miRNA-323a-3p promoted intracranial, aneurysm-induced inflammation via AMPK/NF-κB signaling pathway by AdipoR1

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Conflict of interest
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Abstract

Background. An intracranial arterial wall which locally protrudes outward, typically in the capsule and fusiform, is called an intracranial aneurysm (IA). Among these aneurysms, 1–2% might spontaneously rupture before treatment. Anterior and posterior communicating aneurysms are more likely to rupture than other aneurysms, and an anterior communicating aneurysm is more likely to rupture than a posterior communicating aneurysm.

Objectives. To identify the effects of miRNA-323a-3p expression in intracranial aneurysms and its potential regulatory mechanism.

Materials and methods. Patients with IA and healthy volunteers were enrolled, and their serum samples were extracted for the detection of tumor necrosis factor alpha (TNF-α), interleukin 1β (IL-1β), IL-6, IL-18, and miRNA-323a-3p. Then, the regulatory effects of miRNA-323a-3p on the above inflammatory factors and AdipoR1/AMPK/NF-kb signaling were also detected in vitro.

Results. The downregulation of miRNA-323a-3p reduced the expression of inflammatory factors (TNF-α, IL-1β, IL-6, and IL-18) in an in vitro model in comparison with the control group. The overexpression of miRNA-323a-3p suppressed the protein expression of adiponectin receptor R1 (AdipoR1) and p-AMPK, and induced NF-κB-p65 protein expression in an in vitro model.

Conclusions. We showed that AdipoR1 plasmid, AMPK activator 1 or si-NF-κB reduced the pro-inflammatory effects of miRNA-323a-3p in an in vitro model. The miRNA-323a-3p exacerbated the inflammatory reaction in IA through AMPK/NF-κB signaling by AdipoR1. Our findings suggest that miRNA-323a-3p targeting AdipoR1 is promising in further anti-inflammatory treatment of IAs.

Key words: intracranial aneurysm, NF-κB, ADIPOR1, AMPK, miRNA-323a-3p

Cite as
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Background

An intracranial arterial wall which locally protrudes outward is called an intracranial aneurysm (IA).[1] The incidence rate of IAs in the general population is approx. 5%.[2] Aneurysms can occur in intracranial blood vessels, with up to 90% of aneurysms occurring near the circle of Willis.[2] The most lethal complication is rupture, subsequently leading to subarachnoid hemorrhage (SAH)[2]; aneurysm rupture is also the most common cause of SAH.[3] Approximately, 1–2% of aneurysms might spontaneously rupture before treatment. The anterior and posterior communicating aneurysms are more likely to rupture than other aneurysms, and the anterior communicating aneurysm is more likely to rupture than the posterior communicating aneurysm.[3]

In the last decade, the development of genetics and molecular biology has promoted the development of aneurysm-related research.[4] In this paper, we summarize the molecular biological mechanisms of IA rupture and its association with inflammation.[4] Inflammation is closely correlated with aneurysm formation. The pathogenesis may be a damage in the vascular endothelium of intracranial arteries, leading to an inflammatory response, which ultimately endangers the integrity of the vessel wall.[5] During this process, the key characteristics include the loss of the internal elastic layer, endomysial hyperplasia and ectopic distribution of the vascular smooth muscle.[6]

Adiponectin is a cytokine secreted by mature adipocytes and its biological function is facilitated by its receptors, adiponectin receptor R1 (AdipoR1) and AdipoR2.[7] The adiponectin receptors are mainly expressed in insulin target tissues, including liver, skeletal muscle and pancreatic islet.[8] Studies have found the expression of both AdipoR1 and AdipoR2 in islet cells, with AdipoR1 being dominant.[8] The AdipoR1 acts by activating the AMPK pathway.[7] The NF-κB has been revealed to regulate the expression of various genes during the initiation and progression of AMPK. For example, lipids in food – especially free fatty acids – can activate the transcription factor NF-κB and increase the expression of inflammatory cytokines (including tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), C-reactive protein (CRP), and others), thereby causing inflammatory damage to liver tissue.[9]

To simplify, the activation of NF-κB is a hallmark of inflammation in IA, which can be detected in the infiltrating macrophages and endothelial cells of IA lesions in mouse models in the early stages, subsequently spreading throughout the arterial wall.[10] A specific inhibition of the activation of NF-κB in macrophages can suppress aneurysm formation, macrophage infiltration and expression of pro-inflammatory factors.[10] Upregulated genes in the intima and media of the aneurysm involve proteases, reactive oxygen species (ROS), growth factors, cytokines, complements, adhesion molecules, and pro-apoptotic proteins.[11] Downregulated genes play different roles in the vascular smooth muscle cells and endothelial cells.[12] Thus, the occurrence and development of aneurysms is associated with inflammatory responses, changes in extracellular matrices and apoptosis.[12]

Objectives

The purpose of this work is to identify the effects of miRNA-323a-3p in intracranial aneurysms and their potential regulatory mechanism.

Materials and methods

Patients with intracranial aneurysm

The study was approved by the ethics committee of the First Affiliated Hospital of Soochow University, Suzhou, China. Patients with IA and healthy volunteers were enrolled from November to December 2016. The basic information about the patients with IA is presented in Table 1. The following conditions were reasons for exclusion: severe hepatic and renal dysfunction, heart disease, diabetes, known thyrotoxicosis, and a history of hypersensitivity to iodinated contrast agents. Other exclusion criteria included multiple endovascular atheromatous plaques, obviously tortuous neck vessels, and vascular system hypertension (systolic blood pressure (SBP) ≥180 mm Hg or diastolic blood pressure (DBP) ≥110 mm Hg). Detailed inclusion and exclusion criteria are listed in the study protocol.

We chose to restrict the aneurysm size because the largest 2nd-generation hydrogel coil available when the trial started measured 12 mm.

Table 1. Basic information about patients with intracranial aneurysm

Variable	Healthy volunteers	Patients
Number of subjects	12	12
Sex	M: 6/W: 6	M: 6/W: 6
Age [years]	61–69	62–73
WFNS grading		
I	0	1
II–III	0	5
IV–V	0	6

WFNS – World Federation of Neurosurgical Societies.

ELISA experiment

The serum samples were collected and measured using an enzyme-linked immunosorbent assay (ELISA) kit for TNF-α, IL-1β, IL-6, and IL-18 (Thermo Fisher Scientific, Waltham, USA). Cells were also collected at 1000 g and

4°C for 10 min and used to measure TNF-α (H052), IL-1β (H002), IL-6 (H007), and IL-18 (H015) with an ELISA kit. The absorbance was measured at 450 nm using a Multiskan™ GO spectrophotometer (Thermo Fisher Scientific, Waltham, USA).

qRT-PCR assay

Total RNA was extracted with TRIzol (Sigma-Aldrich, St. Louis, USA), according to the manufacturer's protocol. Then, 1 μg of RNA was reverse-transcribed into cDNA using a reverse reaction kit (Promega, Madison, USA). Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) was performed using SYBR Green (Roche Diagnostics, Basel, Switzerland) and a 7500 real-time PCR system (Applied Biosystems, Mannheim, Germany). The levels of relative expression were calculated and quantified with the $2^{-\Delta\Delta Ct}$ method.

