytosol. When cells were treated with LPLI or insulin, GFP-Dok1 was translocated to plasma membrane, while there was no translocation of GFP under indicated treatment. To further investigate the redistribution of Dok1 under LPLI treatment, fractions of membrane and cytoplasm of insulin-resistant adipocytes were isolated. The western blot result showed that the protein level of Dok1 in the membrane fraction was increased under LPLI treatment, while the level in the cytoplasm fraction was decreased (Fig. 3D).

Phosphorylation of Dok1 at tyrosine 362 is essential for down-regulating MEK/ERK signaling. To confirm the effect of LPLI on Dok1 activity, the phosphorylation of Dok1 was detected. As shown in Fig. 3E, LPLI increased the phospho-Dok1 level, companied with decreased the phospho-ERK level. Similar results were obtained in over-expressed GFP-Dok1 293 T cells (Fig. 3F). To clarify the effect of Dok1 on the ERK phosphorylation under LPLI treatment, Dok1 specific siRNA was delivered into insulin-resistant 3T3-L1 adipocytes. We found that LPLI suppressed the ERK phosphorylation in insulin-resistant adipocytes, while this effect was almost reversed by knocking down Dok1 (Fig. 3G). The release of FFAs (Fig. 3H) and glycerol (Fig. 3I) was also markedly increased after Dok1 knocked down. These results indicate that Dok1 is essential for LPLI to inhibit ERK and prevent lipolysis in insulin-resistant adipocytes.

3.4. LPLI up-regulates transcriptional activity of PPARγ through Dok1/ERK

MEK/ERK has been recognized as a central negative regulator of PPARγ. MEK/ERK phosphorylates PPARγ on Ser84/112, which leads to translocation of PPARγ out of nucleus [12,34] and then phospho-PPARγ is degraded via ubiquitin-proteasome pathway in cytoplasm [35]. To determine whether LPLI could increase the PPARγ protein level in insulin-resistant adipocytes, we measured the phosphorylation

![Fig. 3. Dok1 is essential for LPLI inhibiting of ERK. (A) Time course of Dok1 protein expression in 3T3-L1 cells upon differentiation. (B) Gene expression of Dok1 was detected by real time quantitative PCR. (C) 3T3-L1 adipocytes were transfected with GFP-Dok1 plasmid. The cells were treated with LPLI (10 J/cm²) or insulin (20 nM) and the translocation of GFP-Dok1 was observed by a LSM510 META confocal microscope. (D) 3T3-L1 adipocytes treated with or without LPLI were isolated as fractions of membrane and cytoplasm. The Dok1 redistribution was measured by western blot. (E) 3T3-L1 adipocytes were transfected with GFP-Dok1 plasmid or GFP vector. After 48 h, the cells were treated with LPLI and the phosphorylation of ERK was measured by western blot. (F) 293 T cells were transfected with GFP-Dok1 plasmid and GFP vector. After 48 h, the cells were treated with LPLI and the phosphorylation of ERK was measured by western blot. (G) 3T3-L1 adipocytes were transfected with Dok1 and negative control siRNA. After 72 h, the cells were lysed and the levels of indicated proteins were assessed by western blot. (H) FFA release and (I) glycerol were measured using indicated kits. Phosphorylation protein levels are quantified to total proteins. Data are normalized to untreated group and expressed as mean ± SEM, n ≥ 3. **P < 0.01, ***P < 0.001 vs. indicated group, NS, not significant.](image-url)
of PPARγ and found that LPLI treatment dramatically decreased PPARγ phosphorylation (Fig. 4A). Similar results were obtained by treating with PD98059, an inhibitor of MEK/ERK. To clarify the interaction between PPARγ and phospho-ERK, reciprocal co-immunoprecipitation was performed. We found that the amount of phospho-ERK binding to PPARγ significantly decreased in response to LPLI treatment, which was consistent with the PD98059 treatment (Fig. 4B). The nuclear localization of PPARγ was markedly increased under LPLI or PD98059 treatment detected by cell fractionation experiment (Fig. 4C). The following QPCR results showed that the expression of PPARγ target adipogenic genes was markedly increased under LPLI or PD98059, but neither LPLI nor PD98059 treatment increased PPARγ gene expression (Fig. 4D).
summary, LPLI dramatically inhibits ERK activity, resulting in the decrease of PPARγ phosphorylation. Non-phosphorylated PPARγ maintained in nucleus to promote its downstream adipogenic gene expression.

For validation of the essential role of Dok1 on anti-lipolytic effects of LPLI, insulin-resistant adipocytes were transfected with the Dok1 specific siRNA and then treated with LPLI. As the result shown, LPLI increased PPARγ level in insulin-resistant adipocytes, whereas the protein level

![Image of graphs and figures](image-url)

