CircRNA ACAP2 is Overexpressed in Myocardial Infarction and Promotes the Maturation of miR-532 to Induce the Apoptosis of Cardiomyocyte

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Research

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Abstract

Background: CircRNA ACAP2 and miR-532 both promotes the apoptosis of cardiomyocytes, which contributes to myocardial infarction (MI). Therefore, ACAP2 and miR-532 may interact with each other to participate in MI.

Method: Plasma samples from both MI patients (n=65) and healthy controls (n=65) were subjected to RNA extractions and RT-qPCR to analyze the expression of ACAP2, mature miR-532 and premature miR-532. Correlations among them were analyzed by Pearson's correlation coefficient. Expression of both mature miR-532 and premature miR-532 in cardiomyocytes with ACAP2 overexpression was analyzed by RT-qPCR to study the effects of ACAP2 overexpression on the maturation of miR-532. The role of ACAP2 and miR-532 in regulating the apoptosis of cardiomyocytes induced by hypoxia was analyzed by cell apoptosis assay.

Results: In this study we found that ACAP2 and mature miR-532 were both upregulated in plasma from MI patients. ACAP2 and mature miR-532 were inversely correlated, while ACAP2 and premature miR-532 were not closely correlated. In cardiomyocytes, overexpression of ACAP2 decreased the expression of mature miR-532, but not premature miR-532. Cell apoptosis analysis showed that ACAP2 and miR-532 overexpression promoted the apoptosis of cardiomyocytes induced by hypoxia treatment. In addition, miR-532 inhibitor reduced the effects of ACAP2 overexpression.

Conclusion: Therefore, ACAP2 is overexpressed in MI and may promote the maturation of miR-532 to induce the apoptosis of cardiomyocyte.

Background

Myocardial infarction (MI), commonly known as heart attack, is the death or damage of heart tissues caused by the formation of blood clot that blocks coronary artery, which is responsible for the transportation of oxygen and blood to the heart [1, 2]. Irreversible death of heart cells will occur if restoration of blood does not occur within 20–40 min after the sudden blockage [1, 2]. MI is correlated with high mortality rate. It is estimated that more than 10% of MI patients will die before hospitalization [3, 4]. Even worse, only less than half of the survivors can live longer than 10 years [3, 4]. Open heart surgery, medications and interventional procedures may help to improve MI patients' life quality, while no cure is currently available [5, 6].

The treatment and prevention of MI require the development of novel therapeutic approaches. Studies on the molecular mechanism of MI have showed that nearly all aspects of the development and progression of MI require the involvement of multiple molecular players [7, 8]. Some molecular factors, such as TRAIL, has been proven to be potential molecular targets to treat MI [9, 10]. However, safe and effective targets for molecular targeted MI therapy remain lacking. Circular RNA (circRNAs) are covalently closed RNA transcripts participate in human diseases mainly by regulating gene expression rather that encoding protein products [11]. In effect, circRNAs are potential targets to treat human diseases including MI [12].

However, the function of most circRNAs in MI remains unclear. Previous studies have reported that circRNA ACAP2 and miR-532 both promotes the apoptosis of cardiomyocytes, which contributes to MI [13, 14], suggesting their involvement in MI and the existence of crosstalk between them. This study was therefore carried out to study the interaction between ACAP2 and miR-532 in MI.

Results

MI patients exhibited altered expression of ACAP2 and mature miR-532 in plasma

Plasma samples from both MI patients (n = 65) and healthy controls (n = 65) were subjected to RNA isolations and RT-qPCRs to determine the expression of ACAP2, mature miR-532 and premature miR-532. Compared to control group, MI group exhibited significantly upregulated ACAP2 (Fig. 1A, $p < 0.001$) and mature miR-532 (Fig. 1B, $p < 0.001$). In contrast, no significantly altered expression of premature miR-532 was observed between MI and control groups (Fig. 1C).

ACAP2 and mature miR-532 were inversely correlated cross MI plasma samples

Correlations between ACAP2 and mature miR-532 or premature miR-532 across MI plasma samples were analyzed by Pearson's correlation coefficient. It was observed that ACAP2 and mature miR-532 were inversely and significantly correlated (Fig. 2A), while ACAP2 and premature miR-532 were not closely correlated (Fig. 2B). Therefore, ACAP2 may be correlated with the maturation of miR-532.

Hypoxia upregulated the expression of ACAP2 and mature miR-532 in AC16 cells

To study the effects of hypoxia on the expression of ACAP2, mature miR-532 and premature miR-532, AC16 cells were cultivated under hypoxic condition (1% O_2, 5% CO_2, and 94% N_2) for 24, 48, 72 and 96 h, followed by RNA isolations and RT-qPCRs to determine gene expression. It was observed that hypoxia upregulated the expression of ACAP2 (Fig. 3A, $p < 0.05$) and mature miR-532 (Fig. 3B, $p < 0.05$) in AC16 cells. In contrast, hypoxia treatment failed to significantly alter the expression of premature miR-531 (Fig. 3C).

ACAP2 overexpression suppressed the maturation of miR-532 in AC16 cells

To determine the effects of ACAP2 overexpression on the maturation of miR-532, AC16 cells were transfected with ACAP2 expression vector or miR-532 mimic, followed by the determination of ACAP2 and miR-532 expression every 24 until 96 h. It was observed that ACAP2 and miR-532 were significantly overexpressed between 24 h and 96 h (Fig. 4A, $p < 0.05$). It was observed that overexpression of ACAP2 decreased the expression of mature miR-532 (Fig. 4B, $p < 0.05$), but not premature miR-532 (Fig. 4C, $p < 0.05$). In addition, overexpression of miR-532 failed to significantly alter the expression of ACAP2 (Fig. 4C).

ACAP2 increased hypoxia-induced apoptosis of AC16 cells through miR-532

The effects of transfections on the apoptosis of AC16 cells induced by hypoxic treatment were analyzed by cell apoptosis assay. ACAP2 and miR-532 overexpression promoted the apoptosis of cardiomyocytes induced by hypoxia treatment. In contrast, miR-532 inhibitor suppressed cell apoptosis. In addition, miR-532 inhibitor reduced the effects of ACAP2 overexpression (Fig.5, $p<0.05$).

Discussion

In this study we analyzed the involvement of ACAP2 and miR-532 in MI and explored the interactions between them. We found that ACAP2 and mature miR-532 were both upregulated in MI. In addition, ACAP2 overexpression in MI may promote the maturation of miR-532 in AC16 cells to suppress hypoxia-induced cell apoptosis.