Gene chip profiling

The total RNA was labeled using a miRCURY™ Hy3™/Hy5™ Power labeling kit and hybridized on a miRCURY™ LNA Array (v. 16.0; Exiqon, Vedbæk, Denmark). Scanned images were imported into GenePix Pro v. 6.0 software (Axon Instruments, Foster City, USA) and MEV v. 4.6 software (The Institute for Genomic Research (TIGR), Rockville, USA).

Cell culture and transfection of miR-323a-3p mimics

Human endothelial cells – EA.hy926 (ATCC® CRL-2922™; Fuxiang Biotechnology, Shanghai, China) – were cultured in Dulbecco's modified Eagle's medium (DMEM; Thermo Fisher Scientific) with 10% fetal bovine serum (FBS; Thermo Fisher Scientific), and penicillin (100 U/mL), streptomycin (100 μg/mL) and a 2-mM glutamine mixture at 37°C in a humid, 5% CO_2 atmosphere. The EA.hy926 cells were transfected with miR-323a-3p, anti-miR-323a-3p or negative mimics using Lipofectamine 2000 (Invitrogen, Carlsbad, USA). The EA.hy926 cells were induced with 200 ng of LPS13 into the IA model 48 h after transfection.

Luciferase assay

The 3′UTR of AdipoR1 was cloned into a luciferase reporter vector, pmirGLO (Dual-Luciferase® Reporter Assay System; Promega). The 3′UTR of AdipoR1 and anti-miR-323a-3p mimics were transfected into the cells using Lipofectamine 2000 (Invitrogen). Forty-eight hours after transfection, the luciferase activity in human umbilical vein endothelial cells (HUVECs) was studied with a VICTOR analyzer using a Dual-Glo® Luciferase Assay System (Promega).

Western blotting

The cells were collected and the total proteins were extracted using an radioimmunoprecipitation assay (RIPA). Protein concentration was determined using a bicinchoninic acid protein assay. Equal amounts of protein (50 μg) were resolved on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels, and then transferred to a polyvinylidene difluoride (PVDF) membrane (Merck Millipore, Bedford, USA). The membrane was blocked with 5% skim milk in Tris-buffered saline with Tween (TBST) and probed with antibodies against AdipoR1, p-AMPK, NF-κB-p65, and GAPDH (Cell Signaling Technology, Danvers, USA) at 4°C overnight. After 3 washes with TBST, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (Cell Signaling Technology) for 1 h at 37°C. Immunoreactive bands were visualized using an enhanced chemiluminescence (ECL) detection system (Promega) and analyzed using Image-Pro Plus v. 6.0 software (Media Cybernetics, Inc., Rockville, USA).

Immunohistochemistry

The cells were washed with phosphate-buffered saline (PBS) and fixed with 4% paraformaldehyde for 15 min. The cells were blocked with 5% bovine serum albumin (BSA) and 0.25% Triton-x100 in PBS for 1 h. Then, they were incubated with AdipoR1 overnight at 4°C, followed by incubation with fluorescence-labeled secondary antibodies (Alexa Fluor 594; Cell Signaling Technology) for 2 h. The cells were incubated with 4′,6-diamidino-2-phenylindole (DAPI; Cell Signaling Technology) for 15 min and viewed under a confocal fluorescence microscope (model FV1000; Olympus Corp., Tokyo, Japan).

Statistical analyses

The data are presented as median with the range (when n = 3) or the interquartile range (IQR) (when n = 12). The data were analyzed using Kruskal–Wallis test. A value of $p < 0.05$ was considered statistically significant.

Results

miRNA-323a-3p expression in patients with intracranial aneurysm

To investigate the mechanism of IA, the miRNA expression of patients with IA was measured. There were higher levels of TNF-α (median: 13.08 (IQR: 11.55–14.38) pg/mg of protein compared to median: 113.23 (IQR: 103.7–120.53) pg/mg of protein), IL-1β (median: 8.07 (IQR: 6.33–9.25) pg/mg of protein compared to median: 43.67 (IQR: 42.2–47.45) pg/mg of protein), IL-6 (median:

