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Fibrinogen-like protein 2 aggravates myocardial ischemia/reperfusion injury in mice following sevoflurane anesthetic through ROS production by PPAR

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Abstract

This study aims to investigate the mechanism and effects of Fibrinogen-like protein 2 (FGL2) in myocardial ischemia/reperfusion injury in mice following sevoflurane anesthetic. Mice of SAP group were placed in box with oxygen and anesthetic gas (60 mg/kg, pentobarbital), 2.5% Sevoflurane was pumped into the box for 1 h. H9C2 cells were treated by 3% sevoflurane for 6 h and a mixture of 95% O2 + 5% CO2 for 24 h. Fgl2 mRNA expression was up-regulated in mice of I/R injury following sevoflurane. Fgl2 protein reduced HR, LVDP, dp/dtmax (+) and dp/dtmax (-), increased LVEDP levels, myocardial infarct size and AI in mice of I/R injury following sevoflurane. Fgl2 suppressed PPAR signaling pathway, and promoted ROS production in vivo or vitro model. The activation of PPAR signaling pathway reduced the function of Fgl2 in vivo and vitro model. Fgl2 might serve as a therapeutic target in the treatment of I/R injury following sevoflurane. We hope that our findings will pave a way for future therapies against I/R injury following sevoflurane.

Keywords: FGL2; myocardial ischemia/reperfusion injury; sevoflurane; ROS production; PPAR.

Practical Application: This study aims to investigate the mechanism and effects of Fibrinogen-like protein 2 (FGL2) in myocardial ischemia/reperfusion injury in mice following sevoflurane anesthetic.

1 Introduction

Multiple transient myocardial ischemia/reperfusion can significantly attenuate the myocardial damage in the subsequent prolonged ischemia, also known as ischemic preconditioning (IPC), which is the most potent endogenous protective mechanism discovered so far (Huang et al., 2019a). Moreover, anaesthetics pretreatment has also been found to exert similar myocardial protective effects, however, most studies focus on normal myocardium (Pasqualin et al., 2016; Qi et al., 2019). As a new type of inhalation anesthetics, sevoflurane is widely used in pediatric anesthesia due to its rapid induction and recovery, stable and safe effect (Lavi et al., 2014). Animal experiments show that pretreatment and post-treatment of sevoflurane exert protective effects on the myocardium (Zhang et al., 2018a). In addition, sevoflurane pretreatment has also been investigated in adult cardiac surgery (Dong et al., 2019).

Accumulative experimental results have suggested that ROS is closely associated with cellular events, such as protein oxidation and folding (Guo et al., 2018). Excessive cellular ROS production or changes in the state of redox reactions can directly or indirectly affect the homeostasis of the endoplasmic reticulum and protein folding, thereby inducing endoplasmic reticulum stress (Jun et al., 2019; Li et al., 2018).

PPARγ a ligand-activated transcription factor, belongs to the nuclear hormone receptor superfamily (Qi et al., 2020). It is highly expressed in tissues with vigorous metabolism of fatty acid, including liver, heart and kidney, and also exists in the microvessels of various organs, neurons and glia, including the retina (Rehman et al., 2020). PPARγ plays an important role in regulating glucose and lipid metabolism (Yuan et al., 2019). Recent studies have found that in addition to metabolism regulation, PPARγ and its ligands exert important regulatory effects on oxidative stress, which have therapeutic effects in various disease models (Gao et al., 2020; Li et al., 2020b).

Fibrinogen-like protein 2 (FGL2) also known as fibrin, was first discovered in mice infected with type 3 murine hepatitis virus, with expression in other organs of the human body (Fan et al., 2019). Fgl2 gene silencing has been confirmed to promote the proliferation and migration of cardiac microvascular endothelial cells, suggesting that Fgl2 is involved in the regulation of angiogenesis, which might be associated with the up-regulated expression of Angiopoietin 1 and 2 (Li T et al., 2020; Li et al., 2019).This study aims to investigate the mechanism and effects of FGL2 in myocardial ischemia/reperfusion injury in mice following sevoflurane anesthetic.

2 Materials and methods

2.1 Animal experiment

Male BALB/c mice were kept under special pathogens free (SPF) condition at 22-23 °C with 65-70% relative humidity and 12 h light and dark cycles. All mice (n = 30) were grouped into sham (n = 10), ischemia/reperfusion (I/R) injury (n = 10),

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I/R+SAP group (n = 10). Mice of SAP group were placed in a 30 cm × 30 cm × 30 cm square box with oxygen and anesthetic gas (60 mg/kg, pentobarbital), 2.5% Sevoflurane was pumped into the box for 1 h as literature. All mice of were injected with 1% pentobarbital sodium solution (Sigma-Aldrich USA). Mice were fixed in the supine position, the skin of the neck and thorax were disinfected, and skin of the anterior cervical area was cut using an ophthalmic scissor. The trachea was exposed, and the left coronary artery was ligated as literature (Zhao et al., 2019).

