

Downregulation of MALAT1 alleviates saturated fatty acid-induced myocardial inflammatory injury via the miR-26a/HMGB1/TLR4/NF- κ B axis

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Purpose: The increased level of saturated fatty acids (SFAs) is found in patients with diabetes, obesity, and other metabolic disorders. SFAs can induce lipotoxic damage to cardiomyocytes, but the mechanism is unclear. The long noncoding RNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) acts as a key regulator in palmitic acid (PA)-induced hepatic steatosis, but its role in PA-induced myocardial lipotoxic injury is still unknown. The aim of this study was to explore the role and underlying mechanism of MALAT1 in PA-induced myocardial lipotoxic injury.

Methods: MALAT1 expression in PA-treated human cardiomyocytes (AC16 cells) was detected by RT-qPCR. The effect of MALAT1 on PA-induced myocardial injury was measured by Cell Counting Kit-8, lactate dehydrogenase (LDH), and creatine kinase-MB (CK-MB) assays. Apoptosis was detected by flow cytometry. The activities of cytokines and nuclear factor (NF)- κ B were detected by enzyme-linked immunosorbent assay. The interaction between MALAT1 and miR-26a was evaluated by a luciferase reporter assay and RT-qPCR. The regulatory effects of MALAT1 on high mobility group box 1 (HMGB1) expression were evaluated by RT-qPCR and western blotting.

Results: MALAT1 was significantly upregulated in cardiomyocytes after PA treatment. Knockdown of MALAT1 increased the viability of PA-treated cardiomyocytes, decreased apoptosis, and reduced the levels of LDH, CK-MB, TNF- α , and IL-1 β . Moreover, we found that MALAT1 specifically binds to miR-26a and observed a reciprocal negative regulatory relationship between these factors. We further found that the downregulation of MALAT1 represses HMGB1 expression, thereby inhibiting the activation of the Toll-like receptor 4 (TLR4)/NF- κ B-mediated inflammatory response. These repressive effects were rescued by an miR-26a inhibitor.

Conclusion: We demonstrate that MALAT1 is induced by SFAs and its downregulation alleviates SFA-induced myocardial inflammatory injury via the miR-26a/HMGB1/TLR4/NF- κ B axis. Our findings provide new insight into the mechanism underlying myocardial lipotoxic injury.

Keywords: metastasis-associated lung adenocarcinoma transcript 1, saturated fatty acids, microRNA, high mobility group box-1 protein, inflammation

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Introduction

The excessive accumulation of lipids or lipid intermediates in non-adipose tissues, such as the liver and kidney, leads to cellular dysfunction and death.¹ This pathophysiological process is termed lipotoxicity.¹ It usually develops in patients with diabetes, obesity, and other metabolic disorders.² The heart can also be affected by lipotoxicity, predominantly manifesting as myocardial fibrosis and even heart failure.³ The main cause may be the

deposit of excess saturated fatty acids (SFAs) in cardiomyocytes.³ We and others have provided direct evidence that palmitic acid (PA), a major SFA, leads to myocardial lipotoxic injury in vitro and in vivo.⁴⁻⁶ Although several mechanisms, including inflammation, endoplasmic reticulum stress, alterations in autophagy, and oxidative stress, are responsible for SFA-induced myocardial lipotoxic injury,⁷ the exact mechanism is still not well understood.

Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1, also named NEAT2) is a highly evolutionarily conserved long noncoding RNA (lncRNA) and was initially identified in early-stage non-small cell lung cancer.⁸ MALAT1 is involved in multiple pathophysiological processes, including tumorigenesis and metastasis,^{9,10} ischemic stroke,¹¹ myocardial ischemia reperfusion injury,¹² and pulmonary fibrosis.¹³ Notably, a recent study has found that MALAT1 acts as a key regulator in PA-induced hepatic steatosis by promoting lipid accumulation in hepatocytes.¹⁴ Furthermore, MALAT1 plays a role in the regulation of inflammatory responses.¹⁵⁻¹⁷ However, the role of MALAT1 in SFA-induced myocardial lipotoxic injury is unknown.

Recently, lncRNAs have been reported to bind to microRNAs, as a competitive endogenous RNA (ceRNA), to further regulate target mRNA expression at the post-transcriptional level.¹⁸ Using a bioinformatics approach, we found that MALAT1 transcript sequences contained an miR-26a binding region. Moreover, miR-26a has been demonstrated to bind to high mobility group box 1 (HMGB1) and inhibited HMGB1 expression, resulting in the inhibition of the Toll-like receptor 4 (TLR4)/nuclear factor (NF)- κ B signaling pathway-mediated inflammatory response.¹⁹ Therefore, it is hypothesized that MALAT1 regulates HMGB1 expression through binding to miR-26a in a ceRNA mechanism.

In this study, we evaluated the role of MALAT1 in SFA-induced myocardial lipotoxic injury and its underlying mechanism. Our results indicated that MALAT1 is significantly induced in cardiomyocytes after treatment with PA and the knockdown of MALAT1 alleviates PA-induced myocardial inflammatory injury. Mechanistically, we found that MALAT1 acts as a competing ceRNA to regulate HMGB1 expression by binding to miR-26a, thereby inhibiting the activation of the TLR4/NF- κ B signaling pathway-mediated inflammatory response.