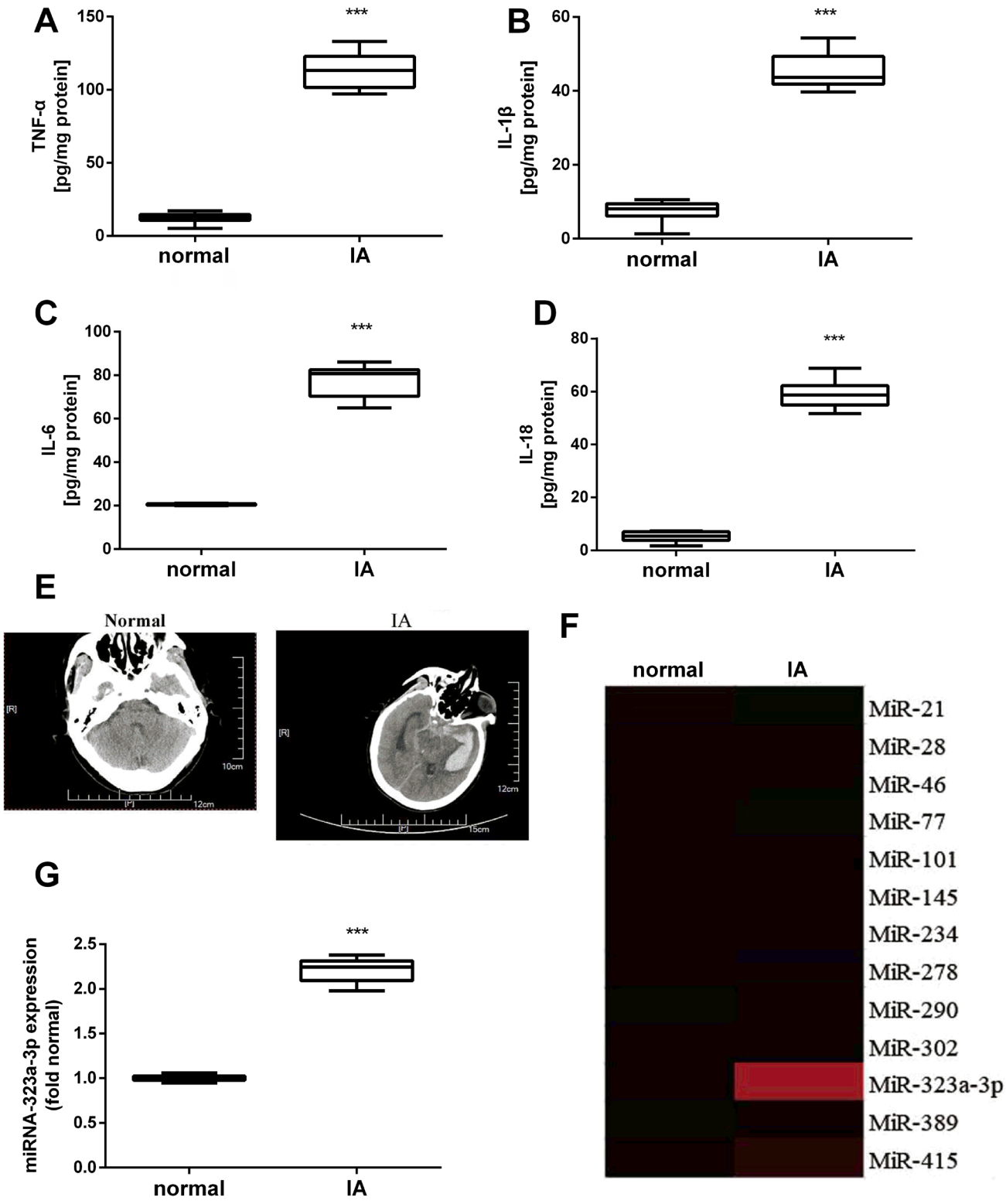

Fig. 1. The miRNA-323a-3p expression in patients with intracranial aneurysm (IA) tumor necrosis factor alpha (TNF-α) (A), interleukin (IL)-1β (B), IL-6 (C), and IL-18 (D) levels; computed tomography (CT) scan of an IA (E); the gene chip (F) and quantitative reverse-transcription polymerase chain reaction (qRT-PCR) (G) for the expression of miRNA-323a-3p in a patient with IA

normal – healthy volunteers; IA – IA patients; ***$p < 0.001$ compared to the sham control group. The Mann–Whitney tests were used for analysis. A–D,G: $U = 0$; $p < 0.001$. Data are presented as median and interquartile range (IQR) ($n = 12$).

20.6 (IQR: 20.16–20.83) pg/mg of protein compared to median: 80.75 (IQR: 72.75–82.3) pg/mg of protein), and IL-18 (median: 5.41 (IQR: 3.88–6.67) pg/mg of protein compared to median: 58.7 (IQR: 55.64–61.57) pg/mg of protein) in the patients with IA than in the control group (Fig. 1A–D). The results of computed tomography

(CT) showed that IA has appeared in patient with IA, but has not appeared in normal group (Fig. 1E). As shown in Fig. 1F,G, the expression of miRNA-323a-3p (median: 1 (IQR: 0.98–1.02) compared to median: 2.25 (IQR: 2.12–2.30)) was higher in the patients with IA than in the control group.

miRNA-323a-3p regulates inflammation in an in vitro model

The study evaluated the effects of miRNA-323a-3p in an in vitro model of IA. We used miRNA-323a-3p mimics to increase the expression of miRNA-323a-3p (median: 1.00 (range: 0.97–1.03) compared to median: 3.33 (range: 3.07–3.53)) in an in vitro model of IA in comparison with the control group (Fig. 2A). The overexpression of miRNA-323a-3p promoted inflammation factors such as TNF-α (median: 76.07 (range: 74.82–84.40) pg/mg of protein compared to median: 279.29 (range: 270.93–330.06) pg/mg of protein), IL-1β (median: 49.89 (range: 47.88–53.43) pg/mg of protein compared to median: 124.32 (range: 117.77–130.61) pg/mg of protein), IL-6 (median 59.75 (range: 59.04–59.78) pg/mg of protein compared to median 102.47 (range: 98.2–104.48) pg/mg of protein), and IL-18 (median: 20.87 (range: 15.66–29.57) pg/mg of protein compared to median: 117.50 (range: 109.98–129.71) pg/mg of protein) in an in vitro model of IA, in comparison with the control group (Fig. 2B–E). However, anti-miRNA-323a-3p mimics showed reduced miRNA-323a-3p expression (median: 1.00 (range: 0.97–1.03) compared to median: 0.41 (range: 0.38–0.44)) in an in vitro model of IA, in comparison with the control group (Fig. 2F). The downregulation of miRNA-323a-3p reduced the levels of TNF-α (median: 78.10 (range: 74.97–79.22) pg/mg of protein compared to median: 21.03 (range: 19.66–23.59) pg/mg of protein), IL-1β (median: 34.22 (range: 31.69–37.29) pg/mg of protein compared to median: 16.62 (range: 14.66–17.42) pg/mg of protein), IL-6 (median: 41.57 (range: 41.10–41.90) pg/mg of protein compared to median: 12.04 (range: 9.69–13.43) pg/mg of protein), and IL-18 (median: 64.91 (range: 64.56–71.74) pg/mg of protein compared to median: 13.03 (range: 8.78–14.37) pg/mg of protein) in an in vitro model of IA, in comparison with the control group (Fig. 2G–J).

miRNA-323a-3p regulates AdipoR1/AMPK/NF-κB-p65 signaling in an in vitro model

To explore the mechanism of miRNA-323a-3p on signaling in IA, a gene chip was used in an in vitro model of IA. As shown in Fig. 3A, the downregulation of miRNA-323a-3p induced AdipoR1 and NF-κB-p65 protein expression in an in vitro model of IA, in comparison with the control group. The 3'UTR of AdipoR1 was targeted by miRNA-323a-3p, and luciferase activity levels (median: 0.99 (range: 0.97–1.04) compared to median: 1.89 (range: 1.86–2.01)) were increased in the downregulation of the miRNA-323a-3p group (Fig. 3B,C). The downregulation of miRNA-323a-3p induced AdipoR1 protein expression in an in vitro model of IA, in comparison with the control group (Fig. 3D). The overexpression of miRNA-323a-3p suppressed AdipoR1 expression (median: 0.97 (range: 0.94–1.09) compared to median: 0.31 (range: 0.23–0.39)) and p-AMPK (median: 1.00 (range: 0.96–1.04) compared to median: 0.36 (range: 0.33–0.39)) protein expression and induced NF-κB-p65 (median: 1.00 (range: 0.98–1.02) compared to median: 2.41 (range: 2.39–2.52)) protein expression in an in vitro model of IA, in comparison with the control group (Fig. 3E–H). The downregulation of miRNA-323a-3p induced AdipoR1 (median: 1.02 (range: 0.78–1.20) compared to median: 2.95 (range: 2.68–3.07)) and p-AMPK (median: 1.05 (range: 0.81–1.14) compared to median: 2.43 (range: 2.34–2.52)) protein expression and suppressed NF-κB-p65 (median: 0.97 (range: 0.88–1.15) compared to median: 0.31 (range: 0.27–0.44)) protein expression in an in vitro model of IA, in comparison with the control group (Fig. 3I–L).