In a recent study, Liu et al. reported that ACAP2 was upregulated in MI rat model and it could sponge miR-29 to induce the apoptosis of cardiomyocytes, thereby promoting disease progression [13]. However, the expression pattern of in ACAP2 patients and its function are unknown. In this study we showed that ACAP2 was upregulated in Mi patients. In addition, overexpression of ACAP2 in AC16 cells was observed after hypoxic treatment. Under hypoxic treatment, ACAP2 overexpression increased the apoptosis of AC16 cell apoptosis. Therefore, the upregulation of ACAP2 in MI is likely induced by hypoxia, and hypoxia-inducible ACAP2 may promote the apoptosis of AC16 cells to aggregate disease condition.

Interestingly, miR-532 was upregulated in diabetic heart and promoted cardiomyocyte apoptosis to promote disease progression [14]. Interestingly, we observed that mature miR-532, but not premature miR-532, was overexpressed in MI. Therefore, the development of MI may accelerate the maturation of miR-532. Consistently, in this study we showed that miR-532 overexpression increased the apoptosis of AC16 cells induced by hypoxia, while miR-532 inhibitor suppressed cell apoptosis. Therefore, inhibition of miR-532 may serve as a potential target for the treatment of MI. However, animal model experiments and clinical trial experiments are needed to further verify our hypothesis.

Interestingly, this study we showed that ACAP2 overexpression increased the expression of mature miR-532, but not premature miR-532 in AC16 cells. Therefore, the increased maturation of miR-532 in AC16

cells may be caused by the upregulation of ACAP2. However, the mechanism of the maturation of miR-532 induced by ACAP2 is unknown. Considering that the maturation of miR-532 require the transportation of premature miR-532 from nucleus to cytoplasm, ACAP2 may suppress the movement of miR-532 to decrease the production of mature miR-532.

Conclusion

In conclusion, ACAP2 is overexpressed in MI and it may promote the maturation of miR-532 to induce hypoxia-induced apoptosis of cardiomyocytes.

Materials And Methods

Research subjects

Chengdu First People's Hospital hospital Ethics Committee approved this study. At this hospital, we enrolled both MI patients (n=65) and healthy controls (n=65) from July 2018 to July 2020 to serve as research subjects. Both groups included 38 males and 27 females, with a mean age of 56.6+/-5.9 years. MI patients were diagnosed through following criteria: 1) biomarkers in serum (increased troponin I or T, and increased CK-MB); 2) ECG signs (pathologic Q waves and ST segment elevation). Heathy controls received systemic physiological exam at the physiological center of the aforementioned hospital and all physiological functions of controls were within normal range. MI patients complicated with other severe diseases (such as diabetes, severe infections, metabolic disorders, cancers, heart diseases, etc) and the ones with initiated therapy were excluded. Healthy controls with a history of MI were also excluded. All MI patients and healthy controls signed informed consent.

Preparation of plasma and cardiomyocytes

Both MI patients (before therapy) and healthy controls were subjected to the extraction of blood (2ml) under fasting conditions. The 2ml blood was transferred to tubes containing 0.2 ml citric acids, followed by centrifuging the mixture for 20 min at 1200g to separate plasma samples. In liquid nitrogen, plasma samples were kept prior to the subsequent experiments.

The cell model used in this study was human cardiomyocyte cell line AC16 from Sigma-Aldrich (USA). Cell culture medium as composed of 12% FBS and 88% DMEM medium. Antibiotics (1% penicillin and streptomycin) were also added. Normal cell culture conditions were 5% CO_2, 95% humidity and 37°C. To study the effects of hypoxia on gene expression, AC16 cells were cultivated under hypoxic condition (1% O_2, 5% CO_2, and 94% N_2) for 24, 48, 72 and 96h prior to the subsequent experiments.

Transient transfections

AC 16 cells were transfected with either ACAP2 expression vector, miR-532 inhibitor or the mimic of miR-532 t. All transfections were performed using lipofectamine 2000 (Invitrogen). ACAP2 expression vector was established with pcDNA3.1 vector (Invitrogen) as backbone. Mimic of miR-532 and negative control (NC) miRNA as well as miR-532 inhibitor and NC inhibitor were both from Invitrogen. NC experiments (empty pcDNA3.1 vector-, NC inhibitor- or NC miRNA-transfected cells) and C experiments (untransfected cells) were included in each transfection. Prior to the subsequent experiments, transfected cells were cultivated in fresh medium for 48h.

Preparation of RNA samples

RNAzol (Sigma-Aldrich) was used for RNA extractions from both plasma samples and AC16 cells. Genomic DNA in all RNA samples was digested by DNase I (Invitrogen) for 90 min at 37°C. RNA integrity was analyzed by 5% urea-PAGE gel electrophoresis. The purity of RNA samples were analyzed by determining the OD260/280 ratios. RNA samples with an OD260/280 ratio close to 2.0 were considered to be pure RNA.

RT-qPCR

RNA samples with satisfactory integrity and purity were used as template to prepare cDNA samples through reverse transcriptions (RTs), which were also performed using SS-IV-RT system (Invitrogen). SYBR® Green Real-Time PCR Master mix (Toyobo) was used to perform all qPCRs with 18S rRNA as internal control.

All-in-One™ miRNA qRT-PCR Detection Kit (GeneCopoeia) was used to determine the expression of both mature miR-532 and premature miR-532. To measure the levels of mature miR-532 expression, poly (A) was added, followed by using poly (T) as reverse primer to perform RTs and qPCRs. To determine the expression of premature miR-532, sequence-specific primers were used to perform both RTs and qPCRs.

Ct values of target genes were normalized to endogenous controls based on $2^{-\Delta\Delta CT}$ method.

Cell apoptosis assay

At 48h post-transfection, cell apoptosis assay was performed to analyze the effects of transfections on cell apoptosis. Briefly, AC16 cells were cultivated in a 6-well cell culture plate with 12000 cells in 2 ml medium per well. Cell were cultivated under hypoxic conditions (1% O_2, 5% CO_2, and 94% N_2) for further 48h, followed by washing with ice-cold PBS. After that, cells were resuspended in binding buffer, followed by staining with Annexin V-FITC and propidium iodide (PI) for 20 min in dark. Finally, cell apoptosis was analyzed by flow cytometry.

Statistical analysis

Comparisons between two groups of participants were performed by unpaired t test. Comparisons among multiple independent transfection groups were analyzed by ANOVA Tukey’s test. Pearson’s correlation coefficient was used to analyze correlations. Differences were statistically significant when $p<0.05$.