Next, All mice (n = 30) were grouped into control (n = 10), Anti-FGL2 (n = 10), Anti-FGL2+PPAR I group (n = 10). All mice were I/R+SAP group, mice of Anti-FGL2 were mice of I/R+SAP model were treated with anti-FGL2 body (1 μg of mice, i.p.), mice of Anti-FGL2+PPAR I group were mice of I/R+SAP model were treated with anti-FGL2 body (1 μg of mice, i.p.) and PPARγ antagonist (GW9662, 1 mg/kg, i.p.).

The heart rate (HR), left ventricular end-diastolic pressure (LVEDP), left ventricular developed pressure (LVDP), maximum rate of rise of left ventricular pressure [dp/dtmax (+)], and maximum rate of decline of the left ventricular pressure [dp/ dtmax (-)] were measured using the arched ST segment as literature (Zhao et al., 2019).

2.2 Histological and immunohistochemistry analysis

After 24 hours of I/R, hearts tissues were cut from the root of the aorta and quickly washed with phosphate buffer saline (PBS). Then, tissue were fixed by paraformaldehyde for 24 h, slicing into pieces at a thickness of 5 μm by using a paraffin microtome.

2.3 Western blot analysis

Proteins were extracted from heart tissue samples or cell samples using RIPA lysis buffer. Proteins were resolved on polyacrylamide gels and transferred onto PVDF membranes.

Membranes were blocked with 5% milk for 1 h and incubated with primary antibodies.

Membranes were washed with TBST for 15 min and incubated with secondary antibodies. Protein blanks were tested by an enhanced chemiluminescence system and densitometry was performed using ImageLab software.

2.4 Quantitative real-time PCR

Total RNA was extracted from liver tissues using TRIzol reagent. total RNA was reservetranscribed into cDNA with a ReverTra Ace qPCR RT Kit. Gene expression was detected using using SYBR Green Real-time PCR Master Mix by a real-time PCR system. Gene expressions were calculated based on the 2-ΔΔCt method.

2.5 Cytokine enzyme-linked immunosorbent assay (ELISA)

Serum samples were collected and centrifuged and measured ROS production, MDA, SOD, GSH and GSH-px levels. ELISA kits were purchased from Shanghai Jingkang Biological Engineering Co., Ltd., (Shanghai, China).

2.6 Cell culture, treatment and lentivirus transduction

H9C2 cells were cultured with DMEM containing 10% FBS at 37 °C in 5% CO2. H9C2 cells were transfected with Fgl2, siFgl2, PPAR, siPPAR, negative mimics using Lipofectamine 2000 (Invitrogen, USA). After 48 h, cells were treated by 3% sevoflurane for 6 h and a mixture of 95% O2 + 5% CO2 for 24 h as literature (Zhao et al., 2019; Kang & Wang, 2019)

2.7 Statistical analysis

Data are expressed as the means ± standard deviation (SD). Values of $p < 0.05$ was considered to be statistically significant. Comparisons among multiple groups were assessed by t-test or one-way analysis of variance (ANOVA).

3 Results

3.1 Fgl2 aggravates myocardial I/R injury following sevoflurane

To address the relevance of Fgl2 expression and I/R injury following sevoflurane, this study analyzed the expression of Fgl2 in mice of I/R injury following sevoflurane. Fgl2 mRNA expression was up-regulated in mice of I/R injury following sevoflurane (Figure 1A). Then, we used Fgl2 protein to investigate the role of fgl2 in I/R injury following sevoflurane. We found that Fgl2 protein reduced HR, LVDP, dp/dtmax (+) and dp/dtmax (-), increased LVEDP levels, myocardial infarct size and AI in mice of I/R injury following sevoflurane, compared with mice of I/R injury following sevoflurane (Figure 1B-1I).