AdipoR1 reduced the pro-inflammation effects of miRNA-323a-3p in an in vitro model

The role of AdipoR1 in the pro-inflammatory function of miRNA-323a-3p was studied in an in vitro model of IA. The AdipoR1 plasmid induced the expression of AdipoR1 (median: 1.04 (range: 0.92–1.04) compared to median: 0.47 (range: 0.46–0.52) compared to median: 0.73 (range: 0.61–0.78)) and p-AMPK (median: 0.99 (range: 0.94–1.08) compared to median: 0.39 (range: 0.36–0.42) compared to median: 0.83 (range: 0.82–0.84)) and suppressed NF-κB-p65 protein expression (median: 1.02 (range: 0.89–1.08) compared to median: 2.47 (range: 2.46–2.62) compared to median: 1.47 (range: 1.32–1.49)) in an in vitro model of IA following overexpression of miRNA-323a-3p, when compared with the overexpression in the miRNA-323a-3p group (Fig. 4A–D). Compared with the overexpression in the miRNA-323a-3p group, the activation of AdipoR1 reduced the pro-inflammatory function of miRNA-323a-3p on the levels of TNF-α (median: 74.88 (range: 71.03–79.08) pg/mg of protein compared to median: 191.81 (range: 187.82–205.36) pg/mg of protein compared to median: 97.33 (range: 91.38–105.70) pg/mg of protein), IL-1β (median: 42.31 (range: 36.85–45.10) pg/mg of protein compared to median: 161.72 (range: 159.50–169.05) pg/mg of protein compared to median: 74.57 (range: 72.72–84.11) pg/mg of protein), IL-6 (median: 53.50 (range: 53.30–55.20) pg/mg of protein compared to median: 187.84 (range: 184.51–188.66) pg/mg of protein compared to median: 65.43 (range: 63.74–65.84) pg/mg of protein), and IL-18 (median: 31.80 (range: 23.02–36.39) pg/mg of protein compared to median: 125.03 (range: 120.93–129.76) pg/mg of protein compared to median: 52.42 (range: 47.13–60.08) pg/mg of protein)

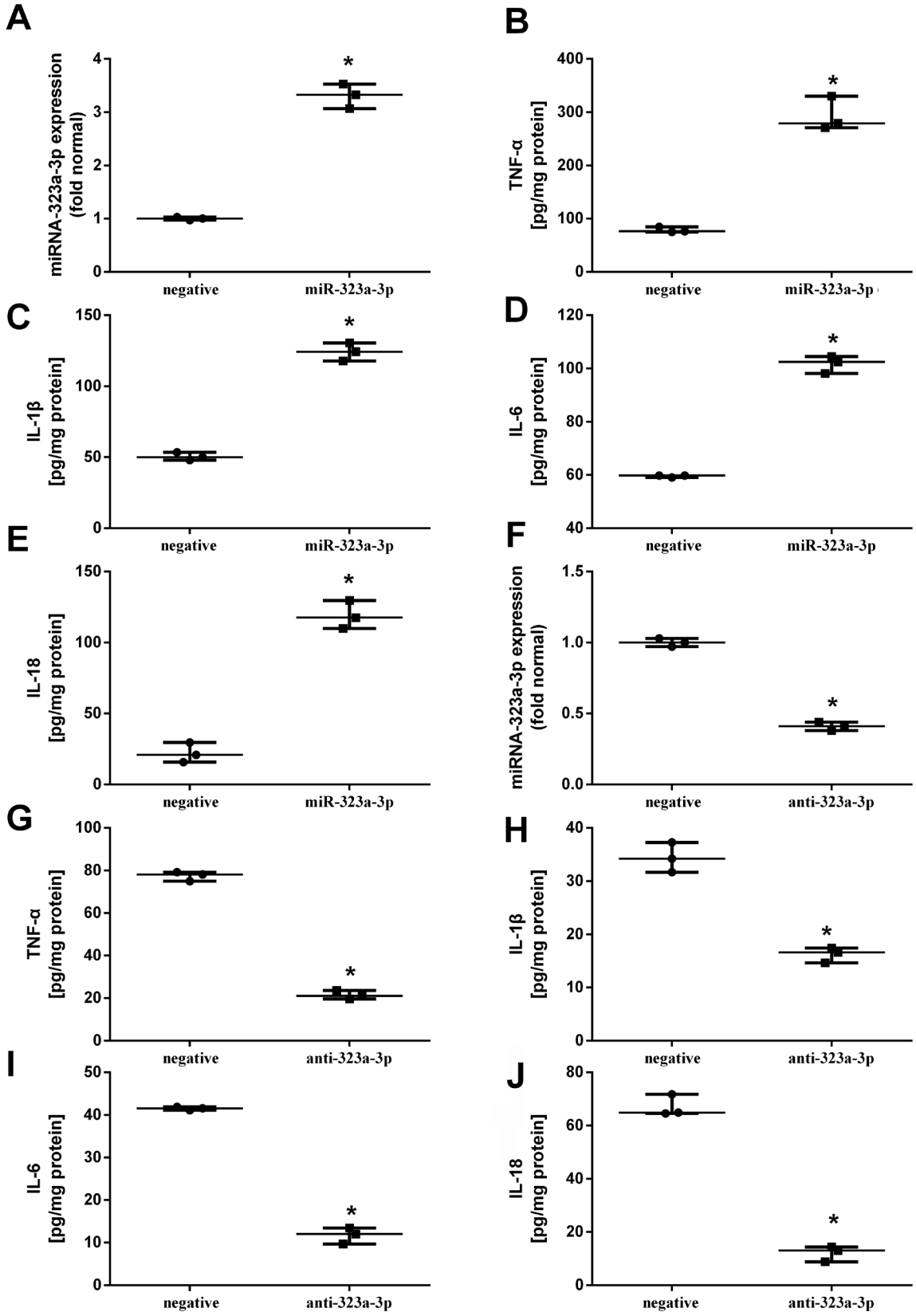

Fig. 2. The miRNA-323a-3p regulates inflammation in an in vitro model. Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) for the expression levels of miRNA-323a-3p (A), tumor necrosis factor alpha (TNF-α) (B), interleukin (IL)-1β (C), IL-6 (D), and IL-18 (E) following the overexpression of miRNA-323a-3p; qRT-PCR for the expression levels of miRNA-323a-3p (F), TNF-α (G), IL-1β (H), IL-6 (I), and IL-18 (J) following the downregulation of miRNA-323a-3p

negative – cells transfected with negative mimics; miR-323a-3p – cells transfected with miR-323a-3p mimics; anti-323a-3p – cells transfected with miR-323a-3p inhibitor; *$p < 0.05$. The Kruskal–Wallis tests were used for analysis. A–J: $\chi^2 = 3.86$; $p = 0.049$. Data are presented as median with range (n = 3).