Declarations

Ethics approval and consent to participate

This study was approved by Ethics Committee of Chengdu First People's Hospital hospital Ethics Committee.

Consent for publication

Not applicable

Availability of data and materials

The datasets used analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Guarantor of integrity of the entire study□Jun Zhang. Study concepts□Jun Zhang. Study design□Yanrong Tang. Definition of intellectual content□Jing Zhang. Experimental studies□Jiyun He. Data acquisition□ Zhenzhen Zhang. Data analysis□Fuqiang Liu. Statistical analysis: Jun Zhang. Manuscript review□Jun Zhang.

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Figures

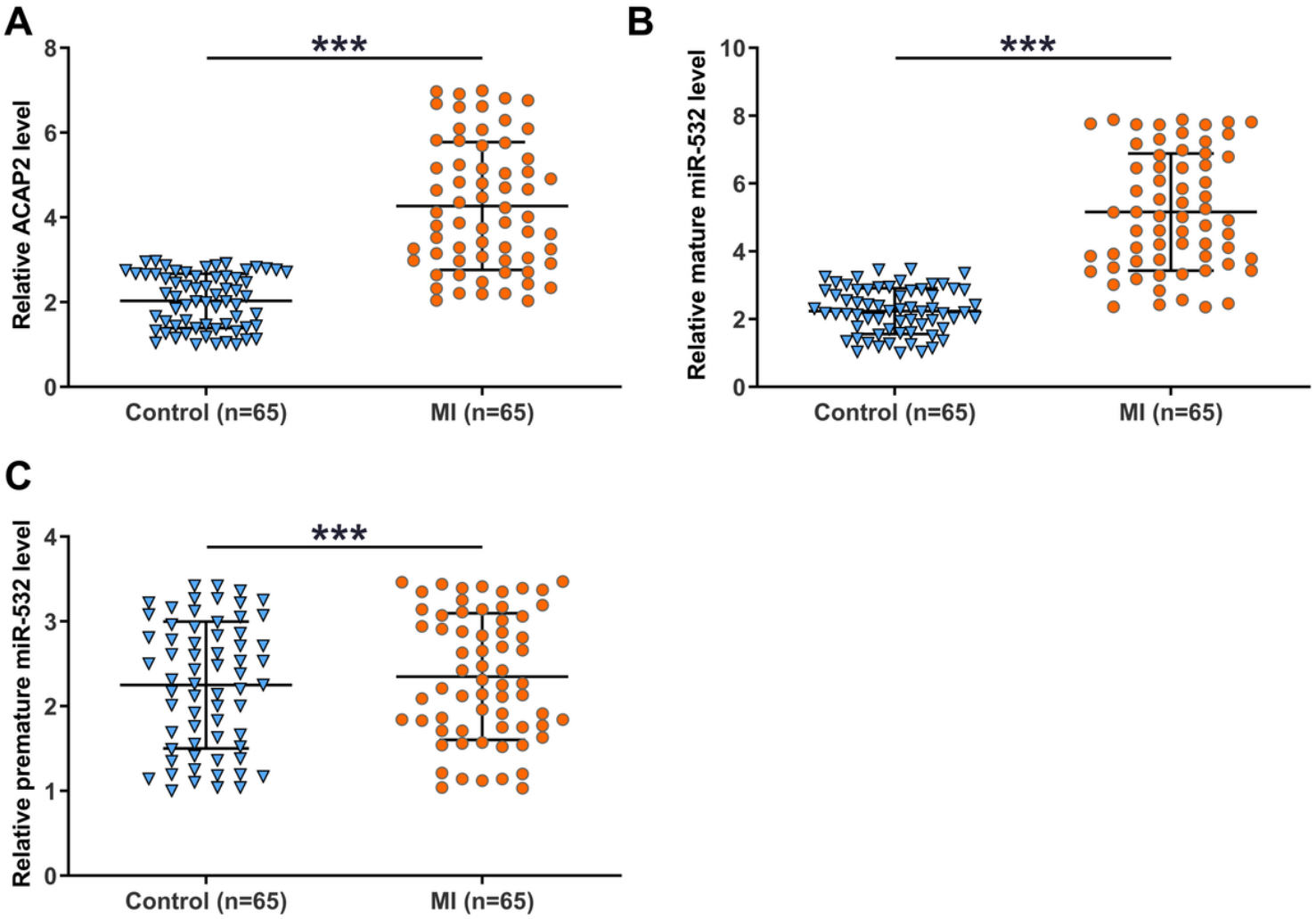
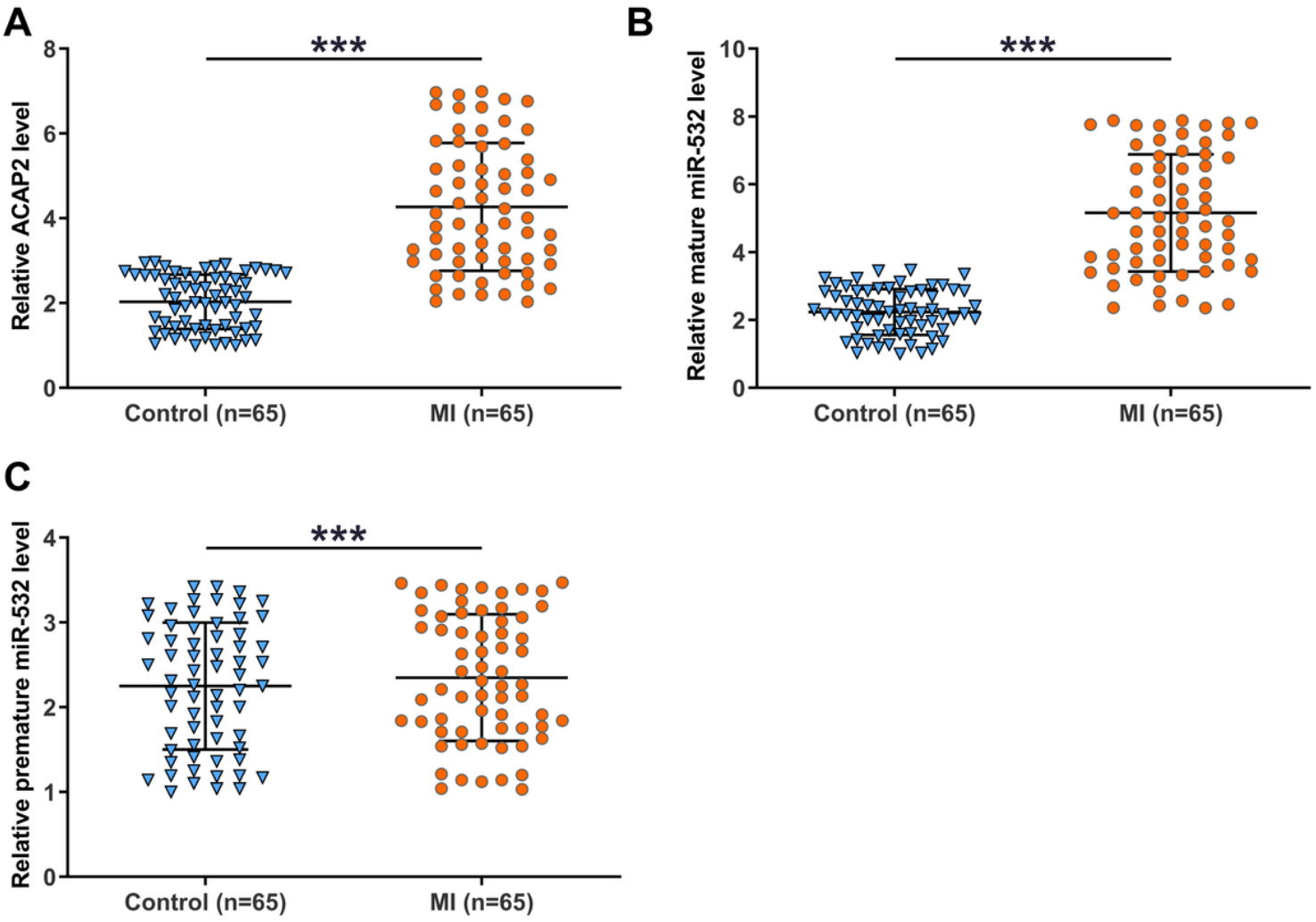


