The Serum of MEF2D Expression in Patients with Myocardial Infarction, May be Clinical Indicators and it Mediated ROS Signaling by HDAC5

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Research

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Abstract

Objectives: In this study, we explain that the function of MEF2D gene in myocardial infarction and its possible mechanism.

Methods: Patients with MI and normal volunteers were collected. The left anterior descending arteries (LAD) of mice were ligated to induce myocardial infarction. H9c2 cells were stimulated with 5% oxygen (O₂) and 5% carbon dioxide (CO₂) and 90% N₂ for 24 h. Quantitative polymerase chain reaction (qPCR), Microarray experiments, Western blot, Enzyme-linked immunosorbent assay (ELISA) and Immunofluorescent staining were used at this experiment.

Results: MEF2D mRNA and protein expressions in heart tissue of patients and mice with myocardial infarction were reduced. MEF2D protein prevented Myocardial infarction in mice of myocardial infarction. The inhibition of MEF2D aggravated Myocardial infarction in mice of myocardial infarction. MEF2D gene promoted ROS-induced oxidative stress in vitro model of myocardial infarction. MEF2D interlinkage HDAC5 to reduced oxidative stress in vivo model or vitro model. The regulation of HDAC5 adjusted the function of MEF2D in vitro model.

Discussion: These data confirmed that the serum of MEF2D expression in patients with Myocardial infarction was reduced, may be clinical indicators and it inhibited ROS-induced oxidative stress signaling by HDAC5. Thus, our results suggest that targeting MEF2D may provide an approach for the treatment of myocardial infarction.

Introduction

The 2017 China Cardiovascular Disease Report published by the National Cardiovascular Disease Center revealed that the mortality rate of cardiovascular disease was higher than that of any other diseases in both urban and rural areas [1]. Among them, myocardial infarction (MI) is one of the important causes of death [2]. The hospitalization cost of acute MI was as high as 15.34 billion RMB in 2019[3] .

Existing studies have reported that excessive activation of oxidative stress response plays a critical role in myocardial cell damage after MI [4]. Oxygen free radicals are massively released during the activation of the oxidative stress response [5]. And oxygen free radicals, with strong oxidative capacity, can attack the lipid of myocardial cell membrane structure, cause lipid oxidation and destroy cell membrane structure [6, 7].

Histone deacetylase 5 (HDAC5) belongs to the class IIa HDAC family, with deacetylase active domain in the carboxyl terminal as well as protein binding domain, nuclear localization and export sequences in the amino terminal [8, 9]. HDAC5 can not only catalyze the deacetylation of histones in the nucleus, but also shuttle between the nucleus and cytoplasm, deacetylate other protein molecules or form complexes with other proteins, thereby regulating physiological processes such as cell division, differentiation, and apoptosis as well as participating in the occurrence of multiple diseases [10, 11].

MEF2D is a member of the MEF2 family. Previous studies on MEF2 were mostly limited to the muscle and nervous system [12]. At present, a large number of studies have confirmed that chromosome translocation in leukemia can cause abnormally high expression of MEF2D, which in turn promote the occurrence and development of leukemia [12, 13]. Meanwhile, MEF2D is specifically expressed in liver tissue, which may also play an important role in ROS formation [14]. In this study, we explain that the function of MEF2D gene in myocardial infarction and its possible mechanism.

Materials And Methods

Clinical research model

30 patients with MI and 30 normal volunteers were collected from Zhongshan People's Hospital. Serum was collected and saved at -80 °C. This experiment was performed in accordance with the Guide for the Care and Use of US National Institutes of Health. Experimental protocols were approved by the Zhongshan People's Hospital. Each cancer patient provided their written informed consent for study participation.

Animals model

Mice were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. C57BL/6 male mice were obtained from the Model Animal Research Center of Nanjing University (Nanjing, China). All aspects of the animal care and experimental protocols were approved by the Zhongshan People's Hospital Committee on Animal Care.

All mice anaesthetized using 50 mg/kg of pentobarbital sodium and the left anterior descending arteries (LAD) were ligated to induce myocardial infarction. Mice were ventilated by a rodent ventilator (Shanghai Alcott Biotech Co., Shanghai, China), then LAD was ligated by an 8.0 suture followed by the thoracotomy. At 2 weeks of modeling, mice were sacrificed and executed other experiment. Left ventricular internal diameter, left ventricular ejection fraction, left ventricular fractional shortening and left ventricular stroke volume were obtained from Millar pressure-volume system (MPVS-400).