3.2 Fgl2 suppressed PPAR signaling pathway in vivo model

The investigate that mechanism of FGL2 in myocardial ischemia/reperfusion injury of mice following sevoflurane, we identified specific genes in ischemia/reperfusion injury with over-expression of FGL2 that are relevant to the pathway analysis (Figure 2A-2B). We found that Fgl2 suppressed PPAR signaling pathway in vivo and vitro model of myocardial ischemia/reperfusion injury following sevoflurane (Figure 2C). Over-expression of FGL2 induced Fgl2 protein expression, and suppressed PPARγ protein expression in vitro model (Figure 2D-2F). Down-regulation of FGL2 suppressed Fgl2 protein expression, and induced PPARγ protein expression in vitro model (Figure 2G-2I). Next, Fgl2 protein also suppressed PPARγ protein expression in myocardial ischemia/reperfusion injury of mice following sevoflurane, (Figure 2J-2K). IF showed that over-expression of FGL2 reduced PPARγ protein expression in vitro model (Figure 2L).

3.3 Fgl2 promoted ROS production in vitro model

To evaluate the function of Fgl2 in mice of I/R injury following sevoflurane, we examined the effects of Fgl2 on ROS production in vitro model. Over-expression of Fgl2 increased ROS production levels and MDA levels, and reduced SOD, GSH and GSH-px levels in vitro model (Figure 3A-3F). Down-regulation of Fgl2 reduced ROS production levels and MDA levels, and increased SOD, GSH and GSH-px levels in vitro model (Figure 3G-3L).

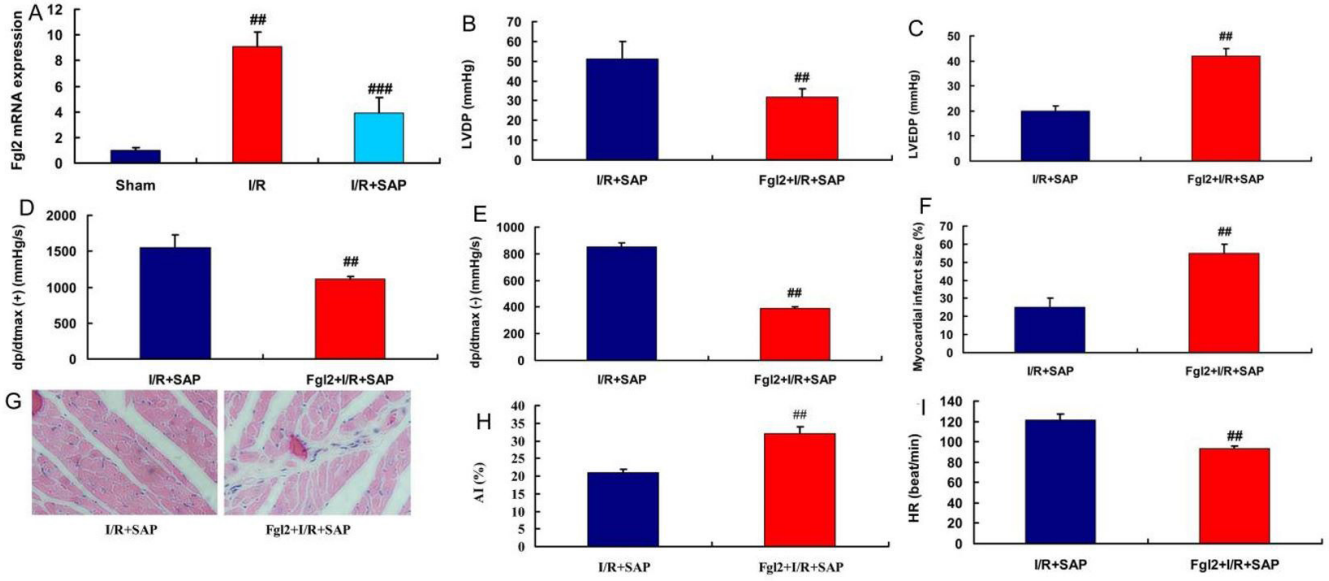

Figure 1. Fgl2 aggravates myocardial I/R injury following sevoflurane. Fgl2 mRNA expression in mice of I/R injury following sevoflurane (A); LVDP (B), LVEDP (C), dp/dtmax (+) (D), dp/dtmax (-) (E), myocardial infarct size (F), myocardial tissues of mice by HE staining (G), AI (H) and HR (I) in mice of I/R injury following sevoflurane by Fgl2 protein. Sham, sham control mice group; I/R, I/R injury mice group; I/R+SAP, I/R injury mice with sevoflurane group; Fgl2+I/R+SAP, I/R injury mice with sevoflurane and Fgl2 protein group. ##p<0.01 compared with sham control mice group or I/R injury mice with sevoflurane group; ###p<0.01 compared with I/R injury mice group.