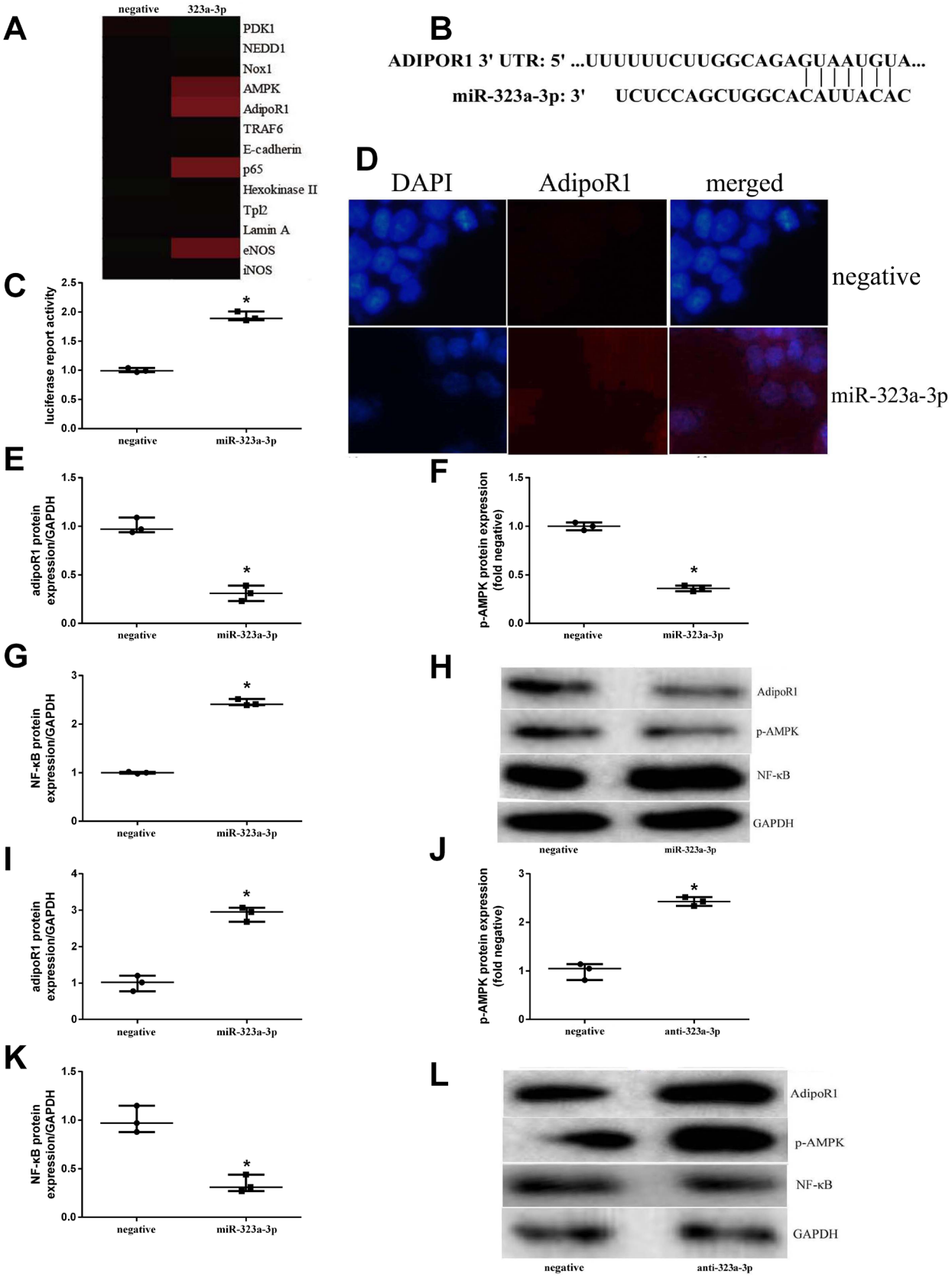

Fig. 3. The miRNA-323a-3p regulates AdipoR1/AMPK/NF-κB-p65 signaling in an in vitro model. A. The gene chip for signaling; B. The 3'UTRs of AdipoR1 was targeted by miRNA-323a-3p; C. Luciferase activity levels; D. Immunohistochemistry for AdipoR1; E–G. AdipoR1, p-AMPK and NF-κB-p65 protein expression using western blot analysis; H. Statistical analysis following the overexpression of miRNA-323a-3p; I–K. AdipoR1, p-AMPK and NF-κB-p65 protein expression using western blot analysis; L. Statistical analysis following the downregulation of miRNA-323a-3p

negative – cells transfected with negative mimics; miR-323a-3p – cells transfected with miR-323a-3p mimics; anti-323a-3p – cells transfected with miR-323a-3p inhibitor. *$p < 0.05$. The Mann–Whitney tests were used for analysis. C, E–G, I–K: $U = 0$; $p = 0.049$. Data are presented as median with range ($n = 3$).