Vitro experimental design

H9c2 rat cardiomyocytes (Cell Bank of the Chinese Academy of Sciences, Shanghai, China) were grown in Dulbecco's modified Eagle medium (DMEM, Gibco, Grand Island, NY, United States), with 5% fetal bovine serum (FBS, Gibco, Grand Island, NY, United States), in a humidified 5% CO₂ incubator at 37°C. HSMECs were performed transfections using Lipofectamine 2000 (Thermo Fisher Scientific). MEF2D (0.4 µg/ml), HDAC5 (0.4 µg/ml), MEF2D (20 nmol/ml) or HDAC5 siRNAs (20 nmol/ml) were transfected in the serum-free and antibiotic-free media. After 48 h of transfection, H9c2 cells were stimulated with 5% oxygen (O₂) and 5% carbon dioxide (CO₂) and 90% N₂ for 24 h.

Quantitative polymerase chain reaction (qPCR)

Total RNAs were isolated with RNA isolator total RNA extraction reagent (Takara) and cDNA was synthesized using PrimeScript RT Master Mix (Takara). qPCR were performed with the ABI Prism 7500 sequence detection system according to the Prime-Script™ RT detection kit. Relative levels of the sample mRNA expression were calculated and expressed as 2- $\Delta\Delta C_t$.

Western blot

Aneurysm tissue or cells samples were lysed with ice-cold RIPA buffer with complete protease and phosphatase inhibitors. The protein concentrations were measured using BCA protein assay kit. Total proteins were separated by SDS–PAGE and transferred onto polyvinylidene difluoride (PVDF) membranes. The membranes were incubated with primary antibodies: MEF2D (1:1000, abcam), HDAC5 (1:1000, Cell Signaling Technology, Inc.) and β -Actin (1:5000, Santa Cruz Biotechnology) after blocking with 5% BSA in TBS, followed by incubation with peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology). The signals were detected with the ECL system and exposed by the ChemiDoc XRS system with Image Labsoftware (Bio-rad).

Enzyme-linked immunosorbent assay (ELISA)

Total protein was diluted using PBS, and measurement was performed according to the instructions of the manufacturer. CK, LDH, SOD, GSH, GSH-PX and MDA levels were measured from cell samples or tissue samples using commercial ELISA kits.

Histological examination

Heart tissue were harvested from each group and immersion fixated in 4% formaldehyde, embedded in paraffin. Heart sections (4 μ m) were cut from Aneurysm tissue and stained with HE. Sections were viewed with a fluorescent microscope.

Microarray experiments

Microarray experiments were performed at the Genminix Informatics (China). Gene expression profiles were analyzed with the Human Exon 1.0 ST GeneChip (Affymetrix).

Immunofluorescent staining.

H9c2 cells were stimulated with 5% oxygen (O₂) and 5% carbon dioxide (CO₂) and 90% N₂ for 24 h. Next, H9c2 cells were fixed with 4% paraformaldehyde for 15 min and incubated with using 0.15% Triton X100 for 15 min at room temperature. H9c2 cells was incubated with MEF2D (1:500, abcam) and HDAC5 (1:500, Cell Signaling Technology, Inc.) at 4°C overnight after blocking with 5% BSA for 1 h. hASMCs was incubated with goat anti-rabbit IgG-cFL 555 antibody (1:100) for 2 h at room temperature and stained with DAPI for 15 min and washed with PBS for 15 min. The images of hASMCs obtained using a Zeiss Axioplan 2 fluorescent microscope (Carl Zeiss AG, Oberkochen, Germany).