3.4 The activation of PPAR signaling pathway reduced the function of Fgl2 in vivo and vitro model

The study rther investigate the role of PPAR signaling pathway the function of Fgl2 in vivo and vitro model. Anti-body of Fgl2 induced PPARγ protein expression, elevated HR, LVDP, dp/dtmax (+) and dp/dtmax (-), reduced LVEDP levels, myocardial infarct size and AI, inhibited MDA levels, and enhanced SOD, GSH and GSH-px levels in mice of I/R injury following sevoflurane (Figure 4). Then, PPARγ antagonist (GW9662, 1 mg/kg, i.p.) suppressed PPARγ protein expression, repressed HR, LVDP, dp/dtmax (+) and dp/dtmax (-),induced LVEDP levels, myocardial infarct size and AI, promoted MDA levels, and restrained SOD, GSH and GSH-px levels in mice by anti-body of Fgl2 of I/R injury following sevoflurane, compared with anti-body of Fgl2 group (Figure 4).

Next, in vitro model, PPARγ plasmid induced PPARγ protein expression, reduced ROS production levels and MDA levels, increased SOD, GSH and GSH-px levels in vitro model by over-expression of Fgl2 group (Figure 5). SiPPARγ suppressed PPARγ protein expression, promoted ROS production levels and MDA levels, decreased SOD, GSH and GSH-px levels in vitro model by over-expression of Fgl2 group (Figure 6).

4 Discussion

Sevoflurane is a commonly used inhalation anesthetics in clinical practice at present (2). It has the advantages of rapid induction, rapid recovery, and mild circulation inhibition (Qiao et al., 2019). A large number of studies have shown that sevoflurane pretreatment can give rise to similar myocardial protection with ischemic preconditioning (Pasqualin et al., 2016; Zhao et al., 2019; Zhang et al., 2018b) Our findings provided evidence strongly suggested that Fgl2 mRNA expression was up-regulated in mice of I/R injury following sevoflurane. Fgl2 protein reduced HR, LVDP, dp/dtmax (+) and dp/dtmax (-), increased LVEDP levels, myocardial infarct size and AI in mice of I/R injury following sevoflurane. Zheng et al. showed that FGL2 knockdown improves heart function in the experimental autoimmune myocarditis rats (Zheng et al., 2018), which may be an effectively protective target for I/R injury following sevoflurane.

Studies have found that myocardial ischemia/reperfusion generally induces oxidative stress in the body to cause inflammatory response, which in turn aggravates cerebral ischemia and hypoxia (Zhao et al., 2018c). Therefore, how to effectively inhibit the oxidative stress response and inflammatory factor secretion in the course of myocardial ischemia/reperfusion is of great clinical significance for the clinical treatment of myocardial damage (Yu et al., 2015). Effectively, it was found that Fgl2 promoted ROS production in vitro or vivo model. Shafik et al. curcumin ameliorative effects against acute pancreatitis via fgl-2 expression (Shafik & Abou-Fard, 2016). Our data suggested that Fgl2 promoted ROS-induced oxidative stress, which facilitated the progression of I/R injury following sevoflurane.

PPAR is a member of the nuclear receptor superfamily and is a ligand-dependent transcription factor (Zhao YB et al., 2019). PPAR can regulate the expression of specific target genes containing PPAR response elements in various promoter regions at the transcription level, and modulate various biological effects, including fatty acid and glucose metabolism, and oxidative stress