Figure 1

MI patients exhibited altered expression of ACAP2 and mature miR-532 in plasma Plasma samples from both MI patients (n=65) and healthy controls (n=65) were subjected to RNA isolations and RT-qPCRs to determine the expression of ACAP2 (A), mature miR-532 (B) and premature miR-532 (C). ***,p<0.001



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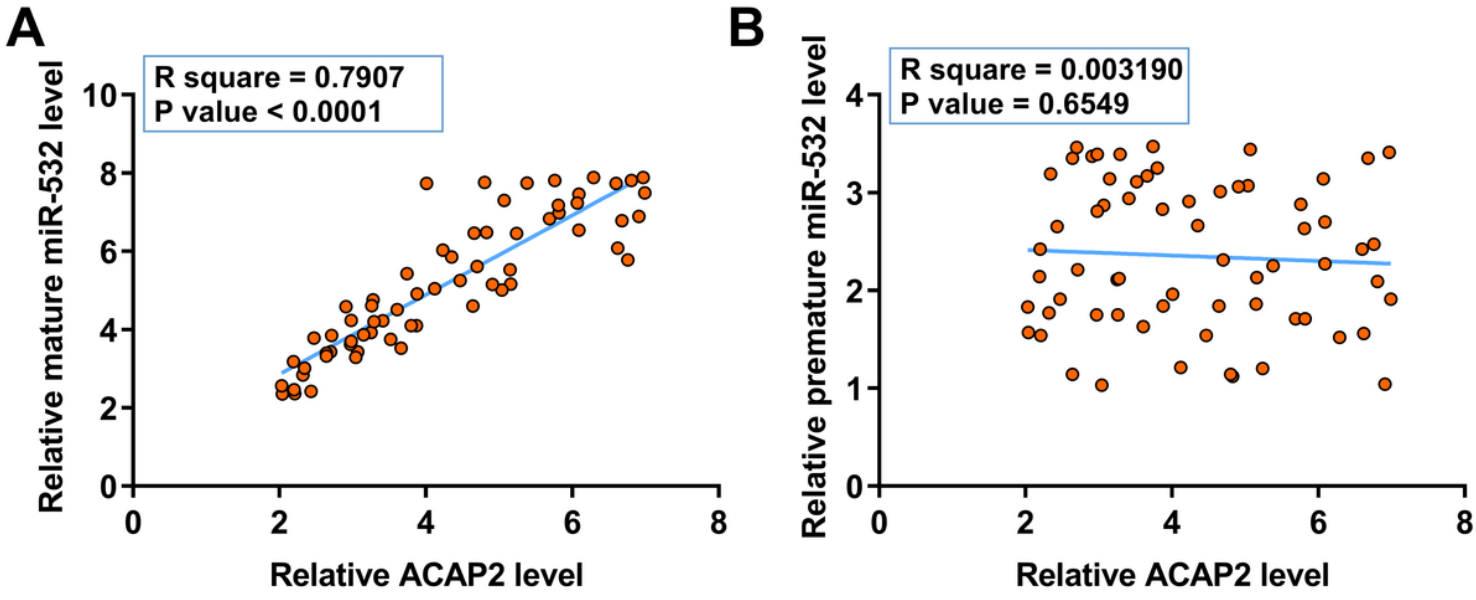
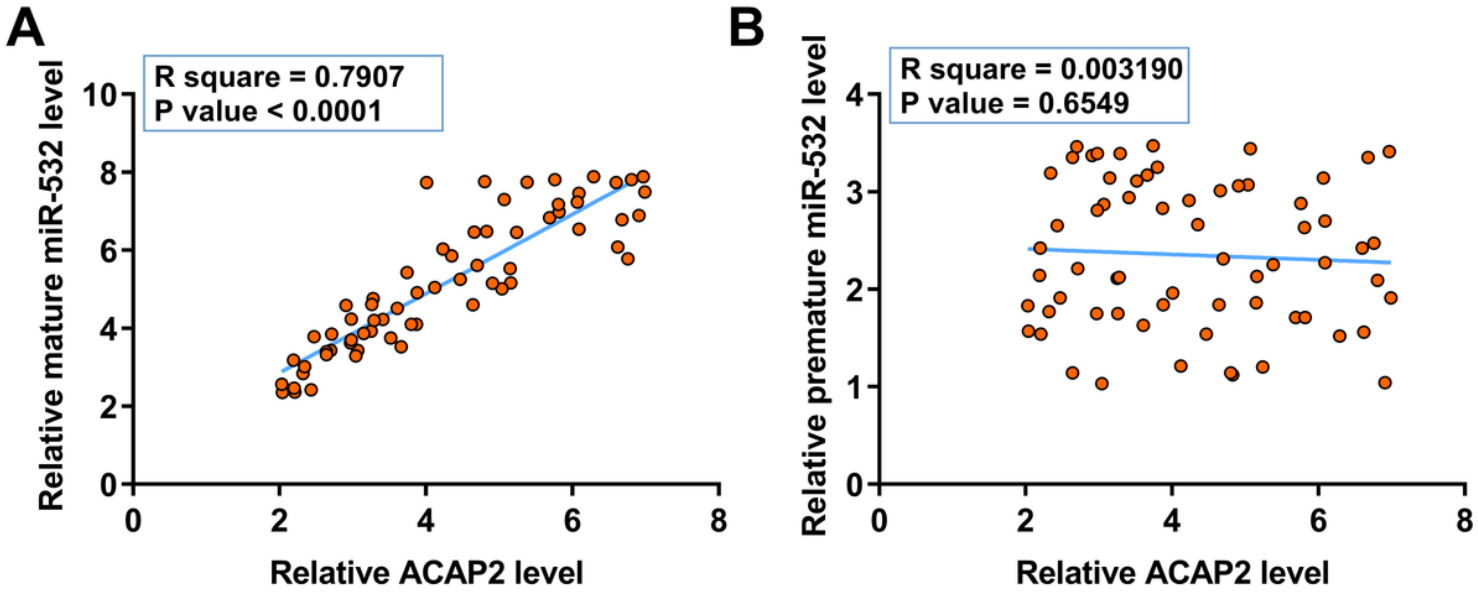


