

## MicroRNA-186-5p is Highly Expressed in Alcohol-Induced Cardiomyocytes and Regulates Apoptosis Level Through Target Gene XIAP Postprint

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### Abstract

**Objective:** To confirm that alcohol can induce apoptosis in AC16 cardiomyocytes and its relationship with alcohol concentration and treatment duration, to investigate the changes in miR-186-5p and X-linked inhibitor of apoptosis protein (XIAP) expression levels as well as cardiomyocyte apoptosis levels under different concentrations of alcohol intervention, and to explore whether miR-186-5p regulates alcohol-induced cardiomyocyte apoptosis by targeting XIAP. **Methods:** Flow cytometry was used to detect apoptosis levels, Western Blot and real-time quantitative PCR were employed to detect changes in miR-186-5p and XIAP expression at protein and gene levels respectively, and dual-luciferase reporter gene assay was performed to verify the targeting relationship between miR-186-5p and XIAP. **Results:** Alcohol induced apoptosis in AC16 cardiomyocytes in a manner positively correlated with alcohol concentration and treatment time; alcohol consumption upregulated miR-186-5p expression and downregulated XIAP expression in AC16 cardiomyocytes; miR-186-5p participated in the process of alcohol-induced AC16 cardiomyocyte apoptosis, while XIAP inhibited alcohol-induced AC16 cardiomyocyte apoptosis; miR-186-5p regulated alcohol-induced cardiomyocyte apoptosis by targeting XIAP. **Conclusion:** After AC16 cardiomyocytes were treated with alcohol, the apoptosis level increased, and further elevated with prolonged alcohol concentration and treatment time; alcohol treatment upregulated miR-186-5p expression and downregulated XIAP expression in cardiomyocytes, with miR-186-5p regulating apoptosis in alcohol-treated cardiomyocytes by targeting XIAP.

## Full Text

### MicroRNA-186-5p is Highly Expressed in Alcohol-Induced Cardiomyocytes and Regulates Apoptosis Through Its Target Gene XIAP

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## Abstract

**Objective:** To confirm that alcohol induces apoptosis in AC16 cardiomyocytes and determine its relationship with alcohol concentration and treatment duration. To investigate the expression levels of miR-186-5p and X-linked inhibitor of apoptosis protein (XIAP) along with changes in cardiomyocyte apoptosis under different alcohol concentrations. To explore how miR-186-5p regulates alcohol-induced cardiomyocyte apoptosis by targeting XIAP.

**Methods:** Flow cytometry was used to detect apoptosis levels. Western blot and real-time quantitative PCR were employed to examine changes in miR-186-5p and XIAP expression at protein and mRNA levels. Dual-luciferase reporter gene assay was performed to verify the targeting relationship between miR-186-5p and XIAP.

**Results:** Alcohol induced apoptosis in AC16 cardiomyocytes in a manner positively correlated with both alcohol concentration and treatment time. Alcohol intake upregulated miR-186-5p expression and downregulated XIAP expression in AC16 cardiomyocytes. miR-186-5p participated in the process of alcohol-induced AC16 cardiomyocyte apoptosis, while XIAP inhibited alcohol-induced AC16 cardiomyocyte apoptosis. miR-186-5p regulated alcohol-induced cardiomyocyte apoptosis by targeting XIAP.

**Conclusion:** Alcohol treatment increased apoptosis levels in AC16 cardiomyocytes, which further elevated with increasing alcohol concentration and prolonged treatment duration. Following alcohol treatment, miR-186-5p expression was upregulated while XIAP expression was downregulated in cardiomyocytes,

with miR-186-5p regulating alcohol-induced cardiomyocyte apoptosis by targeting XIAP.

**Keywords:** cardiomyocyte; apoptosis; microRNA-186-5p; XIAP; alcoholic cardiomyopathy

**Chinese Library Classification Number:** R542.2

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## Introduction

Alcohol exerts toxic effects on the heart, causing cardiomyocyte damage. Chronic heavy alcohol consumption leads to non-ischemic dilated cardiomyopathy known as alcoholic cardiomyopathy (ACM) [1], which is characterized by myocardial hypertrophy and compensatory cardiac contractile dysfunction [2]. Cardiomyocyte apoptosis plays a crucial role in the pathogenesis of ACM. X-linked inhibitor of apoptosis protein (XIAP) is a member of the inhibitor of apoptosis family of proteins (IAP) and represents the most potent IAP protein known in human tissues and cells [3]. XIAP can inhibit apoptosis triggered by viral infection or excessive expression of caspase family proteins.