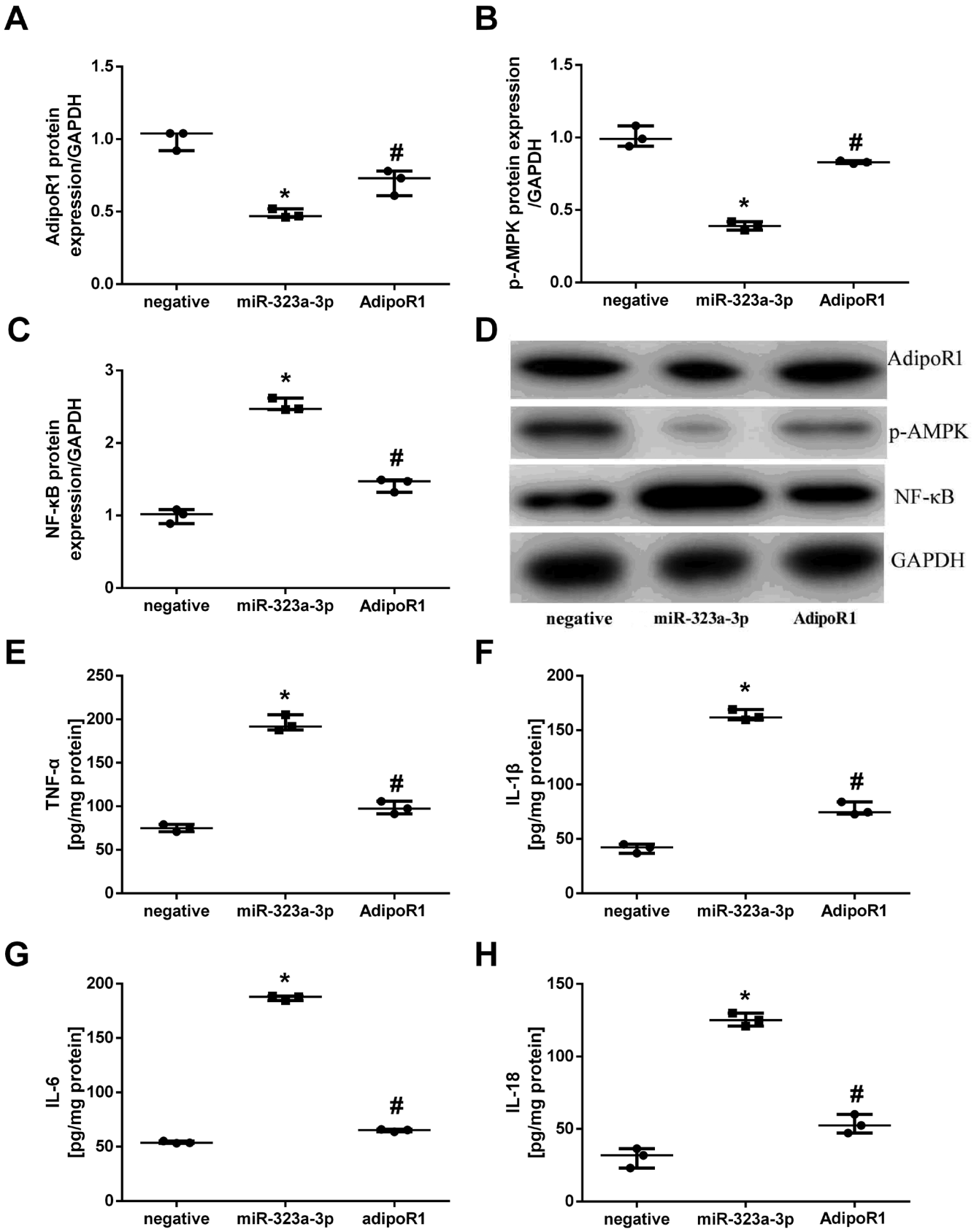

Fig. 4. AdipoR1 reduced the pro-inflammatory effects of miRNA-323a-3p in an in vitro model. AdipoR1, p-AMPK and NF-κB-p65 protein expression using western blot analysis (A, B and C) and statistical analysis (D); tumor necrosis factor alpha (TNF-α) (E), interleukin (IL)-1β (F), IL-6 (G), and IL-18 (H)

negative – cells transfected with negative mimics; miR-323a-3p – cells transfected with miR-323a-3p mimics; AdipoR1 – cells transfected with miR-323a-3p mimics and AdipoR1. The Kruskal–Wallis tests were used for 2 group and multiple group analyses. Multiple group comparisons: A–C, E–H: $\chi^2 = 7.20$; $p = 0.030$. Two group comparisons: *$p < 0.05$; #$p < 0.05$. Data are presented as median with range (n = 3).

in an in vitro model of IA following the overexpression of miRNA-323a-3p, when compared with the overexpression of the miRNA-323a-3p group (Fig. 4E–H).

AMPK reduced the pro-inflammatory effects of miRNA-323a-3p in an in vitro model

The role of AMPK in the pro-inflammatory effects of miRNA-323a-3p was studied in an in vitro model. The AMPK plasmid promoted the protein expression of p-AMPK (median: 1.02 (range: 0.93–1.06) compared to median: 0.21 (range: 0.19–0.24) compared to median: 0.58 (range: 0.44–0.62)), and suppressed NF-κB-p65 expression (median: 0.98 (range: 0.97–1.04) compared to median: 2.95 (range: 2.67– 3.04) compared to median: 1.47 (range: 1.47–1.68)) in an in vitro model of IA following the overexpression of miRNA-323a-3p, when compared with the overexpression of the miRNA-323a-3p group (Fig. 5A–C). The promotion of AMPK reduced the pro-inflammatory effects of miRNA-323a-3p on the levels of TNF-α (median: 74.11 (range: 71.14–82.75) pg/mg of protein compared to median: 193.87 (range: 190.52–209.61) pg/mg of protein compared to median: 90.87 (range: 83.09–99.47) pg/mg of protein), IL-1β (median: 39.30 (range: 36.89–45.07) pg/mg of protein compared to median: 170.35 (range: 163.51–174.39) pg/mg of protein compared to median: 67.60 (range: 66.48–79.31) pg/mg of protein), IL-6 (median: 28.79 (range: 27.36–38.05) pg/mg of protein compared to median: 137.17 (range: 129.12–139.43) pg/mg of protein compared to median: 56.52 (range: 47.13–61.98) pg/mg of protein), and IL-18 (median: 56.64 (range: 54.57–56.79) pg/mg of protein compared to median: 190.36 (range: 186.11–190.64) pg/mg of protein compared to median: 69.50 (range: 67.62–69.88) pg/mg of protein) in an in vitro model, in comparison with the overexpression in the miRNA-323a-3p group (Fig. 5D–G).

Si-NF-κB reduced the pro-inflammatory effects of miRNA-323a-3p in an in vitro model

The role of NF-κB in the pro-inflammatory effects of miRNA-323a-3p was explored in an in vitro model. The si-NF-κB suppressed NF-κB-p65 protein expression (median: 1.07 (range: 0.96–1.07) compared to median: 2.11 (range: 2.03–2.20) compared to median: 1.25 (range: 1.13–1.30)) in an in vitro model of IA following the overexpression of miRNA-323a-3p, when compared with the overexpression of the miRNA-323a-3p group (Fig. 6A,B). The inhibition of NF-κB reduced the pro-inflammatory effects of miRNA-323a-3p on the levels of TNF-α (median: 51.13 (range: 44.35–51.53) pg/mg of protein compared to median: 218.88 (range: 204.50–221.61) pg/mg of protein compared to median: 92.71 (range: 80.86–93.84) pg/mg of protein), IL-1β (median: 31.55 (range: 25.78–33.94) pg/mg of protein compared to median: 200.69 (range: 169.76–209.82) pg/mg of protein compared to median: 77.27 (range: 70.95–83.16) pg/mg of protein), IL-6 (median: 21.57 (range: 19.80–21.63) pg/mg of protein compared to median: 125.91 (range: 124.48–130.60) pg/mg of protein compared to median: 46.34 (range: 41.45–47.21) pg/mg of protein), and IL-18 (median: 38.98 (range: 38.00–50.23) pg/mg of protein compared to median: 163.27 (range: 162.15–170.30) pg/mg of protein compared to median: 71.35 (range: 61.90–74.39) pg/mg of protein) in an in vitro model, when compared with the overexpression in the miRNA-323a-3p group (Fig. 6C–F).