Materials and methods

Cell culture and PA treatment

Human adult ventricular cardiomyocytes (AC16 cell line) purchased from the American Type Culture Collection (Manassas, VA) were grown in Dulbecco's modified Eagle medium (Gibco, Gaithersburg, MD) supplemented with 10% fetal bovine serum (TBD, Tianjin, China), 100 units/ml penicillin, and 100 μ g/ml streptomycin in a humidified atmosphere at 37 °C with 5% CO₂. AC16 cells were treated with 300 μ M PA (Sigma-Aldrich, St. Louis, MO) to induce myocardial lipotoxic injury as described in a previous article.²⁰

Cell transfection

MALAT1-specific small interfering RNA (siRNA), scrambled siRNA (negative control, NC), miR-26a mimics, NC mimics, miR-26a inhibitor, NC inhibitor, and TNF- α -specific siRNA (si-TNF- α) were designed and synthesized by GenePharma Co., Ltd. (Shanghai, China). Cells were transfected with the siRNAs and microRNAs (miRNAs) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Cells were harvested and processed for further analysis after 24 h or 48 h of transfection.

qRT-PCR

Total RNA was extracted from cells with TRIzol reagent (Invitrogen). To detect MALAT1 and mRNA expression, the PrimeScript RT Reagent Kit with gDNA Eraser (TaKaRa, Kusatsu, Japan) and SYBR Premix Ex Taq II (TaKaRa) were used for reverse transcription and quantitative PCR according to the manufacturer's instructions. GAPDH was used as an internal control. To detect miR-26a expression, the Mir-X™ miRNA First Strand Synthesis Kit (TaKaRa) and Mir-X™ miRNA qRT-PCR SYBR® Kit (TaKaRa) were used for reverse transcription and quantitative PCR according to the manufacturer's instructions. U6 was used as an internal control. All oligonucleotide primers were designed by Sangon Biotech Co., Ltd. (Shanghai, China) (Table 1). Relative expression was analyzed using the 2- $\Delta\Delta$ Ct method.

Cell Counting Kit-8 assay

Cell viability was determined using the Cell Counting Kit-8 (CCK-8) assay (Dojindo, Kumamoto, Japan) according to the manufacturer's instructions. Absorbance was detected at 450 nm using the Ultramicro Microporous Plate Spectrophotometer (BioTek, Winooski, VT).

Table 1 The information of primer sequences

Name	Sequences (5' to 3')
MALAT1 forward	GGGTGTTTACGTAGACCAGAACC
MALAT1 reverse	CTTCCAAAAGCCTTCTGCCTTAG
TNF- α forward	TCCTTCAGACACCCCTCAACC
TNF- α reverse	AGGCCCCAGTTTGAATTCTT
IL-1 β forward	GCTGTGCAGGAGATCACAGA
IL-1 β reverse	GGGCTCCATAAAGTCACCAA
miR-26a forward	GCGGCGGTTCAAGTAATCCAGG
HMGB1 forward	ACATCCAAAATCTTGATCAGTTA
HMGB1 reverse	AGGACAGACTTTCAAAATGTTT
GAPDH forward	GCACCGTCAAGGCTGAGAAC
GAPDH reverse	ATGGTGGTGAAGACGCCAGT

Abbreviations: IL-1 β , interleukin β ; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; HMGB1, high mobility group box 1; TNF- α , tumour necrosis factor α .

Lactate dehydrogenase and creatine kinase-MB assays

Lactate dehydrogenase (LDH) and creatine kinase-MB (CK-MB) released from cardiomyocytes into the culture medium were determined using the LDH Assay Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and CK-MB Assay Kit (Nanjing Jiancheng Bioengineering Institute), respectively, according to the manufacturer's protocols. Absorbance was detected at 450 nm using the Ultramicro Microporous Plate Spectrophotometer (BioTek).

Apoptosis assay

Cell apoptosis was determined using the Annexin V-Fluorescein Isothiocyanate (FITC)/Propidium Iodide (PI) Kit (KeyGEN BioTECH, Jiangsu, China) according to the manufacturer's instructions for flow cytometry (BD Biosciences, Franklin Lakes, NJ).

Enzyme-linked immunosorbent assay of cytokine and NF- κ B activity

The concentrations of tumor necrosis factor alpha (TNF- α) and interleukin-1 β (IL-1 β) in the culture medium were detected using a commercial human TNF- α enzyme-linked immunosorbent assay (ELISA) kit (MultiSciences, Hangzhou, China) and human IL-1 β ELISA kit (MultiSciences), respectively, according to the manufacturer's instructions. The activity of NF- κ B in cell lysates was detected using the NF- κ B p65 Transcription Factor Assay Kit (Abcam, Cambridge, UK) according to the recommended experimental protocol.

Luciferase activity assay

Luciferase reporter plasmids (pmirGLO-MALAT1 and pmirGLO-MALAT1-mut) were obtained from Shanghai GenePharma Co., Ltd. The cells were plated in 24-well plates and transfected with pmirGLO-MALAT1 or pmirGLO-MALAT1-mut together with miR-26a mimics or NC mimics. At 48 h post-transfection, luciferase activity was analyzed using the Dual-Glo Luciferase Assay System (Promega, Madison, WI) according to the manufacturer's instructions.

Western blotting analysis

Proteins were extracted from cell lysates using the Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime, Shanghai, China) and the protein concentration was measured using the Enhanced BCA Protein Assay Kit (Beyotime) according to the manufacturer's instructions. Heat-denatured proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred to polyvinylidene fluoride membranes, followed by blocking with 1% bovine serum albumin solution for 1 h. The membranes were then incubated with primary antibodies, including anti-HMGB1 (1:1000; Abcam), anti-TLR4 (1:1000; Abcam), anti-NF- κ B p65 (1:1000; Abcam), anti-cleaved caspase-8 (1:1000; Abcam), anti-cleaved caspase-3 (1:1000; Abcam), anti-GAPDH (1:1000; Zhongshan Jinqiao Biotechnology, Beijing, China), and anti-Lamin A (1:1000; Abcam) at 4 °C overnight, followed by incubation with horseradish peroxidase-conjugated goat anti-rabbit or goat anti-mouse immunoglobulin G (1:4000; EarthOx, Millbrae, CA) at room temperature for 30 min. Protein bands were detected using an enhanced chemi-luminescence kit for western blotting (Beyotime) according to the manufacturer's instructions. GAPDH or Lamin A was used as an internal control. Relative densitometry was calculated using Image J2x analysis software (National Institutes of Health, Bethesda, MD).