MicroRNAs are small regulatory RNA molecules approximately 21-23 nucleotides in length. Current research has identified 13 microRNAs involved in regulating the development and progression of myocardial hypertrophy, suggesting that abnormal microRNA levels play important roles in cardiac hypertrophy, pathological remodeling, and heart failure [4]. Regarding alcohol-induced cardiomyopathy, studies have detected abnormal expression levels of multiple microRNAs in hypertrophic myocardial tissue, including miR-133a, miR-125, and miR-195 [5], indicating that ACM may be associated with abnormal expression of certain microRNAs.

In our previous work, we identified differentially expressed microRNAs associated with ACM (such as miR-186-5p and miR-488-3p). Using TargetScan bioinformatics software, we predicted 949 target genes for miR-186-5p and identified XIAP as a target gene. XIAP is an important anti-apoptotic protein in human tissues and cells, but its function in alcohol-induced cardiomyopathy has not been reported.

In this study, we first analyzed changes in apoptosis levels in human AC16 cardiomyocytes [6] after alcohol treatment using flow cytometry. We then examined changes in miR-186-5p and the apoptosis inhibitor protein XIAP expression levels in alcohol-treated AC16 cardiomyocytes and investigated the specific molecular mechanisms underlying alcohol-induced cardiomyocyte apoptosis.

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## 1. Materials and Methods

### 1.1 Materials

AC16 human cardiomyocytes derived from human left ventricular cardiomyocytes were purchased from Guangzhou Shengyi Biotechnology Co., Ltd. and stored in our hospital's central laboratory. Anhydrous alcohol was purchased from Sinopharm Group. F12 medium was purchased from Gibco (USA). MTT assay kit and RNA extraction reagent TRIzol were purchased from Sigma (USA). Reverse transcription reagents were purchased from TAKARA (Japan). DharmaFECT transfection reagent was purchased from Dharmacon (USA). Luciferase reporter gene plasmid vector (pmirGLO) was purchased from Shanghai GenePharma Co., Ltd. MicroRNA-186-5p mimic/inhibitor was purchased from Guangzhou RiboBio Co., Ltd. miR-186 primers were purchased from Shanghai Shengdian Biological Co., Ltd. XIAP plasmid, quantitative PCR reagent SYBR Master Mix, Lipofectamine 3000, ACTIN antibody, XIAP antibody, goat anti-mouse secondary antibody, goat anti-rabbit secondary antibody, and ECL detection kit were all purchased from Cell Signaling Technology (USA). Luciferase reporter gene assay kit was purchased from Promega. Annexin V/PI kit was purchased from BD (USA).

#### 1.2.1 Flow Cytometry Detection of Apoptosis

Logarithmic growth phase cells were seeded in 6-well plates at 60-70% confluence and treated with alcohol at concentrations of 0 mmol/L for 24h or 48h, 200 mmol/L for 24h or 48h, and 800 mmol/L for 24h or 48h in a CO<sub>2</sub> incubator. After incubation, plates were removed and cells in each well were digested with 0.25% trypsin. Following centrifugation and supernatant removal, cells were washed and resuspended in 100  $\mu$ L of 1 $\times$  binding buffer per tube. After gently mixing, 3  $\mu$ L of Annexin V-FITC was added to each tube, gently mixed again, and incubated in the dark for 15 minutes. Then 200  $\mu$ L of 1 $\times$  binding buffer was added to each tube, mixed gently, and apoptosis was detected and analyzed using flow cytometry. Experiments were repeated five times and average values were calculated.

#### 1.2.2 Dual-Luciferase Reporter Gene Assay for Target Verification

Using bioinformatics software (TargetScan 7.1), we predicted the binding sequence of miR-186 to the XIAP gene and designed synthetic XIAP 3'-UTR sequences and mutated XIAP 3'-UTR sequences. The two synthesized target gene fragments were cloned into pmirGLO luciferase reporter vectors to construct wild-type XIAP 3'-UTR dual-luciferase reporter vector (pmirGLO-wt-XIAP) and mutant vector (pmirGLO-mut-XIAP).

