MicroRNA-17-mediated down-regulation of apoptotic protease activating factor 1 attenuates apoptosome formation and subsequent apoptosis of cardiomyocytes

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ABSTRACT

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Heart diseases such as myocardial infarction (MI) can damage individual cardiomyocytes, leading to the activation of cell death programs. The most scrutinized type of cell death in the heart is apoptosis, and one of the key events during the propagation of apoptotic signaling is the formation of apoptosomes, which further relay apoptotic signals by activating caspase-9. As one of the major components of the apoptosome, apoptotic protease activating factor 1 (Apaf-1) facilitates the formation of apoptosomes with cytochrome c (Cyto-c) and deoxyadenosine triphosphate (dATP). Thus, it may be possible to suppress the activation of the apoptotic program by down-regulating the expression of Apaf-1 using miRNAs. To validate this hypothesis, we selected a number of candidate miRNAs that, based on miRNA targeting prediction databases, were expected to target Apaf-1. Among these candidate miRNAs, we empirically identified miR-17 as a novel Apaf-1 targeting miRNA. The delivery of exogenous miR-17 suppressed the expression of Apaf-1 and consequently attenuated the formation of the apoptosome complex with caspase-9, as demonstrated by co-immunoprecipitation and

immunocytochemistry. Furthermore, miR-17 suppressed the cleavage of procaspase-9 and the subsequent activation of caspase-3, which is downstream of activated caspase-9. Cell viability tests also indicated that miR-17 pre-treatment significantly prevented the norepinephrine-induced apoptosis of cardiomyocytes, suggesting that the down-regulation of apoptosome formation may be an effective strategy to prevent cellular apoptosis. These results demonstrate the potential of miR-17 as an effective anti-apoptotic agent by suppressing Apaf-1 expression and warrant further studies to validate the anti-apoptotic effect of miR-17 *in vivo*.

Key words : Apaf-1, cardiomyocytes, apoptosome, miR-17

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I. INTRODUCTION

Heart diseases such as myocardial infarction (MI) have been the leading cause of death worldwide for many decades [1]. In extremely harsh conditions, such as myocardial ischemia where nutrients and the oxygen supply to the myocardium are deprived, individual cardiomyocytes are subjected to various cell death mechanisms [2]. Among the different types of cardiomyocyte death, apoptosis can be characterized by plasma membrane blebbing, chromatin condensation, nuclear fragmentation, and apoptotic body formation [3]. Apoptosis can be initiated by signals from outside the cell (extrinsic or death receptor pathway) or from inside the cell (intrinsic or mitochondrial pathway), and both pathways lead to the activation of cysteine proteases called caspases [4]. One of the pivotal events of the intrinsic pathway is the formation of the apoptosome.

Damage to mitochondria causes the permeabilization of the outer mitochondrial membrane, compromising membrane integrity [5], which, in turn,

promotes the release of cytochrome c (Cyto-c) from the mitochondria. This released cytochrome c then binds to apoptotic protease activating factor 1 (Apaf-1) to initiate apoptosome formation [6]. In the absence of Cyto-c, the folded WD-40 domain of Apaf-1 keeps Apaf-1 in its inhibited monomeric state [7]. However, in the presence of Cyto-c and deoxyadenosine triphosphate (dATP), the Cyto-c bound WD-40 domain is no longer folded, and the interaction between Apaf-1 and dATP exposes the caspase recruitment domain (CARD) [8]. The binding of dATP also allows the oligomerization of Apaf-1 into an Apaf-1 heptamer that facilitates downstream caspase activation [9]. Exposed CARD domains in the Apaf-1 heptamer interact with the initiator caspase procaspase-9, and as a result, an apoptosome holoenzyme is formed [10]. Once the apoptosome is formed, procaspase-9 is activated by being cleaved, and this step further activates executioner caspases such as casepase-3, triggering caspase-dependent apoptotic signaling cascades [11]. Such a critical role of the apoptosome in mitochondria-dependent apoptosis has made the apoptosome a potent therapeutic target for preventing the apoptosis of cardiomyocytes [12]. In the present study, we attempted to inhibit apoptosome formation by targeting Apaf-1 with microRNAs (miRNAs) to attenuate the apoptosis of cardiomyocytes exposed to norepinephrine (NE), which is known to induce apoptosis of cardiac cells [13,14].