Statistical analysis

Data are expressed as the mean \pm standard error. Statistical analysis was performed using SPSS version 17.0 software (SPSS Inc., Chicago, IL). Differences between groups were first evaluated using one-way analysis of variance, and if the differences were significant, multiple comparison testing was further performed using Fisher's least

significant difference test. *P*-values less than 0.05 were considered statistically significant.

Results

MALAT1 was induced in myocardial cells by PA

Expression of MALAT1 in cardiomyocytes increased by 2.57-, 4.70-, 7.41-, and 2.87-fold after treatment with 300 μ M PA for 6, 12, 24, and 48 h, respectively (Figure 1). These results demonstrated that MALAT1 is induced by PA in AC16 cells.

Knockdown of MALAT1 expression alleviated PA-induced myocardial injury in vitro

Expression of MALAT1 in AC16 cells decreased by 63% after transfection with MALAT1-specific siRNA (Figure 2A), indicating the effective repression of the endogenous expression of MALAT1 in cardiomyocytes. Cell viability increased remarkably (Figure 2B), the activity of LDH (Figure 2C) and CK-MB (Figure 2D) decreased significantly, and the ratio of apoptosis (Figure 2E and F) decreased significantly in response to the downregulation of MALAT1. These results demonstrated that the downregulation of MALAT1 protects against PA-induced myocardial injury in vitro.

Interaction between MALAT1 and miR-26a

LncBase Predicted v.2 tool²¹ (http://carolina.imis.athena-innovation.gr/diana_tools/web/index.php?r=lnbasev2/)

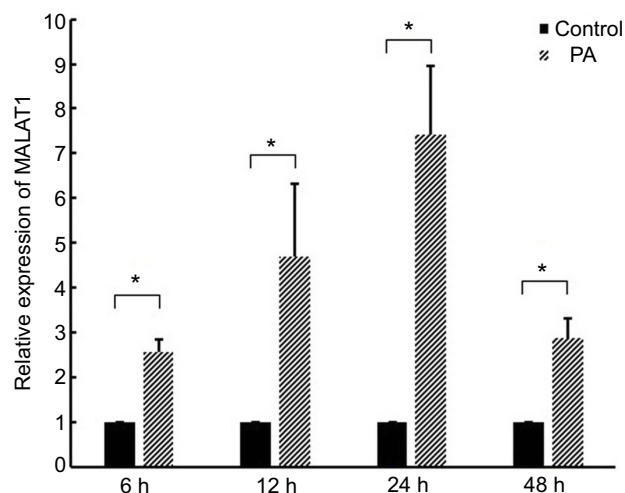


Figure 1 Expression of MALAT1 in cardiomyocytes after treatment with palmitic acid (PA). **P*<0.05. Data are presented as means \pm standard error from three independent experiments.

index-predicted) and starBase v2.0 (<http://starbase.sysu.edu.cn/index.php>) were employed to predict the binding sites between MALAT1 and miRNAs. The result of bioinformatics analysis performed by above two softwares both showed that the MALAT1 transcript sequence contains an miR-26a binding region (Figure 3A). A luciferase reporter assay showed that luciferase activity was 49% lower using the miR-26a mimics than the NC mimics in the pmirGLO-MALAT1 group; miR-26a mimics did not have any inhibitory effects on luciferase activity compared with the NC mimics in the pmirGLO-MALAT1-mut group (Figure 3B). These results demonstrated that MALAT1 specifically binds to miR-26a. Moreover, we found that the downregulation of MALAT1 upregulated miR-26a expression and vice versa (Figure 3C and D). These results further support the reciprocal negative regulatory relationships between miR-26a and MALAT1.

Knockdown of MALAT1 inhibited HMGB1/TLR4/NF- κ B signaling via miR-26a

HMGB1 is a known target of miR-26a;¹⁹ accordingly, we explored whether MALAT1, acting as a ceRNA, regulates HMGB1 expression via miR-26a. The mRNA and protein expression levels of HMGB1 were repressed by transfection with MALAT1 siRNA alone. However, co-transfection with MALAT1 siRNA and an miR-26a inhibitor did not inhibit HMGB1 at the mRNA and protein expression levels (Figure 4A and B). Furthermore, we detected the effect of MALAT1 on the TLR4/NF- κ B signaling pathway. Transfection with MALAT1 siRNA alone decreased the protein expression of TLR4, reduced the protein expression of NF- κ B in the nucleus, inhibited the activity of NF- κ B, and increased the protein expression of NF- κ B in the cytoplasm. However, these effects were abolished by the co-transfection of MALAT1 siRNA and an miR-26a inhibitor (Figure 4C–F). Taken together, these results demonstrated that the knockdown of MALAT1 inhibits the HMGB1/TLR4/NF- κ B signaling pathway via miR-26a.