Using Lipofectamine 3000 transfection reagent, the two recombinant plasmids were co-transfected with miR-186 mimic (miRNA mimics are chemically synthesized molecules that mimic endogenous miRNAs and enhance their function; in this experiment, microRNA-186-5p mimic was purchased from Guangzhou

RiboBio Co., Ltd.) or miR-186 negative control (mimic control) into AC16 cardiomyocytes.

Transfection groups were as follows: - miR-186-5p mimic control + pmirGLO-wt-XIAP-3 -UTR - miR-186-5p mimic + pmirGLO-wt-XIAP-3 -UTR - miR-186-5p mimic control + pmirGLO-mut-XIAP-3 -UTR - miR-186-5p mimic + pmirGLO-mut-XIAP-3 -UTR

Luciferase activity was measured 48 hours after transfection.

### 1.2.3 Western Blot Analysis

Proteins were extracted on ice using RIPA lysis buffer and PMSF. Protein concentration was determined using the Bradford method [7]. Protein samples were prepared using SDS-PAGE loading buffer, and 20  $\mu$ g of each sample was loaded into SDS-PAGE gel wells. The stacking gel concentration was 5% and the separating gel concentration was 10%. Each well received 15  $\mu$ L of sample, with 5  $\mu$ L protein marker loaded in the left and right lanes. Electrophoresis was performed at 80V until the protein marker front entered the separating gel, after which voltage was adjusted to 120V. Electrophoresis was stopped when protein markers were appropriately separated. Proteins were transferred to membranes at 100V for 90 minutes on ice. Membranes were blocked in 1.5% BSA for 2 hours at room temperature. Primary antibodies XIAP (1:1000) and ACTIN (1:400) diluted in 1.5% BSA were incubated overnight at 4°C. Secondary antibodies were diluted according to manufacturer recommendations and incubated for 2 hours at 37°C. After washing, membranes were incubated with secondary antibody on a shaker for 1 hour at room temperature (dilution 1:10,000). ECL detection was performed and images were analyzed using an imaging system. Experiments were repeated five times.

### 1.2.4 Real-Time Quantitative PCR

Total RNA was extracted from cardiomyocytes using the TRIzol one-step method. One microgram of total RNA was reverse transcribed in a 20  $\mu$ L system to synthesize cDNA. Then 0.5  $\mu$ L of the resulting cDNA and target gene primers were added to a 20  $\mu$ L PCR amplification system. Reaction conditions: 37°C for 30 minutes, 85°C for 5 minutes. Primer sequences were as follows: miR-186 forward 5'-GCGCTAAGGCACGCGGT-3', reverse 5'-CAGTGCAGGGTCCGAGGT-3'; XIAP forward 5'-GGCACGAGCAGGGTTTCTT-3', reverse 5'-TCCAAGTCTGAGTCTCCATATTG-3'. Amplification and melting curves were confirmed, and relative gene expression was calculated using the  $2^{-\Delta\Delta Ct}$  method. Experiments were repeated five times.

### 1.2.5 Cell Transfection

One hundred microliters of serum-free medium was mixed with 4.5  $\mu$ L transfection reagent (Lipofectamine 3000) and 1.2  $\mu$ g plasmid, then incubated for 15

minutes. Digested cells were evenly seeded into 6-well plates and cultured until reaching 70% confluence. Transfection was performed as follows: cells were starved with serum-free medium for 2 hours, then the transfection mixture was added dropwise to each well and incubated at 37°C with 5% CO<sub>2</sub> for 5 hours. The transfection medium was then removed and replaced with complete medium containing 10% fetal bovine serum, followed by continued incubation at 37°C with 5% CO<sub>2</sub> for 24 hours. Cells were then collected for subsequent experiments. Experiments were repeated five times.

### 1.2.6 Statistical Analysis

Statistical analysis was performed using SPSS 16.0 software. Data between treatment and control groups were analyzed using one-way ANOVA.  $P < 0.05$  was considered statistically significant.