Figure 2

ACAP2 and mature miR-532 were inversely correlated cross MI plasma samples Correlations between ACAP2 and mature miR-532 (A) or premature miR-532 (B) across MI plasma samples were analyzed by Pearson's correlation coefficient.



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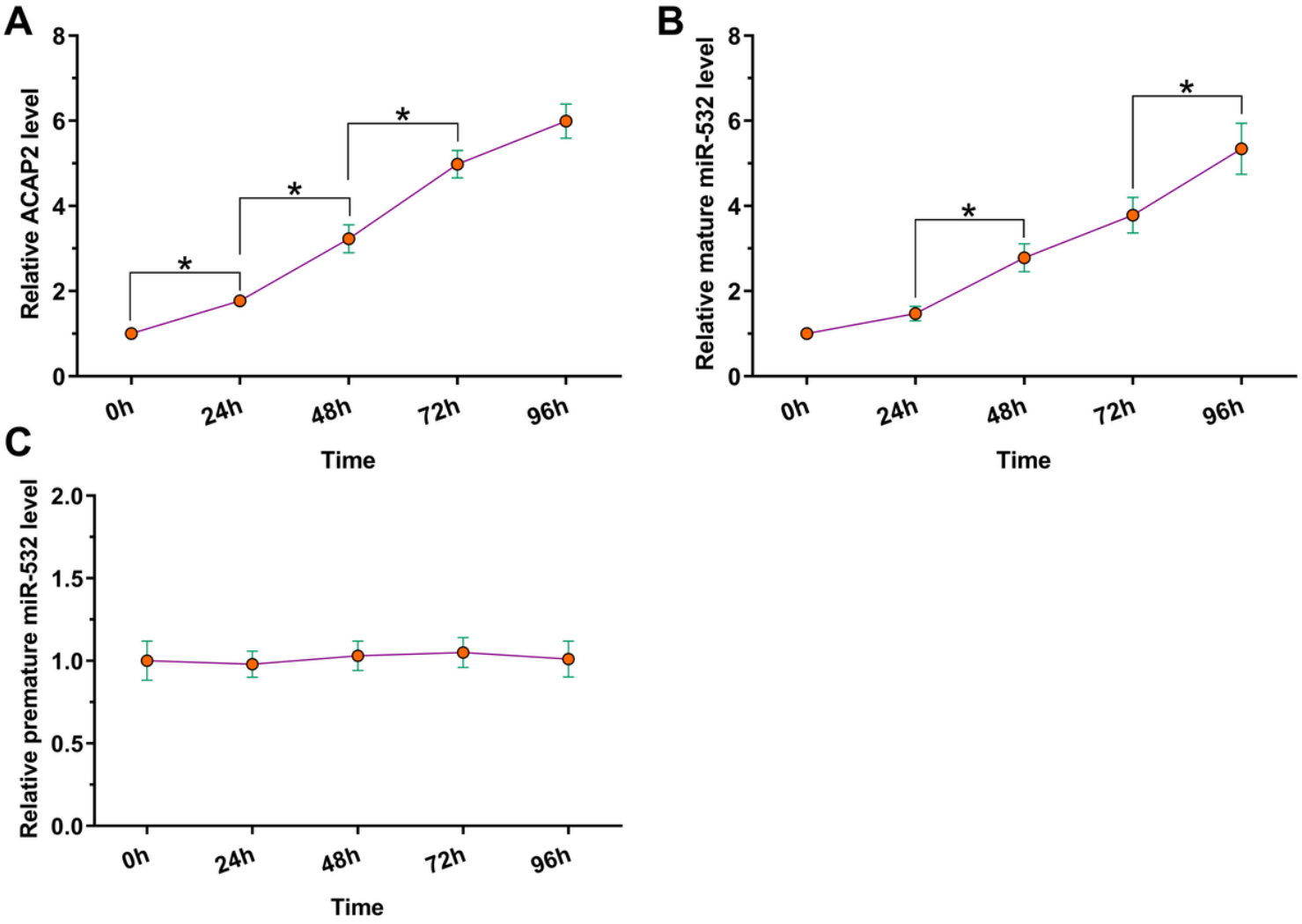
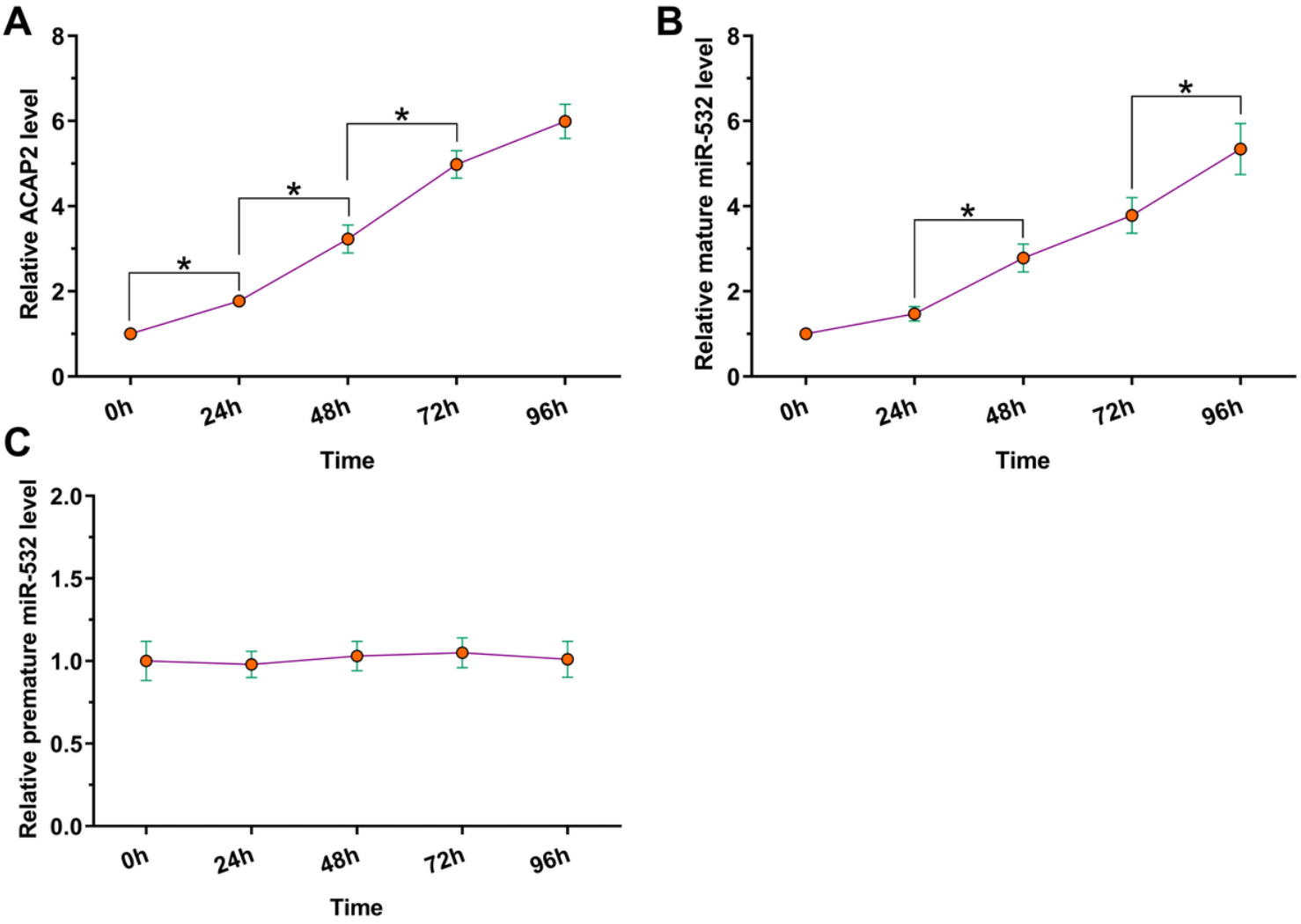