Effect of MALAT1 on PA-induced myocardial inflammation was mediated by miR-26a

To evaluate whether MALAT1 regulates PA-induced inflammation, we detected the mRNA expression levels and concentrations of the pro-inflammatory cytokines TNF- α and IL-1 β in AC16 cells and culture medium, respectively. The mRNA

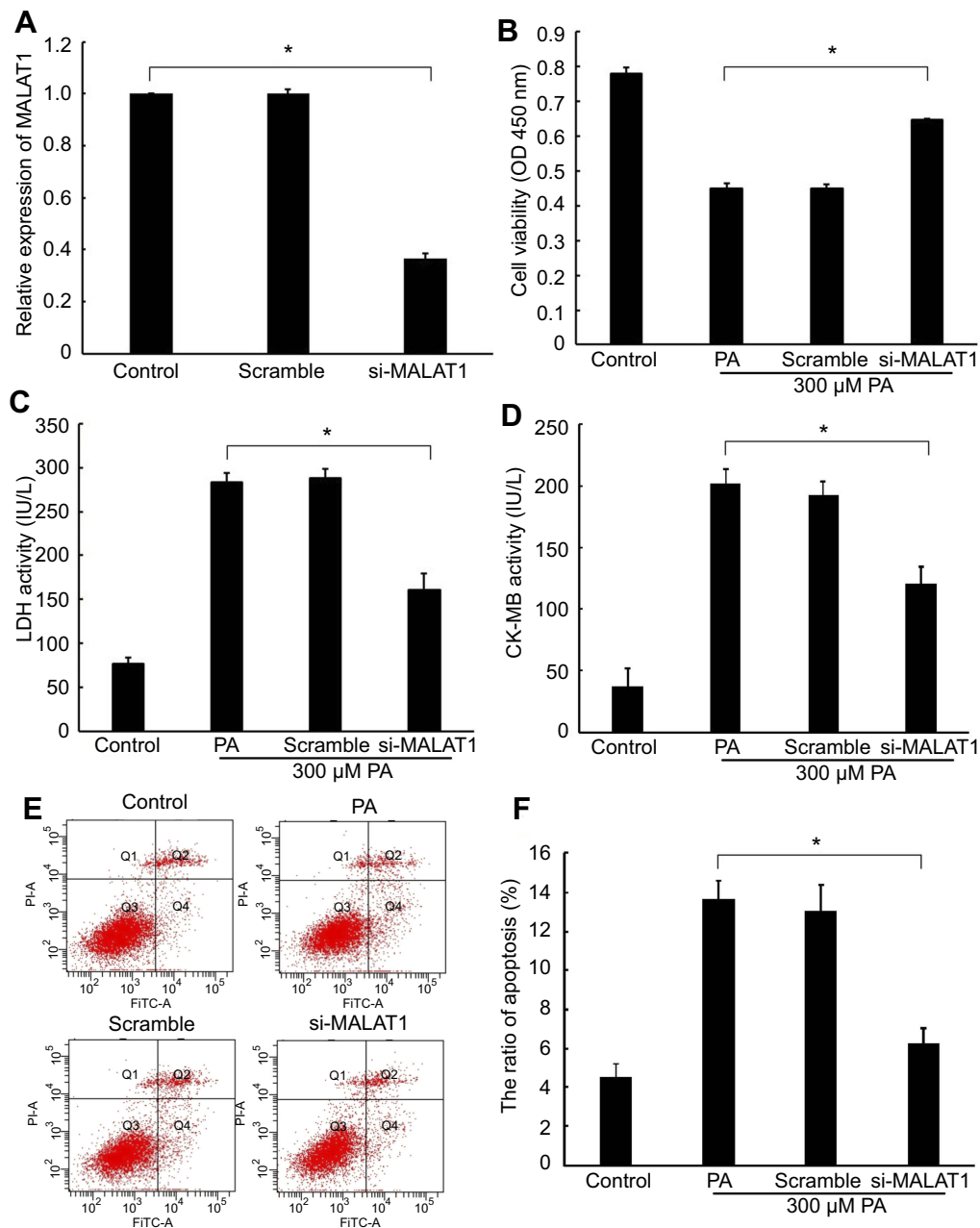


Figure 2 Effect of the knockdown of MALAT1 on palmitic acid (PA)-induced myocardial injury. (A) The expression of MALAT1 in AC16 cells was detected by RT-qPCR after transfection with MALAT1-specific siRNA (si-MALAT1) and a negative control (scramble). (B) Cell viability was determined by a Cell Counting Kit-8 assay. (C) Lactate dehydrogenase (LDH) activity in the culture medium was detected by spectrophotometry. (D) Creatine kinase-MB (CK-MB) activity in the culture medium was detected by spectrophotometry. (E) Apoptosis was measured by flow cytometry. (F) Statistical analysis of the ratio of apoptotic cells. * $P < 0.05$. Data are presented as the mean \pm standard error from three independent experiments.

Abbreviations: FITC, Annexin V-Fluorescein Isothiocyanate; IL-1 β , interleukin 1 β ; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; HMGB1, high mobility group box 1; TNF- α , tumour necrosis factor α .

expression and concentrations of TNF- α and IL-1 β were notably reduced by the downregulation of MALAT1. The repression of TNF- α and IL-1 β by the downregulation of MALAT1 was rescued by an miR-26a inhibitor (Figure 5A–D). Taken together, these results demonstrated that the downregulation of MALAT1 inhibits PA-induced inflammation, and miR-26a mediates the suppressive effect of MALAT1 on PA-induced myocardial inflammation.

MALAT1/miR-26a manipulation regulated PA-induced cell death via the TNF- α -induced apoptosis pathway

Given that the changes in TNF- α and IL-1 β induced by PA are regulated by MALAT1/miR-26a manipulation and TNF- α can induce apoptosis by the death-inducing signaling complex/FADD pathway, we inferred that MALAT1/