Statistical analysis

The data were entered into GraphPad Prism 5.01 Software and represented as mean values $\pm$ SD. Comparisons of data between groups were followed using Student's t test or one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. $P < 0.05$ was considered to denote a statistically significant difference

Results

The serum of MEF2D expression in patients with Myocardial infarction and mice model of myocardial infarction

To investigate the gene expression of MEF2D in Myocardial infarction, we firstly detected the gene expression of MEF2D in patients with myocardial infarction and mice model of myocardial infarction. As shown in Fig. 1A, a significantly lower serum gene expression of MEF2D compared with normal volunteers groups was detected. Serum gene expression of MEF2D was negative correlation with serum CK levels in patients with myocardial infarction and ROC = 0.9011, as shown in Fig. 1B-1C. In myocardial infarction mice models, MEF2D mRNA and protein expressions in heart tissue of mice with myocardial infarction were reduced (Fig. 1D-1E).

MEF2D protein prevented Myocardial infarction in mice of myocardial infarction

Next, we analyzed the correlation between MEF2D expression and oxidative stress in myocardial infarction models. MEF2D recombinant protein reduced left ventricular internal diameter, increased left ventricular ejection fraction, raised left ventricular fractional shortening and left ventricular stroke volume, prevented myocardial fibrosis, weakened CK and LDH activity levels in mice with myocardial infarction (Fig. 2A-2G). In addition, we found remarkably increased activity of SOD, GSH and GSH-PX levels, inhibition of MDA levels in mice with myocardial infarction by MEF2D recombinant protein (Fig. 2H-2K).

The inhibition of MEF2D aggravated Myocardial infarction in mice of myocardial infarction

Next, we found that anti-MEF2D body increased left ventricular internal diameter, decreased left ventricular ejection fraction, abated left ventricular fractional shortening and left ventricular stroke volume, aggravated myocardial fibrosis and heightened CK and LDH activity levels in mice with myocardial infarction (Fig. 3A-3G). When anti-MEF2D body was injected into mice with myocardial infarction, we found that anti-MEF2D reduced SOD, GSH and GSH-PX activity levels, and increased MDA levels in mice with (Fig. 3H-3K).

MEF2D gene promoted ROS-induced oxidative stress in vitro model of myocardial infarction

To investigate the role of MEF2D in the development of MEF2D in vitro model of myocardial infarction, We then transfected MEF2D mimics or mimics control into vitro model of myocardial infarction. MEF2D mimics increased the expression of MEF2D mRNA, aggravated MDA levels and ROS production levels, and decreased SOD, GSH and GSH-PX activity levels in vitro model of myocardial infarction (Fig. 4A-4F). MEF2D siRNA or negative siRNA into vitro model of myocardial infarction, and we found that MEF2D siRNA reduced MEF2D mRNA expression, abated MDA levels and ROS production levels, and promoted SOD, GSH and GSH-PX activity levels in vitro model of myocardial infarction (Fig. 4G-4L).

MEF2D interlinkage HDAC5 to reduced oxidative stress in vivo model or vitro model

To explore the molecular mechanism by which MEF2D in model of myocardial infarction, we examined a range of oxidative stress signaling pathways. Microarray experiments showed that HDAC5 may be a target spot of MEF2D in model of myocardial infarction (Fig. 5A). Network signal diagram showed that downstream passage of MEF2D interlinkage HDAC5 was calculated (Fig. 5B). MEF2D recombinant protein induced HDAC5 protein expression in mice with HDAC5 (Fig. 5C-5D). Anti-MEF2D body suppressed HDAC5 protein expression in mice with HDAC5 (Fig. 5E-5F).

Next, immunofluorescence showed that over-expression of MEF2D induced HDAC5 expression in vitro model of myocardial infarction (Fig. 6A). IP showed that MEF2D interlinkage HDAC5 may be form a complex compound in vitro model (Fig. 6B). Over-expression of MEF2D induced MEF2D and HDAC5 protein expressions in vitro model (Fig. 6C-6E). Down-regulation of MEF2D suppressed MEF2D and HDAC5 protein expressions in vitro model (Fig. 6F-6H).

The regulation of HDAC5 adjusted the function of MEF2D in vitro model

To assess whether LMP10 regulates the expression of HDAC5 in vitro model by in vitro model, siMEF2D and HDAC5 mimics were co-transfected into vitro model, and we found that HDAC5 induced HDAC5 protein expression, reduced MDA level and ROS production levels, and increased SOD, GSH and GSH-PX activity levels in vitro model by siMEF2D mimics (Fig. 7A-7F). Si-HDAC5 and MEF2D mimics were co-transfected into vitro model and we found that si-HDAC5 suppressed HDAC5 protein expression, promoted MDA level and ROS production levels, and reduced SOD, GSH and GSH-PX activity levels in vitro model by siMEF2D mimics (Fig. 7G-7L).