Figure 3

Hypoxia upregulated the expression of ACAP2 and mature miR-532 in AC16 cells To study the effects of hypoxia on the expression of ACAP2 (A), mature miR-532 (B) and premature miR-532 (C), AC16 cells were cultivated under hypoxic condition (1% O2, 5% CO2, and 94% N2) for 24, 48, 72 and 96h, followed by RNA isolations and RT-qPCRs to determine gene expression. *,$p<0.05$.



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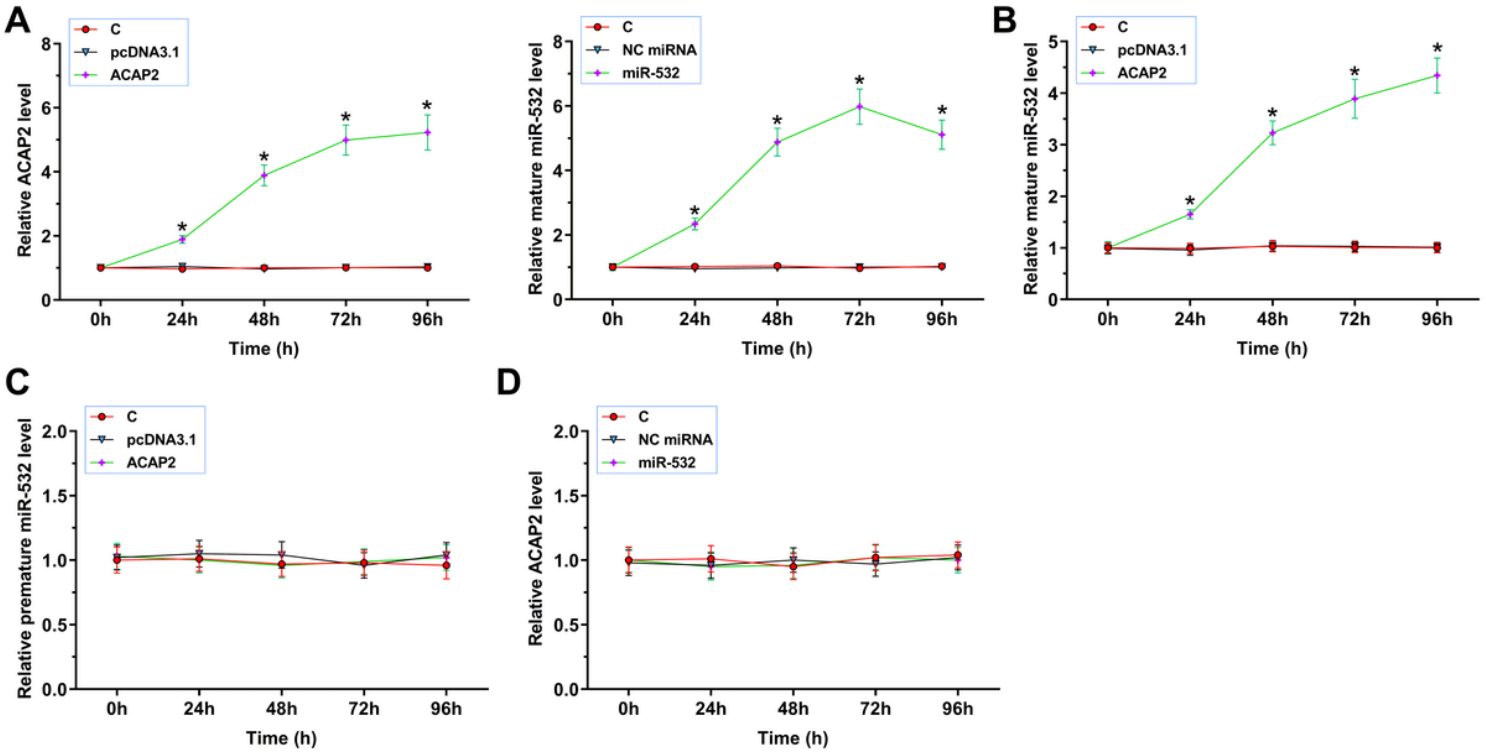
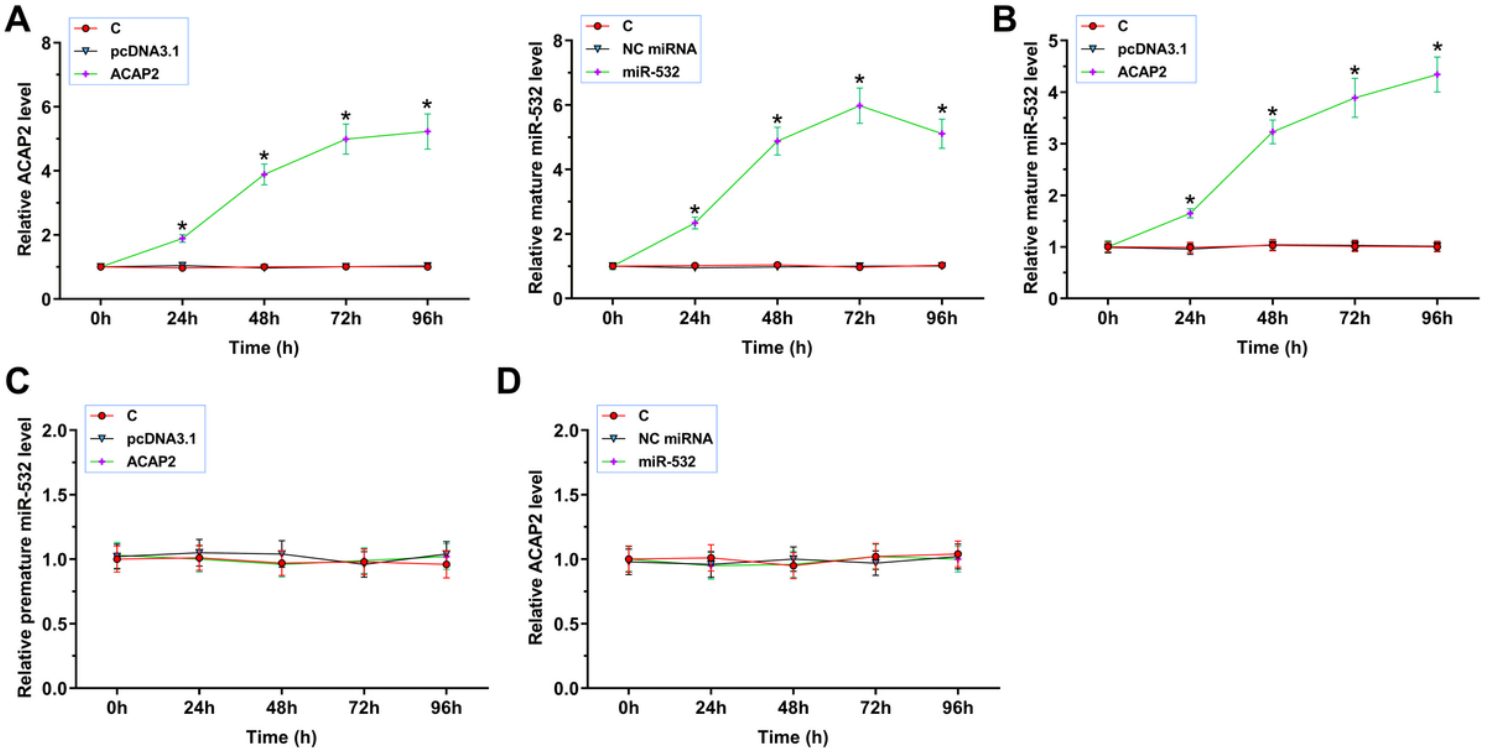


Figure 4

ACAP2 overexpression suppressed the maturation of miR-532 in AC16 cells To determine the effects of ACAP2 overexpression on the maturation of miR-532, AC16 cells were transfected with ACAP2 expression vector or miR-532 mimic, followed by the determination of ACAP2 and miR-532 expression every 24 until 96h (A). The effects of the overexpression of ACAP2 on the expression of mature miR-532 (B) and premature miR-532 (C), and the effects of miR-532 overexpression on the expression of ACAP2 were analyzed by RT-qPCR (C). *,p<0.05.