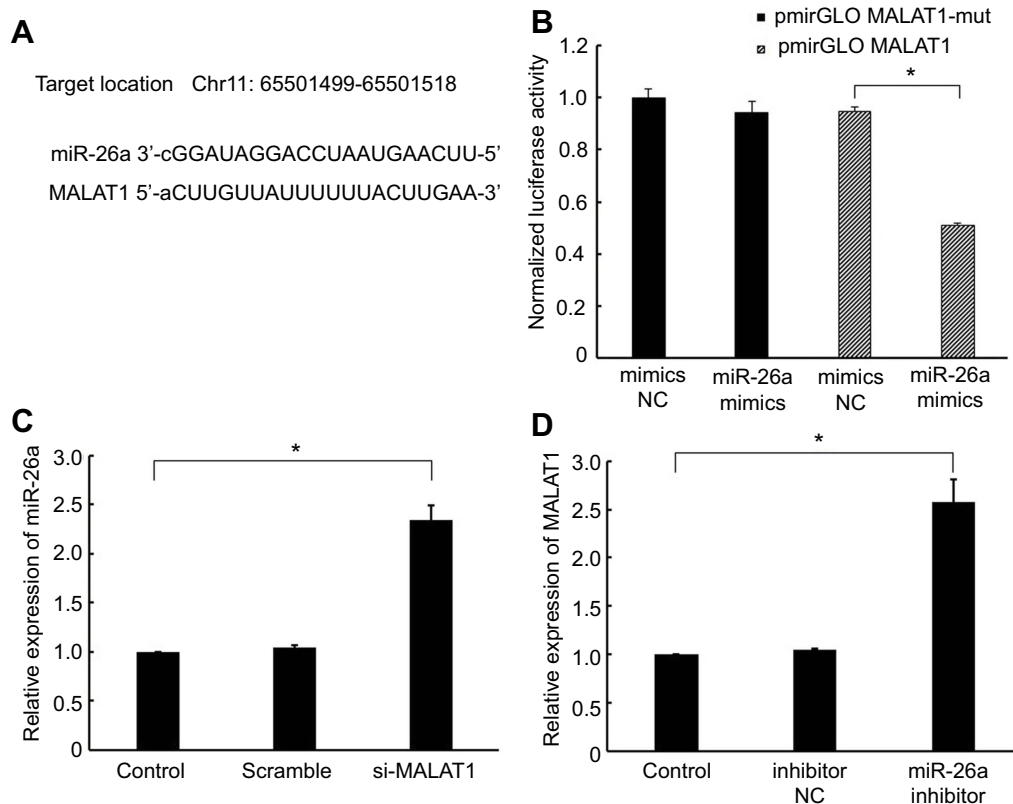


Figure 3 Interaction between MALAT1 and miR-26a. **(A)** miRNA binding sites in the MALAT1 sequence. **(B)** Luciferase reporter gene assay validated the interaction between MALAT1 and miR-26a in AC16 cells. pmirGLO-MALAT1, luciferase vector of MALAT1; pmirGLO-MALAT1-mut, MALAT1 mutant vector. **(C)** Effect of MALAT1 on the level of miR-26a. **(D)** Effect of miR-26a on the level of MALAT1. * $P < 0.05$. Data are presented as the mean \pm standard error from three independent experiments. **Abbreviations:** NC, negative control; MALAT1, metastasis-associated lung adenocarcinoma transcript 1.

miR-26a manipulation may influence PA-induced cell death by regulating the TNF- α -induced apoptosis pathway. As expected, we found that the activation of caspase-8, an essential effector of the TNF- α -induced apoptosis pathway, was downregulated by si-MALAT1, similar to the knockdown of TNF- α (si-TNF- α). The repression of caspase-8 by si-MALAT1 was rescued by an miR-26a inhibitor (Figure 6A). Moreover, the activation of caspase-3 was also regulated by MALAT1/miR-26a manipulation (Figure 6B). These results suggest that the activation of caspase-8/caspase-3 by PA is regulated by MALAT1/miR-26a manipulation. To further determine the effect of MALAT1/miR-26a manipulation on TNF- α -induced apoptosis, we used TNF- α to induce cardiomyocyte apoptosis (Figure 6C). The downregulation of MALAT1 by si-MALAT1 inhibited TNF- α induced apoptosis in cardiomyocytes and the inhibition of apoptosis by si-MALAT1 was rescued by an miR-26a inhibitor (Figure 6D). This result suggests that MALAT1/miR-26a manipulation has a direct regulatory effect on TNF- α -induced apoptosis. Lastly, we found that the downregulation of MALAT1 by

si-MALAT1 increases cell viability and decreases the activity of LDH, consistent with the effects of TNF- α knockdown (si-TNF- α) (Figure 6E and F).

Taken together, these results suggest that MALAT1/miR-26a manipulation may control PA-induced cell death, via the TNF- α -induced apoptosis pathway, at least in part.

Discussion

In our study, we reported the expression and role of MALAT1 on SFA-induced myocardial lipotoxic injury for the first time. MALAT1 is highly conserved among mammals and is ubiquitously expressed in all tissues.²² It is usually induced under various stress conditions, such as ischemia or hypoxia,^{11,12,23} dust exposure,¹³ and hyperglycemia.¹⁶ Thus, we inferred that MALAT1 may be induced in myocardial cells by SFAs. As expected, we found that MALAT1 was upregulated in PA-treated human adult ventricular cardiomyocytes (AC16 cells). We further found that the downregulation of MALAT1 alleviates the extent of PA-induced myocardial injury in vitro. This result is similar to those of Wang et al, who found that the knockdown of MALAT1 expression dramatically suppresses