miRNAs are short, non-coding RNAs that prevent the translation of or degrade target messenger RNAs (mRNAs) by binding to their 3' untranslated

regions (UTRs) [15]. Because miRNAs are known to be involved in a broad range of biological processes, it is not too far-fetched to assume that Apaf-1 could be regulated by miRNAs. In fact, Apaf-1 has been reported to be targeted by number of miRNAs, including miR-23a/b, miR-27a/b, and miR-24a [16,17]. Nevertheless, the multi-targeting nature of miRNAs strongly suggests that there should be more miRNAs targeting Apaf-1. Furthermore, because there is no firmly established way of suppressing apoptosis, particularly by targeting Apaf-1 with miRNAs, efforts to find alternative strategies such as finding different miRNAs targeting Apaf-1 are justified. Therefore, in the present study, we conducted screening for miRNAs targeting Apaf-1 based on both miRNA-targeting prediction databases and empirical data. After the selection of an Apaf-1-targeting miRNA, we further investigated whether this selected miRNA could prevent the apoptosis of cardiomyocytes *in vitro*.

II. MATERIALS AND METHODS

1. Isolation of rat cardiomyocytes

All experimental procedures for these animal studies were approved by the Committee for the Care and Use of Laboratory Animals, Yonsei University College of Medicine and performed in accordance with the Committee's Guidelines and Regulations for Animal Care. Neonatal rat cardiomyocytes from 1-2 day-old Sprague Dawley rat pups were isolated. Briefly, the hearts were minced into small pieces (~1 mm^3) and washed with Dulbecco's

phosphate-buffered saline solution (DPBS, pH 7.4 without Ca^{2+} and Mg^{2+}). The tissues were digested with 5 ml of collagenase II (0.8 mg/ml, 262 units/mg) for 5 min at 37°C. The cells were transferred to a new tube containing cell culture medium (a-MEM, containing 10% fetal bovine serum, Gibco BRL) and centrifuged at 1600 rpm for 3 min at room temperature (RT). The cell pellets were re-suspended in 5 ml of cell culture medium. The above processes were repeated 7–9 times until most of the tissue was removed. The cell suspensions were collected and incubated in 100-mm tissue culture dishes for 2 h to reduce fibroblast contamination. The non-adherent cells were collected and seeded. The cells were treated with 0.1 μM 5-bromo-20-deoxyuridine (BrdU, Sigma-Aldrich) to increase the purity of the cardiomyocytes. The cells were cultured with 10% FBS α-MEM in a 5% CO_2 incubator at 37°C. To induce cardiomyocyte apoptosis, the cells were treated with 100 μM of NE for 24 h [14].

2. Immunoprecipitation (IP)

For IP, cells were lysed in IP lysis buffer (Thermo, Waltham, MA, USA) for 30 min on ice. The cell lysates were centrifuged at 10,000 g for 20 min, and the supernatant was retained. Protein concentrations were determined using the BCA Protein Assay Kit (Thermo). After quantification, the lysates were incubated with Apaf-1 antibody (Santa Cruz Biotechnology, Dallas, TX, USA), rotating the tube for 1 h at 4°C. After 1 h, Dynabeads Protein G (Life Technologies, Carlsbad, CA, USA) were added to the lysates and incubated

overnight at 4°C. The Dynabeads-antibody-Apaf-1 complexes were centrifuged at 2,500 g for 30 min at 4°C, and the antibody-Apaf-1 complexes were eluted using an elution buffer containing 0.2 M Tris (pH 6.8), 2% SDS, 0.04% Coomassie blue, 40% glycerol and β-mercaptoethanol. The eluted proteins were subjected to Western blotting using primary antibodies specific for Apaf-1 (Santa Cruz Biotechnology) and Caspase-9 (Sigma, St. Louis, MO, USA).