Discussion

Malignant ventricular arrhythmia (including ventricular tachycardia, ventricular tachycardia, ventricular fibrillation and ventricular fibrillation) is the main cause of death in patients after MI, accounting for over half of the deaths in patients after MI [15]. The excessive regeneration of sympathetic nerves after MI plays an important role in the occurrence of ventricular arrhythmias after MI, which provides the electrical substrate for the occurrence of malignant arrhythmias after MI [16]. Therefore, effective inhibition of the excessive regeneration of sympathetic nerves after MI may be one of the effective ways to prevent and to treat arrhythmia after MI [17]. Here, we found that MEF2D mRNA and protein expressions in heart tissue of mice with myocardial infarction were reduced in patients and mice model. Estrella et al. showed that MEF2D deficiency triggers programmed cell death in neonatal cardiomyocytes [18]. Thus, these results suggest that the down-regulation of MEF2D may play a role in myocardial infarction.

MI leads to abnormal intracellular environment caused by insufficient energy supply [19]. The compensatory increase in cardiac contraction can cause the increase in ROS level caused by NADPH in the cell membrane [20]. More seriously, the increased ROS levels triggers the mitochondria to produce a large amount of ROS ("oxygen triggered-oxygen release" mechanism in mitochondria) [21]. Oxidative stress can not only attack cell membrane and organelles, but also cause inflammation by interacting with inflammatory factors, further aggravating the myocardial damage caused by MI [22]. Therefore, increasing the level of antioxidant enzymes and decreasing the content of ROS are generally considered as one of the important therapeutic ideas for MI [23]. We demonstrated that MEF2D protein prevented Myocardial infarction in mice of myocardial infarction, and MEF2D reduced ROS-induced oxidative stress in vitro model of myocardial infarction. Chen et al. demonstrated that MEF2D control the effects of methylene blue against glutamate-induced oxidative damage in HT22 cells [18]. Therefore, our results revealed that MEF2D overexpression could protect myocardial infarction by the inhibition of ROS-induced oxidative stress.

Massive studies have demonstrated that HDAC5 can inhibit cardiac hypertrophy by binding to MEF2 (a key transcription factor for muscle cell differentiation) in the nucleus [24, 25]. However, stimuli such as hypoxia and inflammation can activate PKC, PKD and CaMK to phosphorylate HDAC5 to cause nucleoplasmic shuttling, thereby abolishing the inhibitory effect on MEF2 [25]. We revealed that MEF2D interlinkage HDAC5 to reduced oxidative stress in vivo model or vitro model of myocardial infarction. Shi et al. revealed that MEF2D regulated HDAC5 signaling pathway and participates in the oxidative stress after cerebral ischemia [26]. Therefore, MEF2D -induced HDAC5 might contribute to the inhibition of ROS-induced oxidative stress in myocardial infarction.

Our data revealed a novel anti-oxidation function for MEF2D in myocardial infarction. We identified a regulatory mechanism of MEF2D inhibited ROS-induced oxidative stress, leading to an induction of HDAC5 in myocardial infarction model. Thus, our results suggest that targeting MEF2D may provide an approach for the treatment of myocardial infarction.

Declarations

Ethics approval and consent to participate

This study was support by the Ethics Committee of Zhongshan People's Hospital.

Consent for publication

Not applicable.

Availability of data and material

Not applicable.

Funding

None.

Competing interests

There are no potential conflicts of interest to disclose.

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Figures

Figure 1

The serum of MEF2D expression in patients with Myocardial infarction and mice model of myocardial infarction Serum of MEF2D mRNA expression and CK levels (A and B), sensitivity analyze (C) in patients with myocardial infarction; MEF2D mRNA expression (D) and protein expression (E) in mice model of myocardial infarction. Normal, normal volunteers group; Patients, Patients with myocardial infarction; Sham, sham control group; Model, mice with myocardial infarction. **p<0.01 compared with normal volunteers group or sham control group.