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## 2. Results

### 2.1 Alcohol Induces Apoptosis in AC16 Cardiomyocytes

AC16 cardiomyocytes were treated with different alcohol concentrations (0 mmol/L, 200 mmol/L, 800 mmol/L) for different durations (24 h, 48 h), and flow cytometry was used to analyze the effects of various alcohol concentrations and treatment times on AC16 cell apoptosis. As shown in [Figure 1: see original paper], Q4-1 represents necrotic and mechanically damaged cells, Q4-2 represents late-stage apoptotic cells, Q4-3 represents viable cells, and Q4-4 represents early-stage apoptotic cells. The 0 mmol/L groups (24 h or 48 h) served as controls, while the 200 mmol/L (24 h or 48 h) and 800 mmol/L (24 h or 48 h) groups were experimental groups. Results showed that alcohol treatment significantly increased apoptosis levels in AC16 cardiomyocytes compared to control groups ( $P < 0.05$ ), with highly significant differences at  $P < 0.01$ . These findings indicate that alcohol induces apoptosis in AC16 cardiomyocytes in a manner positively correlated with both alcohol concentration and treatment duration.

### 2.2 Verification of XIAP as a Direct Target Gene of miR-186

Bioinformatics software TargetScan 7.1 predicted that the 332-338 base position in the XIAP gene mRNA 3'-UTR is a potential binding site for miR-186. The miRanda database also identified complementary binding sequences between XIAP gene mRNA 3'-UTR and miR-186, suggesting stable binding and a high likelihood of miR-186 downregulating XIAP expression.

To further confirm whether XIAP is a direct target gene of miR-186-5p, we performed dual-luciferase reporter assays and real-time quantitative PCR for target verification. We constructed wild-type pmirGLO-wt-XIAP-3'-UTR and mutant

pmirGLO-mut-XIAP-3 -UTR vectors, which were co-transfected with miR-186-5p mimic control or miR-186-5p mimic into AC16 cardiomyocytes ([Figure 2: see original paper]). Results showed that fluorescence intensity significantly decreased when wild-type pmirGLO-wt-XIAP-3 -UTR was combined with miR-186-5p mimic compared to the other three groups ( $P < 0.05$ ), while no significant differences were observed among the other three groups ( $P > 0.05$ ). These findings indicate that miR-186-5p has a direct binding site in the XIAP 3'-UTR region and can targetedly inhibit XIAP expression, confirming that XIAP is a direct target gene of miR-186-5p.

### 2.3 Alcohol Intake Upregulates miR-186-5p Expression in AC16 Cardiomyocytes

AC16 cardiomyocytes were treated with alcohol, and miR-186-5p expression levels were detected using real-time quantitative PCR. As shown in [Figure 3: see original paper], Figure 3a demonstrates miR-186-5p RNA expression levels after 24 h of alcohol intervention at different concentrations. The 0 mmol/L group served as control, while 200 mmol/L and 800 mmol/L groups were experimental groups. Compared to the control group, miR-186-5p RNA expression levels were significantly elevated in experimental groups ( $P < 0.05$ ). Figure 3b shows miR-186-5p RNA expression levels after treatment with 800 mmol/L alcohol for different durations. The 0 h group served as control, while 24 h and 48 h groups were experimental groups. Experimental groups showed significantly higher miR-186-5p RNA expression compared to control ( $P < 0.05$ ). These results indicate that alcohol upregulates miR-186-5p expression in AC16 cardiomyocytes, with expression levels further increasing as alcohol concentration and treatment duration increase.

### 2.4 Alcohol Intake Downregulates XIAP Expression in AC16 Cardiomyocytes

To elucidate the specific mechanism of alcohol-induced cardiomyocyte apoptosis, we examined apoptosis-related protein expression levels in alcohol-treated AC16 cardiomyocytes using Western blot. As shown in [Figure 4: see original paper], Figure 4a presents XIAP protein expression levels after 24 h treatment with different alcohol concentrations. The 0 mmol/L group served as control, while 200 mmol/L and 800 mmol/L groups were experimental groups. XIAP protein expression was reduced in experimental groups compared to control. Figure 4b shows XIAP protein expression levels after treatment with 800 mmol/L alcohol for different durations. The 0 h group served as control, while 24 h and 48 h groups were experimental groups. XIAP protein expression was reduced in experimental groups compared to control. Figures 4c and 4d quantify XIAP protein expression based on grayscale values: XIAP protein expression was significantly reduced in experimental groups compared to controls ( $\#P < 0.05$ ). Figure 4e shows XIAP RNA expression levels after 24 h treatment with different alcohol concentrations, with significantly reduced expression in experimental

groups compared to control ( $P < 0.05$ ). Figure 4f shows XIAP RNA expression levels after treatment with 800 mmol/L alcohol for different durations, with significantly reduced expression in experimental groups compared to control ( $P < 0.05$ ). These findings indicate that alcohol downregulates both protein and RNA levels of XIAP in AC16 cells, with expression levels further decreasing as alcohol concentration and treatment duration increase.