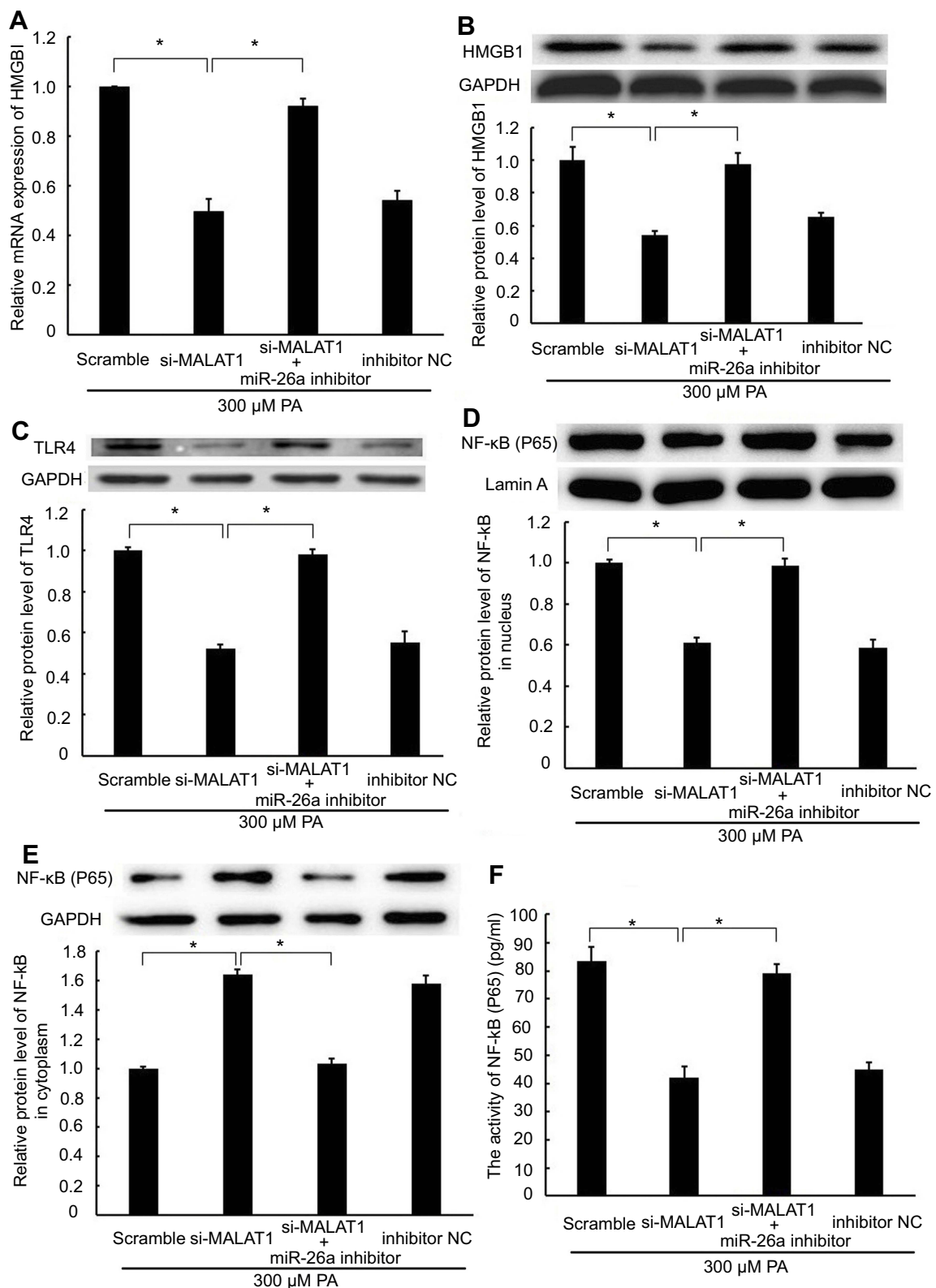


Figure 4 The regulatory effect of MALAT1 on the HMGB1/TLR4/NF- κ B signaling pathway was mediated by miR-26a. MALAT1 negative control (scramble) and MALAT1-specific siRNA (si-MALAT1) alone or in combination with an miR-26a inhibitor or miR-26 negative control (inhibitor NC) was transfected into AC16 cells, followed by palmitic acid treatment. **(A)** The mRNA expression of HMGB1 was detected by RT-qPCR. **(B)** The protein expression of HMGB1 was detected by western blotting. **(C)** The protein expression of TLR4 was detected by western blotting. **(D)** The protein expression of NF- κ B (P65) in the nucleus was detected by western blotting. **(E)** The protein expression of NF- κ B (P65) in the cytoplasm was detected by western blotting. **(F)** The activity of NF- κ B was detected by ELISA. * P <0.05. Data are presented as the mean \pm standard error from three independent experiments.

Abbreviations: NC, negative control; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; HMGB1, high mobility group box 1; TLR4, Toll-like receptor 4.