Figure 2

MEF2D protein prevented Myocardial infarction in mice of myocardial infarction Left ventricular internal diameter (A), left ventricular ejection fraction (B), left ventricular fractional shortening (C), left ventricular stroke volume (D), myocardial fibrosis (E), CK and LDH activity levels (F and G), SOD (H), GSH (I), GSH-PX (J) and MDA (K) levels MI, mice with myocardial infarction; MI+MEF2D, mice with myocardial infarction by MEF2D recombinant protein. **p<0.01 compared with mice with myocardial infarction.

Figure 3

The inhibition of MEF2D aggravated Myocardial infarction in mice of myocardial infarction Left ventricular internal diameter (A), left ventricular ejection fraction (B), left ventricular fractional shortening (C), left ventricular stroke volume (D), myocardial fibrosis (E), CK and LDH activity levels (F and G), SOD (H), GSH (I), GSH-Px (J) and MDA (K) levels MI, mice with myocardial infarction; MI+anti-MEF2D, mice with myocardial infarction by anti-MEF2D body. ** $p < 0.01$ compared with mice with myocardial infarction.

Figure 4

MEF2D gene promoted ROS-induced oxidative stress in vitro model of myocardial infarction MEF2D mRNA expression (A), MDA and ROS production levels (B and C), SOD (D), GSH (E) and GSH-PX (F) in vitro model of myocardial infarction by over-expression of MEF2D; MEF2D mRNA expression (G), MDA and ROS production levels (H and I), SOD (J), GSH (K) and GSH-PX (L) in vitro model of myocardial infarction by down-regulation of MEF2D. Negative, negative mimics group; MEF2D, over-expression of MEF2D group; Negative-si, si-negative mimics group; MEF2D-si-1, down-regulation of MEF2D-1 group; MEF2D-si-2, down-regulation of MEF2D-2 group; MEF2D-si-3, down-regulation of MEF2D-3 group; MEF2D-si, down-regulation of MEF2D group. ** $p < 0.01$ compared with negative mimics group or si-negative mimics group.

Figure 5

MEF2D interlinkage HDAC5 to reduced oxidative stress in vivo model Heat map and analysis result (A), network signal diagram showed that downstream passage of MEF2D interlinkage HDAC5 (B), HDAC5 protein expression (C and D) in vivo model in mice with myocardial infarction by MEF2D recombinant protein, HDAC5 protein expression (E and F) in vivo model in mice with myocardial infarction by anti-MEF2D body. MI, mice with myocardial infarction; MI+anti-MEF2D, mice with myocardial infarction by anti-MEF2D body; MI+MEF2D, mice with myocardial infarction by MEF2D recombinant protein. ** $p < 0.01$ compared with mice with myocardial infarction.

Figure 6

HDAC5 may be a target spot of MEF2D in vitro model of myocardial infarction HDAC5 and MEF2D expression (IF, A), HDAC5 and MEF2D protein (IP, B); HDAC5 and MEF2D protein expression in vitro model by over-expression of HDAC5 (C, D and E); HDAC5 and MEF2D protein expression in vitro model by down-regulation of HDAC5 (F, G and H). Negative, negative mimics group; MEF2D, over-expression of MEF2D group; Negative-si, si-negative mimics group; MEF2D-si, down-regulation of MEF2D group. ** $p < 0.01$ compared with negative mimics group or si-negative mimics group.

Figure 7

The regulation of HDAC5 adjusted the function of MEF2D in vitro model HDAC5 protein expression (A), MDA levels (B), SOD (C), ROS production levels (D), GSH (E) and GSH-PX (F) in vitro model of myocardial infarction by down-regulation of MEF2D and over-expression of HDAC5; HDAC5 protein expression (G), MDA levels (H), SOD (I), ROS production levels (J), GSH (K) and GSH-PX (L) in vitro model of myocardial infarction by over-expression of MEF2D and down-regulation of HDAC5. Negative, negative mimics group; MEF2D, over-expression of MEF2D group; Negative-si, si-negative mimics group; MEF2D-si, down-regulation of MEF2D group; MEF2D+siHDAC5, over-expression of MEF2D and down-regulation of HDAC5 group; MEF2D-si+HDAC5, down-regulation of MEF2D and over-expression of MEF2D group. ** $p < 0.01$ compared with negative mimics group; ## $p < 0.01$ compared with over-expression of MEF2D group or down-regulation of MEF2D group.