3. Immunocytochemistry

Cells were cultured in four-well slide chambers, washed twice with PBS, and fixed in 1% paraformaldehyde solution for 10 min. The cells were then washed twice with PBS before permeabilization using 0.1% Triton X-100 for 10 min. Next, the cells were blocked for 1 h in blocking solution (2% bovine serum albumin and 10% horse serum in PBS) and incubated with Apaf-1 antibody (Santa Cruz Biotechnology) and Cytochrome C antibody (Santa Cruz Biotechnology). FITC-conjugated mouse, rabbit, and goat (Jackson ImmunoResearch Laboratories) secondary antibodies were then used. Immunofluorescence was detected by confocal microscopy (LSM710; Carl Zeiss).

4. Delivery of microRNA

Transfections of miRNA mimics were performed using the TransIT-X2 system (Mirus Bio LLC, Madison, WI, USA). Mature miRNA mimics and

miRNA inhibitors (Genolution Pharmaceuticals, Inc., Korea) were used at final concentrations of 100 nM and 50 nM, respectively. After 4 h of incubation in a CO_2 incubator at 37°C, the medium was changed to fresh medium.

5. Cell viability assay

To measure cell viability, cell counting kit-8 reagent (CCK-8, Dojindo) was added to each well to a final concentration of 0.5 mg/mL, and the cells were incubated at 37°C for 2 h. The absorbance at 450 nm was measured using a microplate reader.

6. Luciferase reporter assay

The 3'UTR sequence of Apaf-1 was amplified using Apaf-1 primers. The primer sequences were as follows: Apaf-1 sense, 5′- CTA GCT AGC AAA TGA GAG CTC ATT GCG TTA TGC-3′ and anti-sense, 5′- CCG CTC GAG GGC AAG CGC TCT ACC ACT AGC TA -3'. The 3'UTR fragment containing the miR-17 binding site was then cloned into the pmirGLO vector. Chinese hamster ovary (CHO) cells were plated at a density of 1 x 10^5 cell/well in a 12-well plate and then transfected with either pmirGLO control vector or pmirGLO vector with Apaf-1 3'UTR using Lipofectamine 2000. After 48 h, the relative luciferase activity was measured using the Dual Luciferase assay kit (Promega, Fitchburg, WI, USA) according to the manufacturer's instructions. Renilla luciferase was used for normalization.

7. Co-localization image analysis

The degree of co-localization was measured by ZEN 2009 Light Edition software (Carl Zeiss Microimaging GmbH, Germany) that calculated the percentage of co-localized pixels out of all pixels. The correlation R value can range from -1 to 1. A value of 1 indicates that the fluorescence patterns of the two molecules are perfectly matched [18].

8. Western blot

The cells were washed in PBS and lysed in lysis buffer (Danvers, MA, USA, Cell Signal Technology). Protein concentrations were determined using the BCA Protein Assay Kit (Waltham, MA, Thermo Science). Equal amounts of proteins were separated on a sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) and transferred to a polyvinylidene difluoride membrane (Millipore, Billerica, MA, USA). After blocking the membrane using Tris-buffered saline/Tween 20 (TBS-T, 0.05% Tween 20) and 10% skim milk for 1 h at RT, the membranes were incubated with the appropriate primary antibodies overnight at 4°C. The membrane was washed three times with 0.05% TBS-T for 10 min and then incubated for 1 h at RT with horseradish peroxidase-conjugated secondary antibodies. The bands were detected with an enhanced chemiluminescence (ECL) reagent (Santa Cruz Biotechnology). The band intensities were quantified using NIH Image J version 1.34e software.

9. Caspase activity

Caspase activity was measured using Caspase-Glo 3/7 Assay (Promega) according to the manufacturer's instructions. In brief, the cells were plated at a density of 3 x 10^3 cells/well in a 96-well plate. The cells were transfected with the microRNA mimic and microRNA inhibitor as previously described. After transfection, the 96-well plates containing the cells were removed from the incubator and equilibrated to RT. Caspase-Glo 3/7 reagent (100 μm) was added to each well of the 96-well plate containing 100 μl of blank. After 2 h, the contents of each well were gently mixed at 300-500 rpm for 30 sec and incubated for 2 h at RT. The luminescence of each sample was measured in a microplate using a Single-Tube Luminometer (Promega) as directed.