### **2.5 miR-186-5p Participates in Alcohol-Induced AC16 Cardiomyocyte Apoptosis**

Previous experiments observed apoptosis in alcohol-treated AC16 cardiomyocytes and found upregulated miR-186-5p expression. To investigate the role of miR-186-5p in alcohol-induced cardiomyocyte apoptosis, AC16 cardiomyocytes were treated with alcohol (800 mmol/L, 24 h) and apoptosis levels were measured by flow cytometry. Additionally, miR-186-5p mimic was transfected into alcohol-treated (800 mmol/L, 24 h) AC16 cardiomyocytes, and apoptosis levels were measured by flow cytometry. As shown in [Figure 5: see original paper], Q2-1 represents necrotic and mechanically damaged cells, Q2-2 represents late-stage apoptotic cells, Q2-3 represents viable cells, and Q2-4 represents early-stage apoptotic cells. Results showed that alcohol treatment (800 mmol/L, 24 h) significantly increased AC16 cardiomyocyte apoptosis compared to the 0 mmol/L control group ( $P < 0.05$ ). Transfection with miR-186-5p mimic, which upregulates miR-186-5p expression, further increased cardiomyocyte apoptosis ( $P < 0.01$  vs. control). Compared to the alcohol-only treatment group, miR-186-5p mimic transfection significantly increased apoptosis ( $\#P < 0.05$ ). These findings indicate that alcohol induces cardiomyocyte apoptosis and that miR-186-5p promotes further increases in alcohol-induced cardiomyocyte apoptosis.

### **2.6 Upregulated XIAP Expression Inhibits Alcohol-Induced AC16 Cardiomyocyte Apoptosis**

To further investigate the role of XIAP in alcohol-induced cardiomyocyte apoptosis, AC16 cardiomyocytes were treated with alcohol (800 mmol/L, 24 h) and apoptosis levels were measured by flow cytometry. Additionally, XIAP plasmid was transfected into alcohol-treated (800 mmol/L, 24 h) AC16 cardiomyocytes, and apoptosis levels were measured by flow cytometry. As shown in [Figure 6: see original paper], Q2-1 represents necrotic and mechanically damaged cells, Q2-2 represents late-stage apoptotic cells, Q2-3 represents viable cells, and Q2-4 represents early-stage apoptotic cells. Results showed that both alcohol treatment (800 mmol/L, 24 h) and XIAP plasmid transfection increased AC16 cardiomyocyte apoptosis compared to the 0 mmol/L control group ( $P < 0.05$ ). However, XIAP plasmid transfection significantly reduced apoptosis levels compared to the alcohol-only treatment group ( $\#P < 0.05$ ). These findings indicate that alcohol induces cardiomyocyte apoptosis and that XIAP can partially reverse alcohol-induced cardiomyocyte apoptosis.

## 2.7 miR-186-5p Regulates XIAP Expression in AC16 Cardiomyocytes

The above experiments confirmed that both miR-186-5p and XIAP affect apoptosis in alcohol-treated AC16 cardiomyocytes. Luciferase reporter assays demonstrated that miR-186-5p can directly bind to the 3'-UTR (332-338) of XIAP mRNA, thereby inhibiting XIAP expression. We therefore hypothesized that XIAP functions as a target gene of miR-186-5p to regulate apoptosis in AC16 cardiomyocytes. To test this hypothesis, we transfected miR-186-5p mimic into AC16 cardiomyocytes to upregulate miR-186-5p expression and measured XIAP expression levels using Western blot and real-time quantitative PCR. As shown in [Figure 7: see original paper], Figure 7a demonstrates that transfection with miR-186-5p mimic and alcohol treatment (800 mmol/L, 24 h) reduced XIAP protein expression compared to control. Figure 7b shows that transfection with miR-186-5p inhibitor and alcohol treatment (800 mmol/L, 24 h) increased XIAP protein expression compared to control. Figures 7c and 7d quantify XIAP protein expression: miR-186-5p mimic significantly reduced XIAP protein expression compared to the 800 mmol/L-24 h control group ( $\#P < 0.05$ ), while miR-186-5p inhibitor significantly increased XIAP protein expression compared to control ( $\#P < 0.05$ ). Figure 7e shows that miR-186-5p mimic transfection reduced XIAP RNA expression compared to control ( $P < 0.05$ ). Figure 7f shows that miR-186-5p inhibitor transfection increased XIAP RNA expression compared to control ( $P < 0.05$ ). These findings indicate that miR-186-5p reduces XIAP expression levels in alcohol-treated AC16 cardiomyocytes, while miR-186-5p inhibitor can partially reverse alcohol-induced downregulation of XIAP expression.