Discussion

Intracranial aneurysms are a common cerebrovascular disease, though their pathogenesis is unclear.[2,14] It is currently believed that the mechanisms of IA include endothelial injury, inflammatory response, dysregulation of the phenotype in vascular smooth muscle, extracellular matrix remodeling, apoptosis, and breakdown of the vessel wall.[14] In the current study, we demonstrated that the expression of miRNA-323a-3p was higher in patients with IA. The overexpression of miRNA-323a-3p promoted an increase of TNF-α, IL-1β, IL-6, and IL-18 levels in an in vitro model of IA.

The formation of IA is closely related to immune inflammation, though its origin and mechanism are still not fully understood.[15] The NF-κB is a major regulator of inflammatory cytokines.[16] Relevant studies have shown that NF-κB signaling transduction plays an important role in endothelial cell injury-mediated vascular diseases, and AMPK could regulate NF-κB signaling transduction.[15,16] In this study, we observed that the overexpression of miRNA-323a-3p suppressed the expression of AdipoR1 and p-AMPK and induced NF-κB-p65 protein expression in an in vitro model of IA. The si-NF-κB reduced the pro-inflammatory effects of miRNA-323a-3p in an in vitro model.

The AMPK is a key regulator of energy metabolism in organisms, and can be activated by various factors, including stress, exercise, hormones, and substances which affect cell metabolism.[17] Energy metabolism is also closely associated with immune regulation.[7] In the case of an insufficient supply of nutrients and a lack of energy, the immune function of the body is significantly worsened.[17] As a "cell energy-regulating receptor", when signaling transduction is impaired, AMPK could cause metabolic syndromes such as obesity, diabetes, insulin resistance, etc., which are often accompanied by chronic inflammation (including sepsis, rheumatoid arthritis and cardiovascular disease), seriously endangering human health.[18] In this study, we observed that the activation of AMPK reduced the pro-inflammatory effects of miRNA-323a-3p in an in vitro model.

When AdipoR2 binds to AdipoR2, it activates the downstream AMPK signaling pathway mediated by APPL1 and

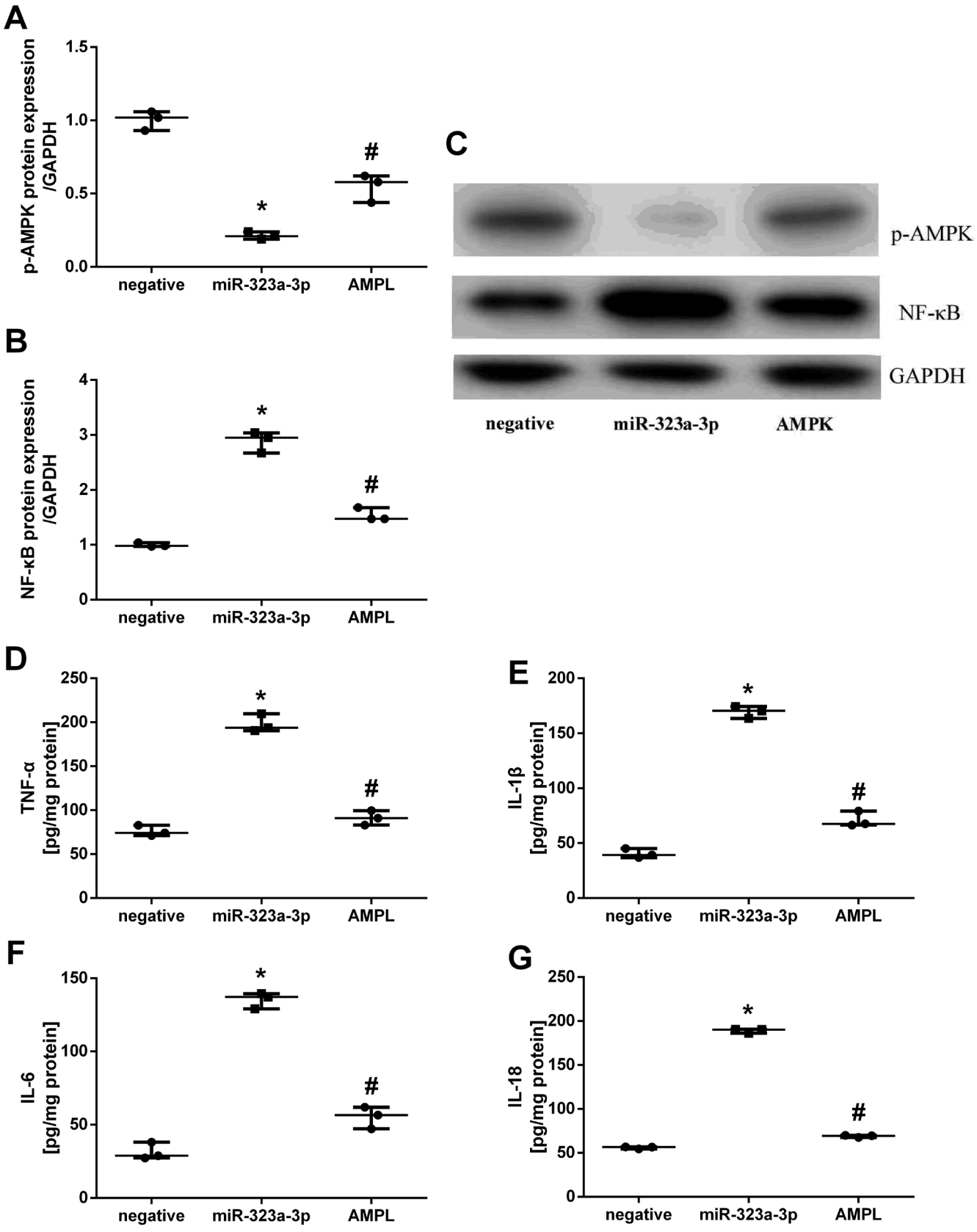

Fig. 5. AMPK reduced the pro-inflammatory effects of miRNA-323a-3p in an in vitro model. The expression of p-AMPK and NF-κB-p65 using western blot analysis (A and B) and statistical analysis (C); tumor necrosis factor alpha (TNF-α) (D), interleukin (IL)-1β (E), IL-6 (F), and IL-18 (G)

negative – cells transfected with negative mimics; miR-323a-3p – cells transfected with miR-323a-3p mimics; AMPK – cells transfected with miRNA-323a-3p mimic and AMPK. The Kruskal–Wallis tests were used for 2 group and multiple group analyses. Multiple group comparisons: A, B, D–G: $\chi^2 = 7.20$; $p = 0.030$. Two group comparisons: $^*p < 0.05$; $^\#p < 0.05$. Data are presented as median with range (n = 3).