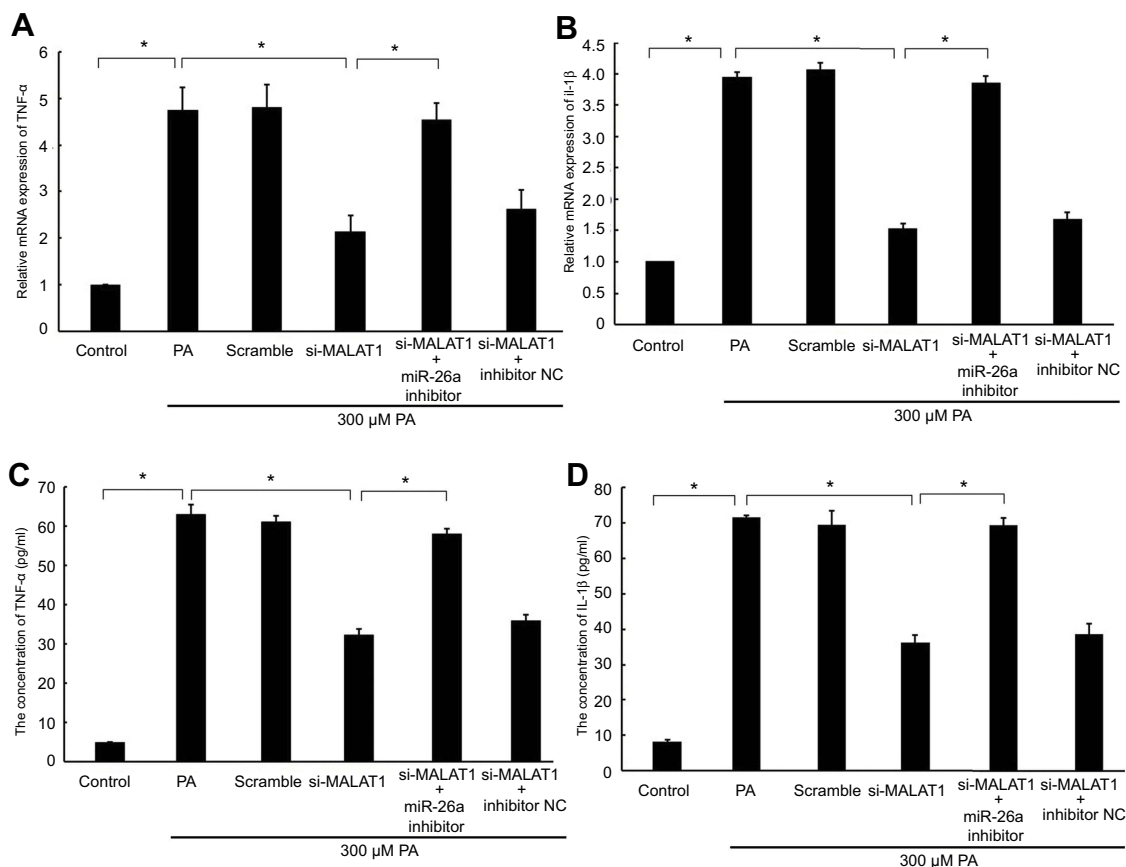


Figure 5 The effect of MALAT1 on PA-induced myocardial inflammation was mediated by miR-26a. MALAT1 negative control (scramble) and MALAT1-specific siRNA (si-MALAT1) alone or in combination with an miR-26a inhibitor or miR-26 negative control (inhibitor NC) and TNF- α -specific siRNA (si-TNF- α) were transfected into AC16 cells, followed by palmitic acid (PA) treatment. **(A)** The mRNA expression of TNF- α was detected by RT-qPCR. **(B)** The mRNA expression of IL-1 β was detected by RT-qPCR. **(C)** The concentration of TNF- α in the culture medium was detected by ELISA. **(D)** The concentration of IL-1 β in the culture medium was detected by ELISA. * $P < 0.05$. Data are presented as the mean \pm standard error from three independent experiments.

Abbreviations: IL-1 β , interleukin β ; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; TNF- α , tumour necrosis factor α ; NC, negative control.

PA-induced lipid accumulation in HepG2 cells.¹⁴ Taken together, MALAT1 is a new putative therapeutic target for SFA-induced myocardial lipotoxic injury.

There is growing evidence that myocardial lipotoxic injury induced by SFAs, especially PA, is involved in the activation of inflammatory and innate immune responses.^{5,6} PA can promote the development of myocardial injury by direct interactions with myeloid differentiation protein 2, a TLR4 accessory protein, thereby resulting in the activation of TLR4/NF- κ B signaling for the regulation of pro-inflammatory molecules.⁵ Several studies have demonstrated that MALAT1 is a modulator of hyperglycemia or the lipopolysaccharide-induced inflammatory response and the overexpression of MALAT1 upregulates the expression of pro-inflammatory molecules, including TNF- α , IL-1 β , and IL-6.^{16,17} In the present study, we demonstrated that the downregulation of MALAT1 inhibits PA-induced

inflammatory responses in myocardial cells by repressing the levels of TNF- α and IL-1 β . Thus, our results provide additional evidence that MALAT1 is a modulator of the inflammatory process and regulates the expression of pro-inflammatory molecules.

Like other lncRNAs, MALAT1 could regulate gene expression by various mechanisms. For instance, MALAT1 regulates alternative splicing by modulating SR splicing factor phosphorylation.²⁴ Moreover, MALAT1 could also bind to several miRNAs, such as miR-144,⁹ miR-125,²⁵ miR-142,²⁶ and miR-129,²⁶ to regulate target gene expression by a ceRNA regulatory mechanism. In the present study, a luciferase reporter assay and RT-qPCR confirmed that MALAT1 specifically binds to miR-26a and there exists a reciprocal negative regulatory relationship between miR-26a and MALAT1. These findings lay a foundation for further analyzes of a potential ceRNA regulatory network.