**Fig. 4.** LPLI up-regulates transcriptional activity of PPARγ through Dok1/ERK pathway. (A) The levels of phospho-PPARγ and PPARγ were detected by western blot. (B) Reciprocal co-immunoprecipitations with antibodies against PPARγ or ERK was used to pull-down the immunocomplex containing PPARγ and phospho-ERK. The levels of PPARγ and phospho-ERK in immunocomplex and whole cell lysate (WCL) were detected by western blot. (C) The phospho-ERK and PPARγ were detected in cytoplasmic and nuclear fractions respectively. (D) Gene expression of PPARγ, ap2, adiponectin, FAT, adipsin and perilipin were detected by real time quantitative PCR. β-Actin and histone were used as loading controls and markers for the cytosolic and nuclear fractions, respectively. (E) 3T3-L1 adipocytes were transfected with Dok1 and negative control siRNA. After 72 h, the cells were lysed and the levels of indicated proteins were assessed by western blot. (F) 3T3-L1 adipocytes were treated with or without LPLI and PD98059 and then were lysed. The levels of indicated proteins were assessed by western blot. (G) Intracellular triglyceride level was measured by indicated kit. (H) FFA release was measured using indicated kit. Protein levels are quantified to β-actin, phosphorylation protein levels are quantified to total proteins. Data are normalized to untreated group and expressed as mean ± SEM, n ≥ 3. *P < 0.05, **P < 0.01, ***P < 0.001 vs. indicated group. NS, not significant.
of PPARγ was dramatically reduced by Dok1 knockdown in insulin-resistant adipocytes with or without LPLI (Fig. 4E). Next, the phosphorylation of Dok1 in insulin-resistant 3T3-L1 adipocytes was detected and we found that LPLI or PD98059 treatment increased the phosphorylation of Dok1. Correspond with this result, the level of phospho-ERK was dramatically decreased and the level of PPARγ was increased under LPLI treatment (Fig. 4F). All of those demonstrated that LPLI rescued PPARγ from ERK depends on Dok1. We further measured the intracellular triglyceride level (Fig. 4G) and FFA release (Fig. 4H) under indicated treatments. The results indicated that the fat storage capacity was increased after inhibiting ERK by LPLI or PD98059, and the antilipolytic effects of LPLI disappeared after knockdown Dok1 (Fig. 4G and H). Taken together, LPLI inhibits excessive lipolysis in insulin-resistant adipocytes by activating Dok1/ERK/PPARγ signaling axis.

4. Discussion

Insulin resistance is defined as the impairment of metabolic action of insulin in peripheral tissues, resulting in suppression of glucose transport, glycogen synthesis and anti-lipolysis [32]. Insulin resistance develops within hours after an acute increase in plasma FFA levels in mammals [36,37]. However, treatments of insulin resistance triggered by excessive FFAs are hampered by the lack of suitable methods to measure and lower the plasma FFA level [36]. In the present study, the effects of LPLI on FFA release and the relevant molecular mechanisms were investigated in insulin-resistant 3T3-L1 adipocytes. Firstly, we observed that LPLI prevented excessive lipolysis in insulin-resistant adipocytes through inhibition of ERK activity (Fig. 2). Furthermore, we verified that Dok1 is essential for LPLI inhibiting ERK (Fig. 3). Finally, we demonstrated that the effect of LPLI on Dok1/ERK pathway lessens phosphorylation of PPARγ and restores PPARγ genomic function (Fig. 4).

Peplow, P. V. et al. [38] have shown that irradiation of left inguinal region in diabetic mice with 810 nm laser has potential to ameliorate diabetes as shown by decreased blood plasma fructosamine. Kenji Ryotokuji et al. [39] have shown that pinpoint plantar long-wavelength infrared light irradiation (PP-LILI) can normalize blood glucose levels by reducing stress hormones and by improving insulin sensitivity. Our recent study confirmed that He–Ne laser can promote glucose uptake of 3T3-L1 adipocytes through activating PI3-K/Akt/GLUT4 pathway [24]. Nevertheless, whether LPLI could ameliorate lipid metabolic disorders remains unclear. In the present study, we showed for the first time that LPLI markedly suppressed excessive FFA release from insulin-resistant 3T3-L1 adipocytes.

The decrease of fat storage capacity in diabetes individuals is partly due to the degradation of PPARγ, while phosphorylated PPARγ on serine 84/112 by ERK cascades accelerates its degradation [34]. KR-62776, one of PPARγ partial agonist, can inhibit adipocyte differentiation via activating ERK [40]. Silencing of MAP4K by specific siRNA results in folds increased of PPARγ protein level in 3T3-L1 adipocytes [41]. Those
studies give the insights that the adipolytic function of ERK is closely related to its negative regulation of PPARγ. Rescue of PPARγ by inhibiting ERK may be a very effective means to improve adipogenesis and remodel fat storage capacity of adipocytes under insulin resistance. Our present study verified that LPLI can suppress excessive FFA efflux from insulin-resistant 3T3-L1 adipocytes by inhibiting ERK and then reducing the interaction between ERK and PPARγ.

The results seem to be paradoxical with our previous research, which reported that LPLI promotes cell proliferation by activating ERK [23]. The inconformity results may due to Dok1, the natural negative regulator for MEK/ERK, is highly expressed in adipocytes. Dok1 can trigger Ras GTPase-activating protein (Ras-GAP) interaction with the vicinity of Ras and result in the attenuation of Ras activity, followed by the decrease of MEK/ERK activity [17]. In this study, we showed that LPLI can activate Dok1 in insulin-resistant 3T3-L1 adipocytes, accompanied with down-regulation of ERK activity. Knockdown of Dok1 inhibited the effects of LPLI on ERK, suggesting that activation of Dok1 is essential for LPLI restraining ERK cascades. Understanding the mechanism of LPLI preventing lipolysis may stand out Dok1/ERK/PPARγ signaling axis as a novel target in adipose tissue. Our research extends the knowledge into the biological mechanisms induced by LPLI. We do not eliminate the possibility that other signaling such as P38/Akt which may also act on improving lipid metabolism disorders under LPLI in the present study. Further studies are in progress to illustrate the effects of LPLI on ameliorating metabolic syndrome in insulin-resistant state.

5. Conclusions

In conclusion, the current investigation shows that LPLI may act as a novel approach on prevention of excessive lipolysis in insulin-resistant adipocytes. The anti-lipolytic effect of LPLI is based on inhibition of ERK through Dok1, accompanied with up-regulation of genomic function of PPARγ (Fig. 5). Our research extends the knowledge into the biological mechanisms induced by LPLI. More importantly, our results suggest that Dok1/ERK/PPARγ signaling axis may be a potential target to ameliorate lipid metabolism disorders in insulin-resistant state.

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