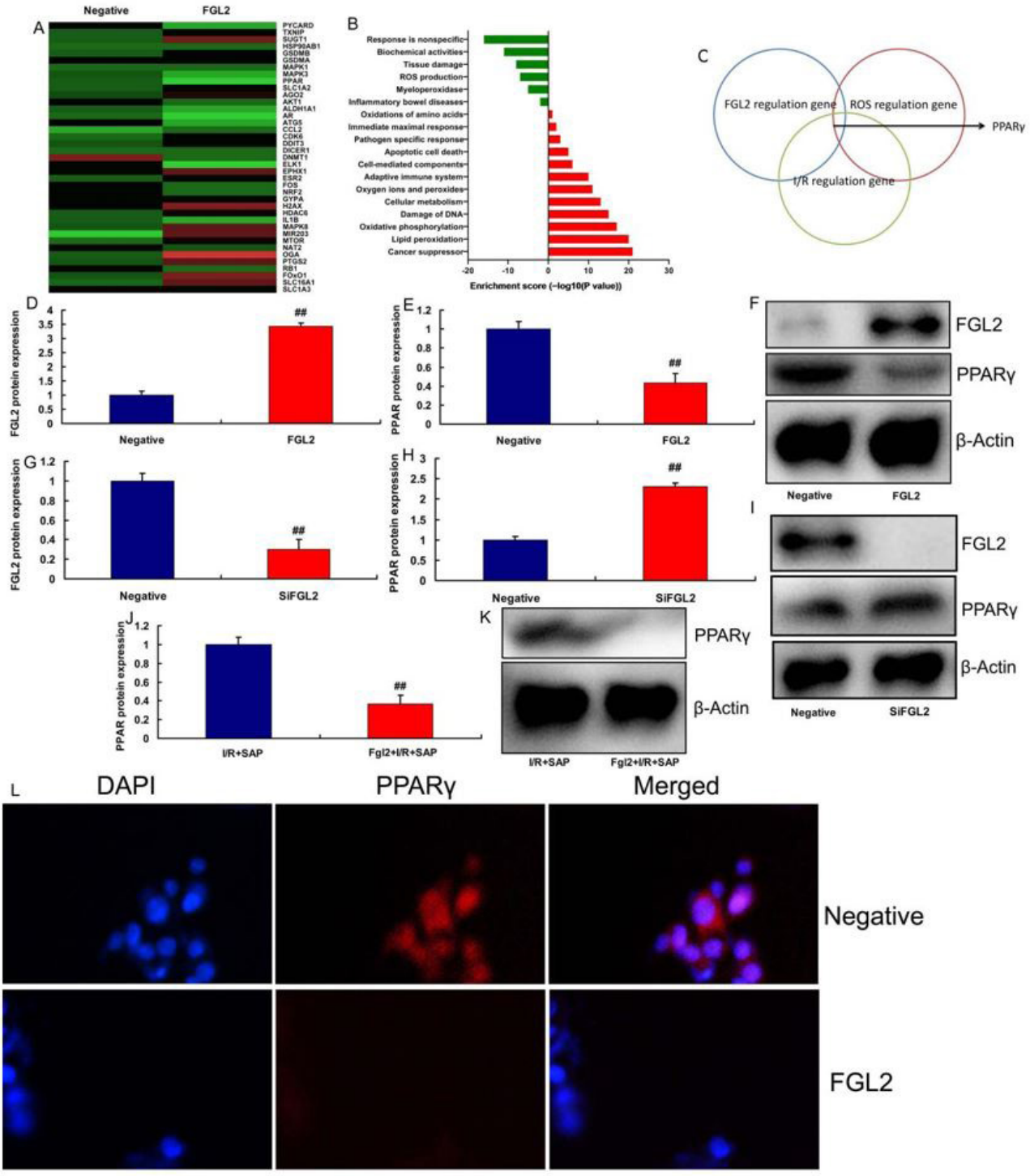

Figure 2. Fgl2 suppressed PPAR signaling pathway in vivo model. Heat map, results figure and refine results of gene chip (A, B and C); Fgl2 and PPARγ protein expression in vitro model by over-expression of Fgl2 (D, E and F); Fgl2 and PPARγ protein expression in vitro model by down-regulation of Fgl2 (G, H and I); PPARγ protein expression in mice of I/R injury following sevoflurane by Fgl2 protein (J and K); PPARγ protein expression in vitro model by over-expression of Fgl2 (IF, L). Negative, negative mimics group; Fgl2, over-expression of Fgl2 group; SiFgl2, down-regulation of Fgl2 group; I/R injury mice with sevoflurane group; Fgl2+I/R+SAP, I/R injury mice with sevoflurane and Fgl2 protein group. ##p<0.01 compared with negative mimics group or I/R injury mice with sevoflurane group.

inhibition (Xu et al., 2005; Zhu et al., 2018). In the later part of the study, we found that Fgl2 suppressed PPAR signaling pathway in vivo and vitro model; The activation of PPAR signaling pathway reduced the function of Fgl2 in vivo and vitro model. Hu et al. (2020) suggest that Fgl2 aggravates nonalcoholic steatohepatitis via interaction with PPAR (Hu et al., 2020; Momchilova et al.,