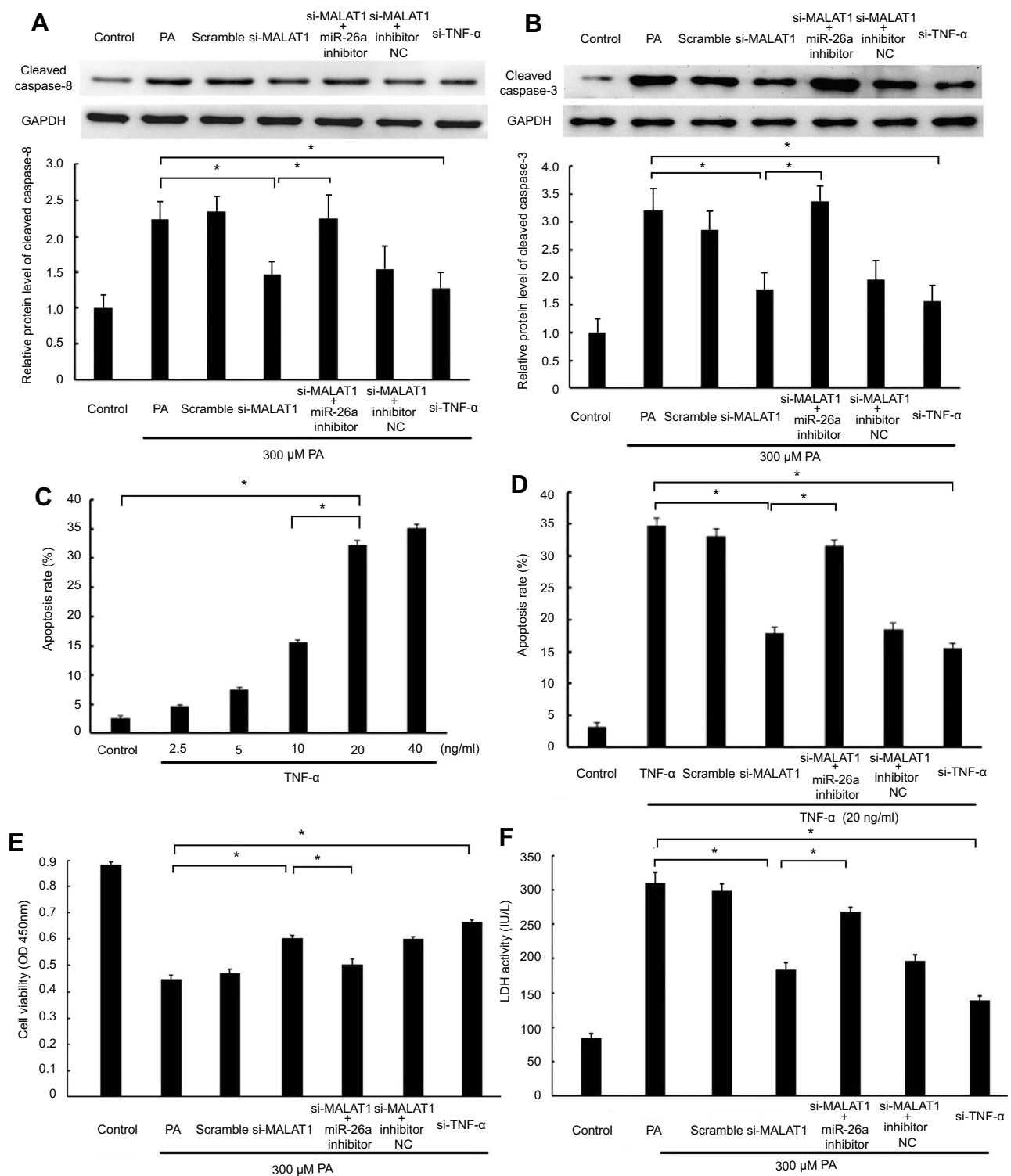


Figure 6 MALAT1/miR-26a manipulation regulated PA-induced cell death via a TNF- α -induced apoptosis pathway. MALAT1 negative control (scramble) and MALAT1-specific siRNA (si-MALAT1) alone or in combination with an miR-26a inhibitor or miR-26 negative control (inhibitor NC) and TNF- α -specific siRNA (si-TNF- α) were transfected in AC16 cells, followed by palmitic acid (PA)/TNF- α treatment. (A) The protein expression of cleaved caspase-8 was detected by western blotting. (B) The protein expression of cleaved caspase-3 was detected by western blotting. (C) AC16 cells were treated with different concentrations of TNF- α for 12 h to induce apoptosis. (D) MALAT1/miR-26a manipulation regulated TNF- α -induced apoptosis. (E) Cell viability was determined by CCK8 assay. (F) Lactate dehydrogenase (LDH) activity in the culture medium was detected by spectrophotometry. * $P < 0.05$. Data are presented as the mean \pm standard error from three independent experiments.

Abbreviations: si-TNF- α , TNF- α -specific siRNA; NC, negative control, MALAT1, metastasis-associated lung adenocarcinoma transcript 1; CCK8, cell counting kit-8.

HMGB1 is a known target gene of miR-26a and miR-26a inhibits HMGB1 expression, resulting in the inhibition of the TLR4/NF- κ B-mediated inflammatory response.¹⁹ Accordingly, we hypothesized that MALAT1 regulates HMGB1 expression by acting as a ceRNA for miR-26a. As expected, we found that the downregulation of MALAT1 inhibited the expression of HMGB1, leading to the repression of the TLR4/NF- κ B signaling pathway. These repressive effects of the downregulation of MALAT1 were mediated by miR-26a. Similarly, Liu et al reported that MALAT1 regulates the expression of HMGB1 via miR-142-3p and miR-129-5p in osteosarcoma cells.²⁶ We consider that MALAT1 regulates HMGB1 expression by miR-26a binding, thereby inhibiting the activation of the TLR4/NF- κ B signaling pathway. Furthermore, we found that the phenotypes of myocardial inflammatory injury by the downregulation of MALAT1 were rescued by an miR-26a inhibitor, which confirmed that miR-26a is required for the suppressive effect of MALAT1 on PA-induced myocardial inflammatory injury. Taken together, these results demonstrated that MALAT1 regulates PA-induced myocardial inflammatory injury via the miR-26a/HMGB1/TLR4/NF- κ B axis.

However, this study had two limitations. First, the role of MALAT1 in PA-induced myocardial injury was only confirmed in a single myocardial cell line. Thus, these results should be verified in more myocardial cell lines and animal models. Second, we did not illuminate how PA induces MALAT1 expression in cardiomyocytes. Previous studies have suggested that various transcription factors, such as specificity protein 1 and hypoxia-inducible factor-2a,^{27,28} could recognize the promoter region of MALAT1 to induce expression. Thus, further studies should evaluate whether PA promotes the interaction between transcription factors and the promoter region of MALAT1.

Conclusion

We demonstrated that MALAT1 is induced in cardiomyocytes by SFAs. The knockdown of MALAT1 alleviates SFA-induced myocardial inflammatory injury via the miR-26a/HMGB1/TLR4/NF- κ B axis. Our findings provide new insight into the mechanism underlying myocardial lipotoxic injury.

Acknowledgments

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Disclosure

The authors report no conflicts of interest in this work.

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