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## Discussion

Alcoholic cardiomyopathy (ACM) is a myocardial disease characterized by cardiac dilation, arrhythmia, and congestive heart failure resulting from chronic heavy alcohol consumption. Studies have demonstrated that alcohol-induced cardiomyocyte apoptosis plays a key role in ACM pathogenesis [8-11]. In this study, we treated AC16 cardiomyocytes with alcohol and analyzed its effects on cardiomyocyte apoptosis. We found that alcohol treatment increased cardiomyocyte apoptosis levels, which continued to rise with prolonged alcohol exposure and treatment duration. These findings are consistent with previous research [12-15].

Regarding the mechanisms of alcohol-induced cardiomyocyte apoptosis, studies have shown that TNF- $\alpha$ , apoptotic protein Bax, and Caspase-3 levels are significantly elevated in ACM [16]. In this study, we found that alcohol intake induced abnormal XIAP expression in cardiomyocytes. XIAP is a member of the inhibitor of apoptosis family of proteins (IAP) and represents the most potent IAP protein known in human tissues and cells [17-19]. XIAP can inhibit apoptosis triggered by viral infection or excessive expression of caspase

family proteins. We found that XIAP expression levels were downregulated in AC16 cardiomyocytes after alcohol treatment. To further confirm whether XIAP plays a role in alcohol-induced cardiomyocyte apoptosis, we upregulated XIAP expression in cardiomyocytes using plasmid transfection and found that cardiomyocyte apoptosis was inhibited.

In recent years, microRNA research has focused on disease pathogenesis, progression, and prognosis. In this study, we screened microRNA expression levels in alcohol-treated cardiomyocytes and found that miR-186-5p expression was significantly upregulated. Furthermore, transfection with miR-186-5p mimic to upregulate miR-186-5p expression in alcohol-induced cardiomyocytes further increased apoptosis levels, confirming that miR-186-5p plays an important promoting role in alcohol-induced cardiomyocyte apoptosis.

We simultaneously detected abnormal expression levels of both miR-186-5p and XIAP in alcohol-treated cardiomyocytes. Using dual-luciferase reporter assays and real-time quantitative PCR for target verification, we found that wild-type pmirGLO-wt-XIAP-3'-UTR combined with miR-186-5p mimic showed reduced fluorescence intensity compared to other groups, indicating that miR-186-5p has a direct binding site in the XIAP 3'-UTR region and can targetedly inhibit XIAP expression. Therefore, XIAP is a direct target gene that co-regulates alcohol-induced cardiomyocyte apoptosis with miR-186-5p. To verify this inference, we transfected miR-186-5p mimic into alcohol-treated cardiomyocytes to upregulate its expression and found that XIAP expression was downregulated at both protein and RNA levels. Conversely, transfection with miR-186-5p inhibitor into alcohol-treated AC16 cardiomyocytes upregulated XIAP expression at both protein and gene levels.

In summary, alcohol intake induces apoptosis in AC16 cardiomyocytes in a dose- and time-dependent manner. The underlying mechanism involves upregulated miR-186-5p expression and downregulated XIAP expression in alcohol-treated cardiomyocytes, with miR-186-5p targeting XIAP to co-regulate alcohol-induced cardiomyocyte apoptosis. This study provides new therapeutic targets for ACM prevention and treatment.