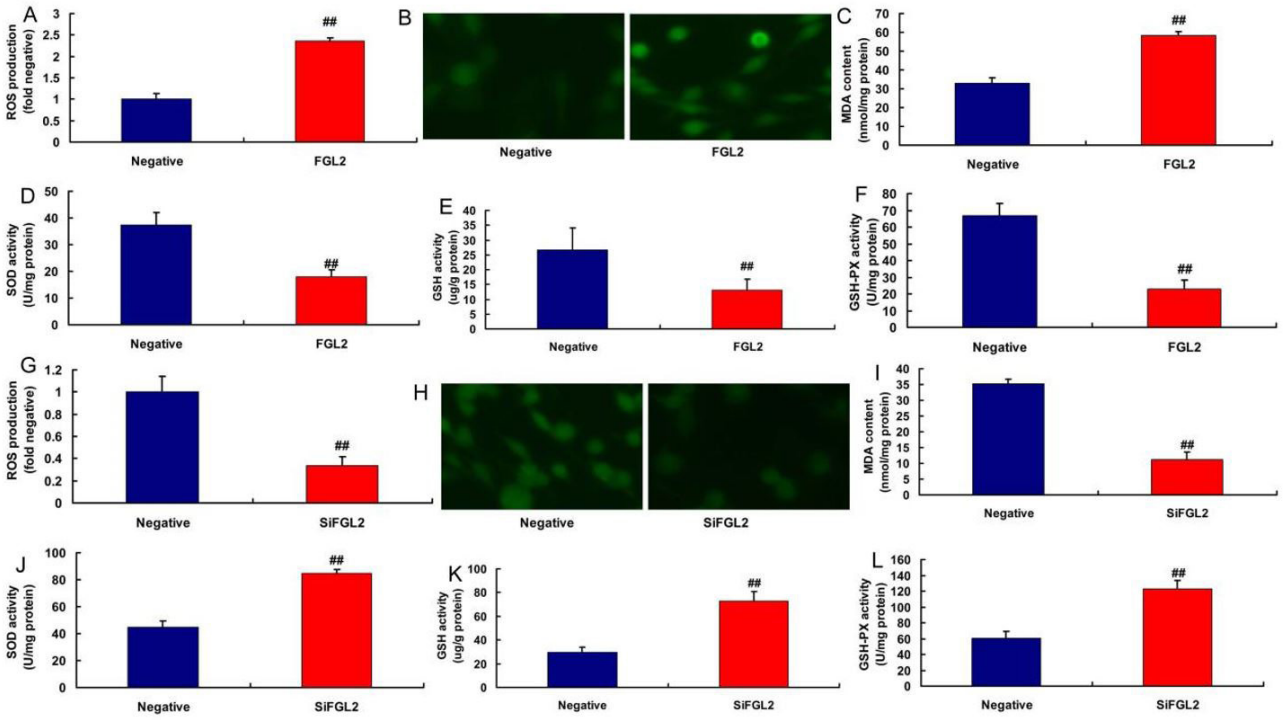

Figure 3. Fgl2 promoted ROS production in vitro model. ROS production (A and B), MDA (C), SOD (D), GSH (E) and GSH-px (F) levels in vitro model by over-expression of Fgl2; ROS production (G and H), MDA (I), SOD (J), GSH (K) and GSH-px (L) levels in vitro model by down-regulation of Fgl2. Negative, negative mimics group; Fgl2, over-expression of Fgl2 group; SiFgl2, down-regulation of Fgl2 group. ##p<0.01 compared with negative mimics group.