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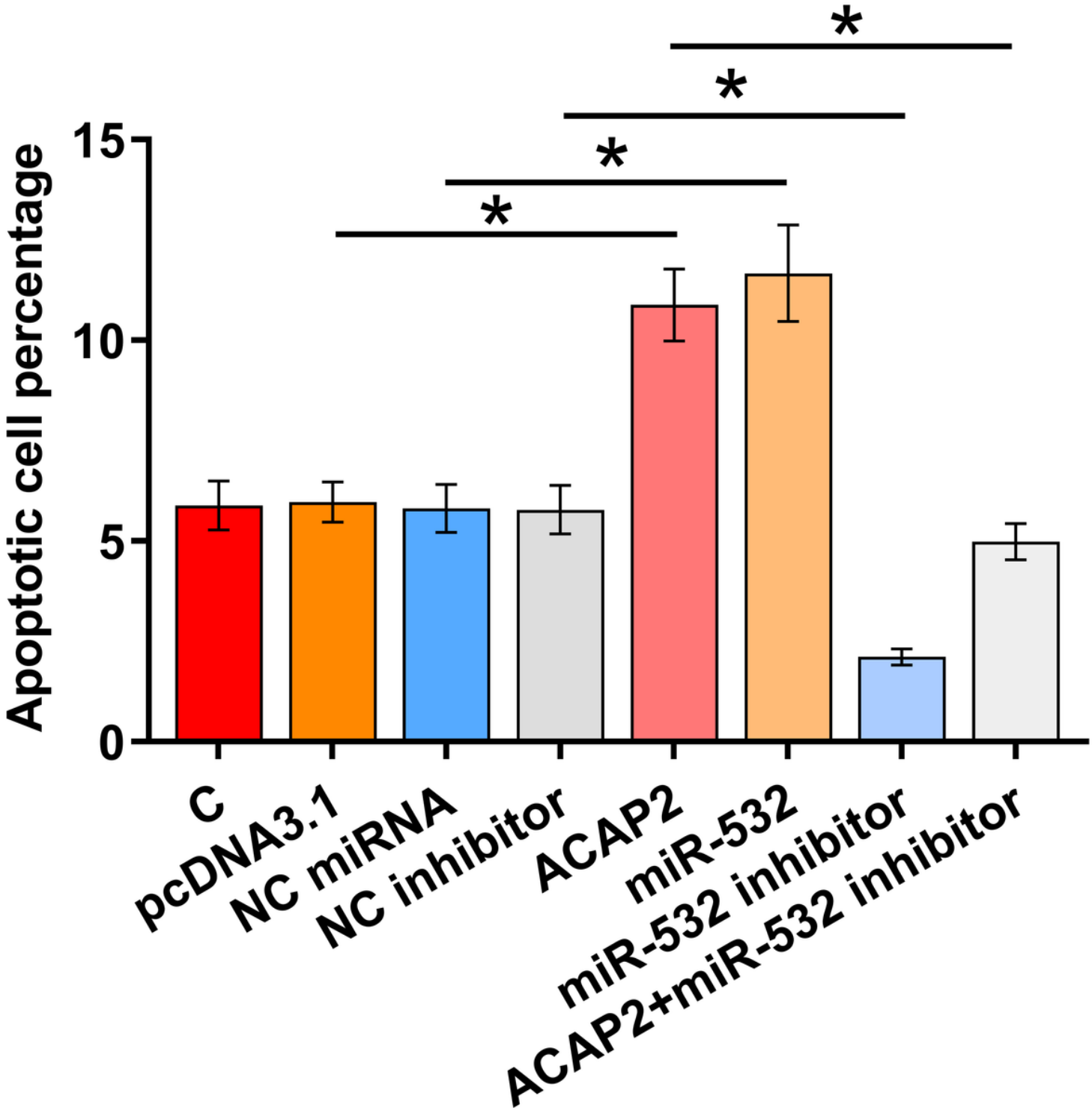
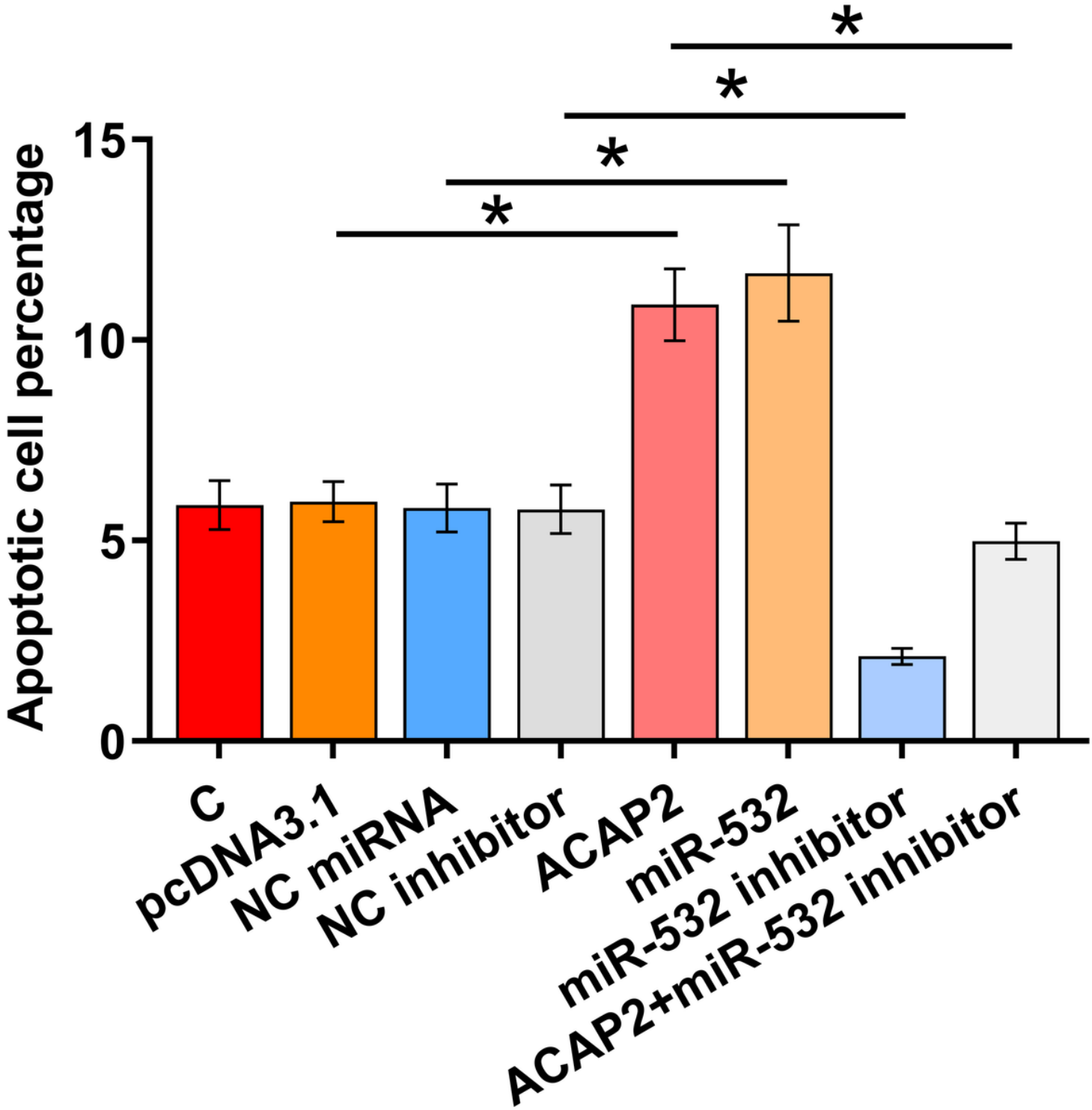


Figure 5

ACAP2 increased hypoxia-induced apoptosis of AC16 cells through miR-532 The effects of transfections on the apoptosis of AC16 cells induced by hypoxic treatment were analyzed by cell apoptosis assay. *,p<0.05.



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