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## Conclusions

1. Alcohol induces cardiomyocyte apoptosis, with apoptosis levels further increasing as alcohol concentration and treatment duration increase.
2. miR-186-5p regulates alcohol-induced cardiomyocyte apoptosis by targeting XIAP, functioning as a negative regulator that directly inhibits XIAP expression to modulate alcohol-induced AC16 cardiomyocyte apoptosis.
3. Alcohol intake upregulates microRNA-186-5p expression and downregulates XIAP expression in AC16 cardiomyocytes in an alcohol concentration- and time-dependent manner.

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## References

- [1] Naimi TS, Nelson DE, Brewer RD, et al. The intensity of binge alcohol consumption among U.S. adults. *American Journal of Preventive Medicine*, 2010, 38(2).
- [2] Steiner JL, Lang CH. Etiology of alcoholic cardiomyopathy: Mitochondria, oxidative stress and apoptosis. *International Journal of Biochemistry and Cell Biology*, 2017, 89:125-135.
- [3] Wang Z, Song J, Zhang L, et al. Increased expression of microRNA-378a-5p in acute ethanol exposure of rat cardiomyocytes. *Cell Stress and Chaperones*, 2017, 22(2).
- [4] Jing L, Lin C, Lu Y, et al. Investigation of microRNA expression profiles associated with human alcoholic cardiomyopathy. *Cardiology*, 2015, 130(4):223-233.
- [5] Lai Y, Guo H, Li J, et al. Comparison of surgical results in patients with hypertrophic obstructive cardiomyopathy after classic or modified Morrow septal myectomy. *Medicine (Baltimore)*, 2017, 96(51):9371.
- [6] Jiang J, Mo H, Liu C, et al. Inhibition of miR-186-5p contributes to high glucose-induced injury in AC16 cardiomyocytes. *Experimental and Therapeutic Medicine*, 2018, 15(1):627-632.
- [7] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 1976, 72:248-254.
- [8] Ji F, Liu Q, Feng Z. Genetic association between 1425G/A SNP in PRKCH in a Chinese population. *Oncotarget*, 2017.
- [9] Mu J, Zhang G, Xue D, et al. Sudden cardiac death owing to arrhythmogenic right ventricular review. *Medicine (Baltimore)*, 2017, 96(47):8808.
- [10] Dahraoui S, Uwingabiye J, Belarj B, et al. Unexpected discovery of multiple myeloma following cardiomyopathy. *Clinical Case Reports*, 2018, 6(1):86-90.
- [11] Adachi K, Ashiguchi S, Saito M, et al. Detection and management of cardiomyopathy in female dystrophinopathy carriers. *Journal of the Neurological Sciences*, 2017:74-80.
- [12] Baltrūnienė V, Bironaitė D, Kažukauskienė I, et al. The role of serum adiponectin for outcome prediction in patients with dilated cardiomyopathy and advanced heart failure. *BioMed Research International*, 2017:1-13.
- [13] Bollen IA, van der Meulen M, de Goede K, et al. Cardiomyocyte hypocontractility and reduced myofibril density in end-stage pediatric cardiomyopathy. *Frontiers in Physiology*, 2017, 8:1-12.

- [14] Buccheri D, Zambelli G. The link between spontaneous coronary artery dissection and takotsubo cardiomyopathy: Analysis of the published cases. *Journal of Thoracic Disease*, 2017, 9(12).
- [15] Fu KY, Zamudio R, Henderson-Frost J, et al. Association of caspase-1 polymorphisms with Chagas cardiomyopathy among individuals in Santa Cruz, Bolivia. *Revista da Sociedade Brasileira de Medicina Tropical*, 2017, 50(4):516-523.
- [16] Edison N, Curtz Y, Paland N, et al. Degradation of Bcl-2 by XIAP and ARTS promotes apoptosis. *Cell Reports*, 2017, 21(2):442-454.
- [17] Cesa LC, Shao H, Srinivasan SR, et al. X-Linked inhibitor of apoptosis protein (XIAP) is a client of heat shock protein 70 (Hsp70) and a biomarker of its inhibition. *Journal of Biological Chemistry*, 2018, 16(2):7.
- [18] Yang W, Zhou H, Yan Y. XIAP underlies apoptosis resistance of renal cell carcinoma cells. *Molecular Medicine Reports*, 2018, 17(1):125-130.
- [19] Edison N, Curtz Y, Paland N, et al. Degradation of Bcl-2 by XIAP and ARTS promotes apoptosis. *Cell Reports*, 2017, 21(2):442-454.

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