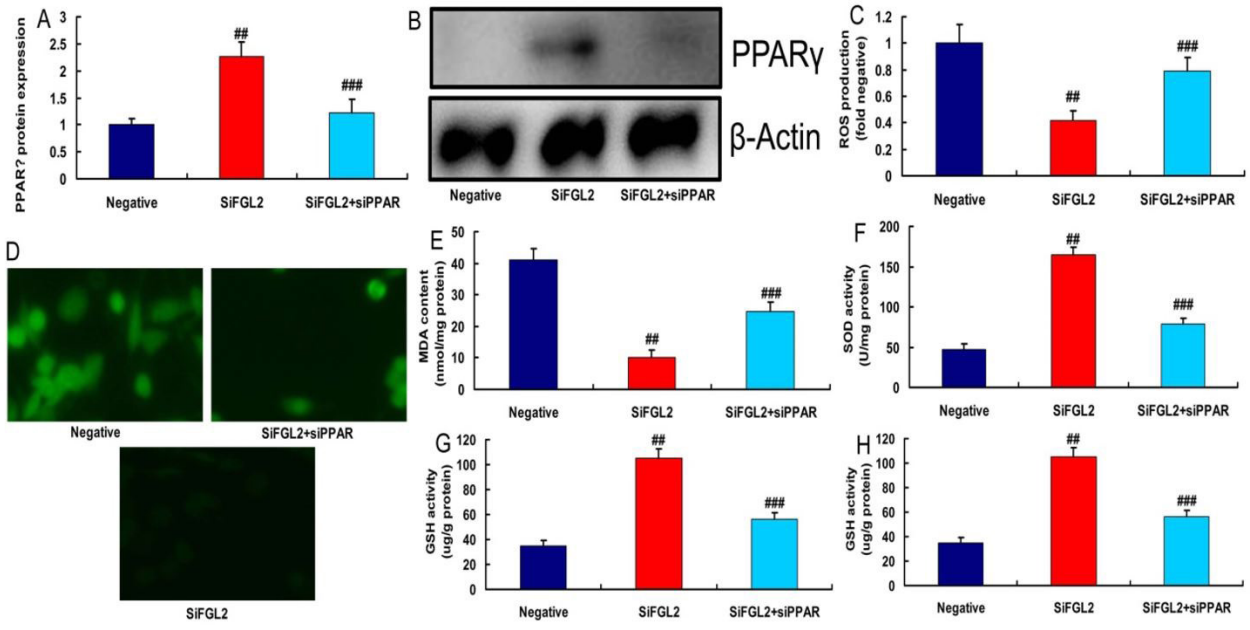

Figure 4. The activation of PPAR signaling pathway reduced the function of Fgl2 in vivo and vitro model. PPARγ protein expression (A and B), HR (C), LVDP (D), LVEDP (E), dp/dtmax (+) (F), dp/dtmax (-) (G), myocardial infarct size (H), myocardial tissues of mice by HE staining (G), AI (I) and HR (J), MDA (K), SOD (L), GSH (M) and GSH-px levels (N) in mice of I/R injury following sevoflurane. Control, I/R injury mice with sevoflurane group; Anti-Fgl2, I/R injury mice with sevoflurane by anti-Fgl2 group; Anti-Fgl2+PPAR i, I/R injury mice with sevoflurane by anti-Fgl2 and GW9662 group. **##p<0**.01 compared with I/R injury mice with sevoflurane group; ###p<0.01 compared with I/R injury mice with sevoflurane by anti-Fgl2 group.

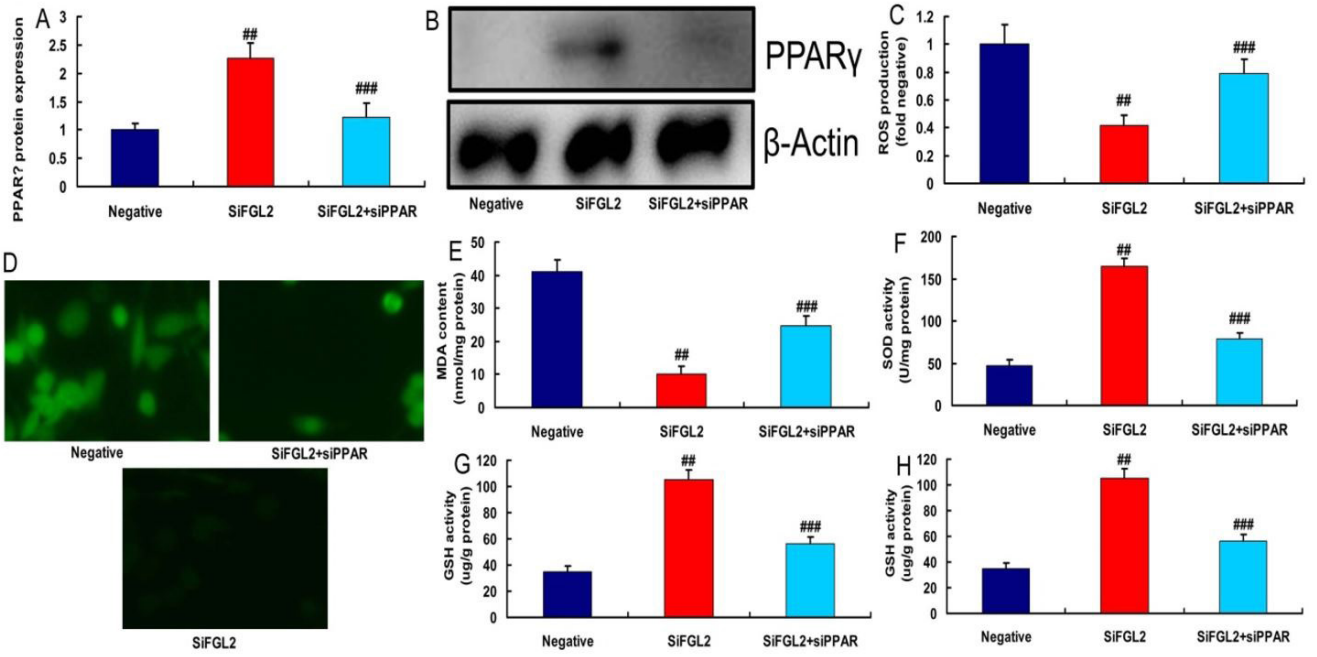

Figure 5. The inactivation of PPAR signaling pathway reduced the function of siFgl2 in vitro model. PPARγ protein expression (A and B), ROS production (C and D), MDA (E), SOD (F), GSH (G) and GSH-px (H) levels. Negative, negative mimics group; SiFgl2, down-regulation of Fgl2 group; SiFgl2+siPPAR, down-regulation of Fgl2 and PPAR group. ##p<0.01 compared with negative mimics group; ###p<0.01 compared with down-regulation of Fgl2 group.