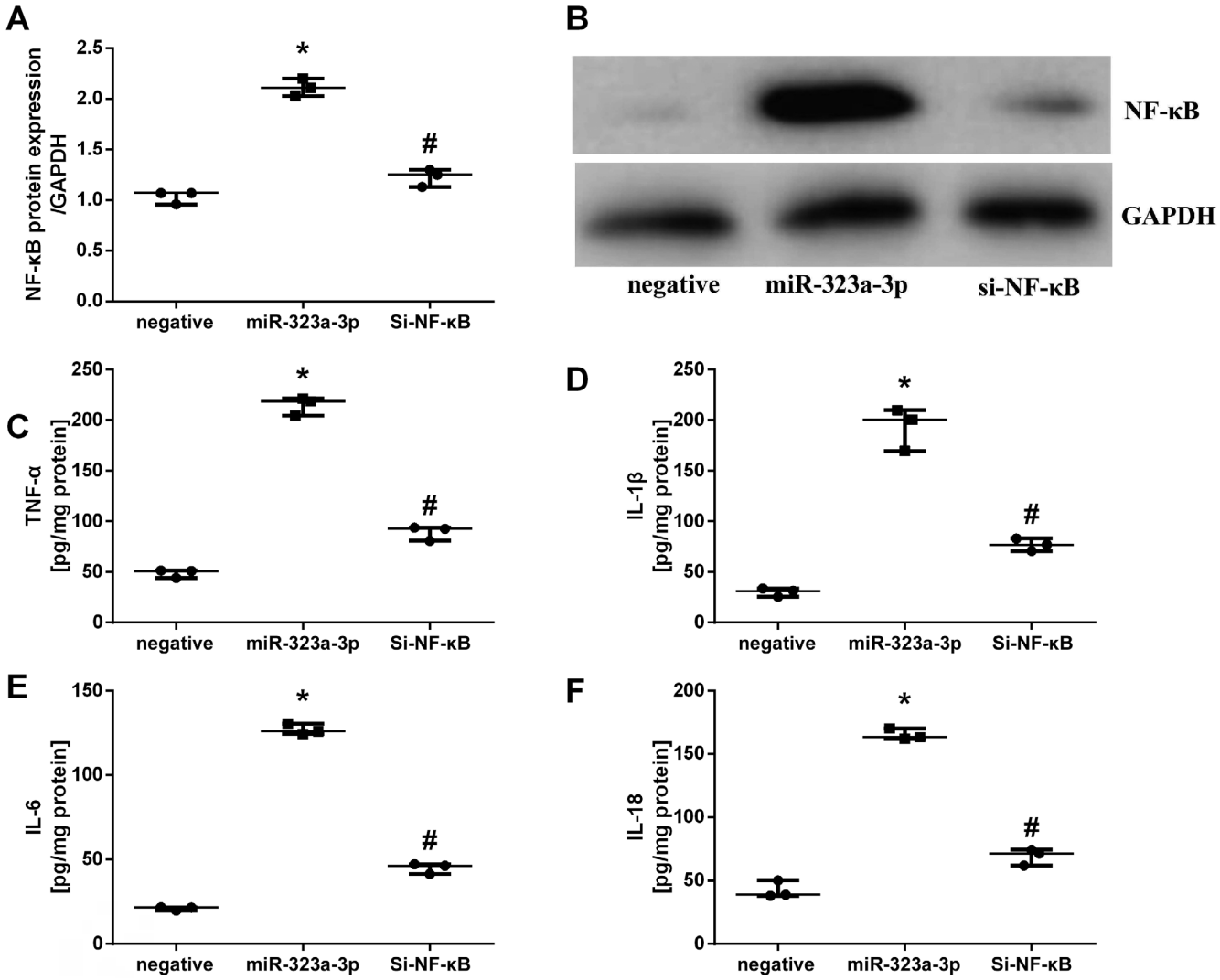

Fig. 6. Si-NF-κB reduced the pro-inflammatory effects of miRNA-323a-3p in an in vitro model. The NF-κB-p65 protein expression using western blot analysis (A) and statistical analysis (B); tumor necrosis factor alpha (TNF-α) (C), interleukin (IL)-1β (D), IL-6 (E), and IL-18 (F)

negative – cells transfected with negative mimics; miR-323a-3p – cells transfected with miR-323a-3p mimics; si-NF-κB – cells transfected with miRNA-323a-3p mimic and NF-κB antagonist. The Kruskal–Wallis tests were used for 2 group and multiple group analyses. Multiple group comparisons: A–H: $\chi^2 = 7.20$; $p = 0.030$. Two group comparisons: $^*p < 0.05$; $^\#p < 0.05$. Data are presented as median with range (n = 3).

participates in glycolipid metabolism; such process can reduce body fat and improve hepatic insulin sensitivity and liver steatosis.[19] Meanwhile, adiponectin decreases the secretion of inflammatory factors such as IL-6 and TNF-α by inhibiting Kupffer cells from activating hepatic stellate cells and mediating the IKK-b/NF-κB pathway by attenuating the nuclear translocation of NF-κB; such action further suppresses the inflammatory response and antagonizes the progression of NAFLD.[20] We found that the activation of AdipoR1 or AMPK reduced the pro-inflammatory effects of miRNA-323a-3p in an in vitro model.

Limitations

This study sill has several limitations. First, due to the small sample size, a stratified analysis of gene expression based on patients' World Federation of Neurosurgical Societies (WFNS) scores was not performed. Second, due to ethical requirements, we were unable to detect the expression of miRNAs and inflammatory factors in tissue samples from aneurysms.

Conclusions

In summary, our study demonstrated that the expression of miRNA-323a-3p was higher in patients with IA. The overexpression of miRNA-323a-3p raised TNF-α, IL-1β, IL-6, and IL-18 levels in an in vitro model of IA via AdipoR1/AMPK/NF-κB. Our findings suggest that miRNA-323a-3p targeting AdipoR1 is promising in further anti-inflammation treatment of IA.

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