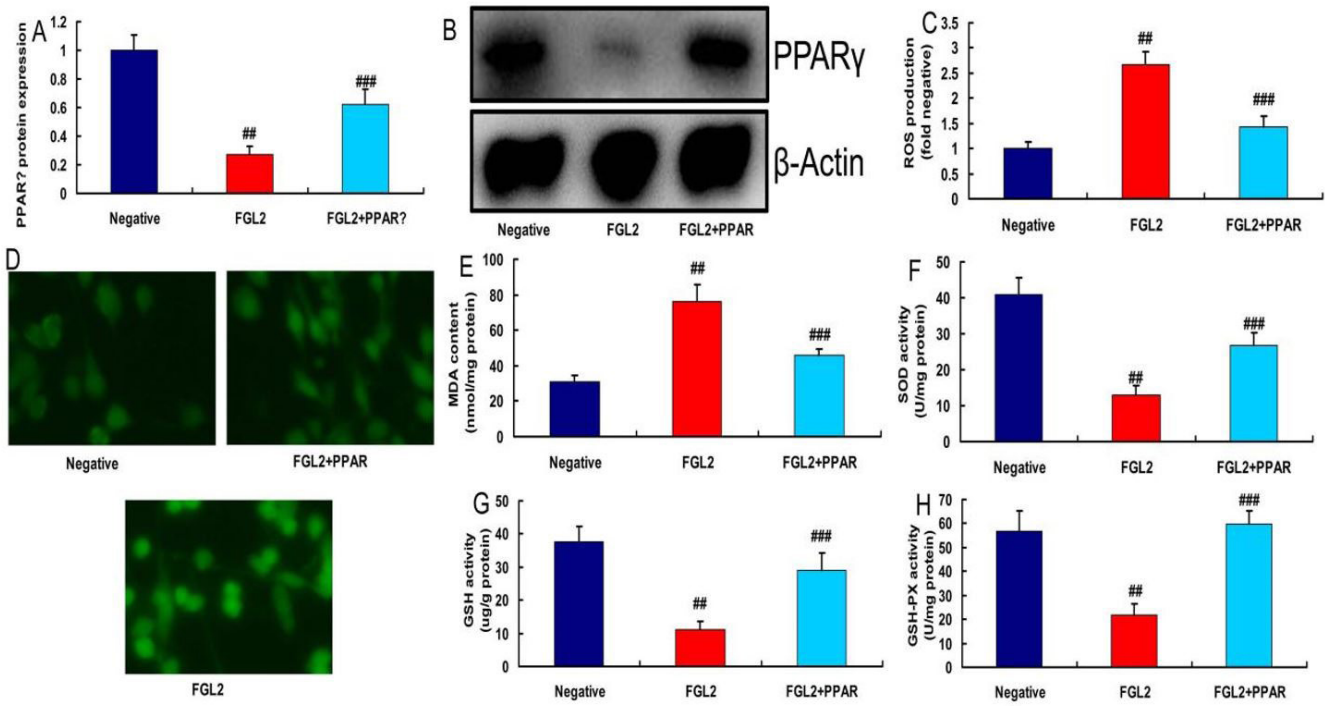

Figure 6. The activation of PPAR signaling pathway reduced the function of Fgl2 in vivo and vitro model. PPARγ protein expression (A and B), ROS production (C and D), MDA (E), SOD (F), GSH (G) and GSH-px (H) levels. Negative, negative mimics group; Fgl2, over-expression of Fgl2 group; Fgl2+PPAR, over-expression of Fgl2 and PPAR group. ##p<0.01 compared with negative mimics group; ###p<0.01 compared with over-expression of Fgl2 group.

2020; Huang et al., 2019b). These data suggested that fgl2 may cooperate with PPAR signaling pathway in the progression of I/R injury following sevoflurane.

In conclusion, our results demonstrate thatFgl2 promoted ROS-induced oxidative stress in I/R injury following sevoflurane by PPARγ signaling pathway. Thus, Fgl2 might serve as a therapeutic target in the treatment of I/R injury following sevoflurane. We hope that our findings will pave a way for future therapies against I/R injury following sevoflurane.

Ethics approval and consent to participate

This study was approved by the Ethics committee of the Zhongshan People's Hospital.

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Not applicable.

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