10. Tetra methyl rhodamine methyl ester (TMRM) staining

Mitochondrial membrane potential (Dcm) was determined using a tetramethylrhodamine methyl ester (TMRM) fluorescent dye (Invitrogen). Six hours after treatment, the cells were loaded with 200 nM TMRM for 30 min at 37°C in medium. Then, the cell pellets were washed with pre-warmed with PBS and re-suspended in PBS for analysis. The data were acquired using a BD ACCURI C6 cytometer (BD Biosciences) and analyzed with BD Accuri C6 Software, a flow cytometry program.

11. Annexin V/PI apoptosis assay

To determine the apoptosis of cells, the Annexin V-FITC/propidium iodide (PI) apoptosis detection kit (BD Biosciences) was used. Briefly, cells were collected and re-suspended in 200 ml of buffered medium. Annexin V solution (10 ml) was added to the cell suspension, which was then incubated for 15 min in the dark at RT. Then, 5 μl of PI was added, and the cells were analyzed by flow cytometry (BD ACCURI C6 cytometer, BD Biosciences). The number of cells per group was 10^4. Annexin V/PI double negative cells represented viable cells, Annexin V positive/PI negative cells represented early apoptotic cells, and Annexin V/PI double positive cells represented late apoptotic cells.

12. Statistical analysis

Quantitative data were expressed as the means ± S.E.M of at least 3 independent experiments. For statistical analysis, one-way ANOVA with Bonferroni correction was performed using the OriginPro 8 SR4 software (ver. 8.0951, OriginLab Corporation, Northampton, MA, USA) if there were more than 3 groups. A p value of less than 0.05 was considered to be statistically significant.

III. RESULTS

Figure.1

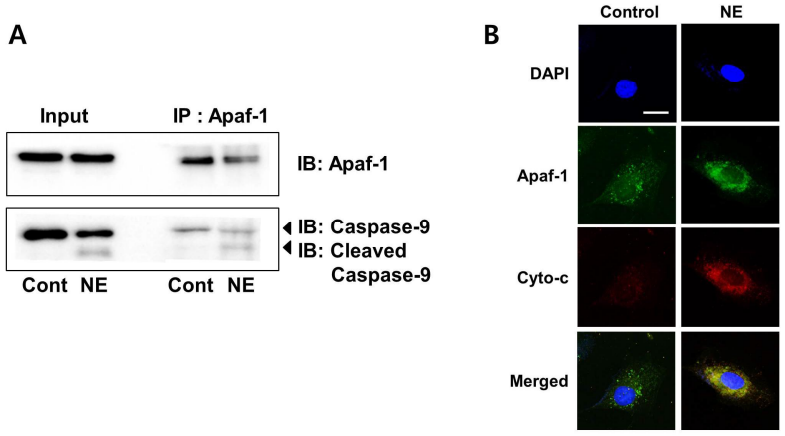

Fig.1 Apoptosome formation under apoptotic condition. A) Rat cardiomyocytes were treated with norepinephrine (NE, 100μM, 24 hr), and the cells were lysed and subjected to IP by using anti-Apaf-1 antibodies. The precipitates and 5% of the lysate (input) were subjected to Western blotting using anti-caspase-9 antibodies that detect both pro (uncleaved) casepase-9 and activated (cleaved) caspase-9. IP: immunoprecipitation, IB:immunoblotted. B) Immunocytochemisty for detecting Apaf-1 and cytochrome c (Cyto-c). DAPI was used to stain nucleus. Scare bar = 20μm.

1. Apaf-1-mediated apoptosome formation during apoptosis

The IP results indicated that the NE treatment activated caspase-9, as shown by an increase in the cleaved form of casepase-9 (Fig. 1A). Immunocytochemical analysis of the cells treated with NE showed the co-localization of Apaf-1 and Cyto-c, indicating NE-induced apoptosome formation in cardiomyocytes (Fig. 1B). These results demonstrated that NE induced apoptosome formation in cardiomyocytes, increasing the amount of activated caspase-9.

2. miR-17 targets Apaf-1

Figure.2

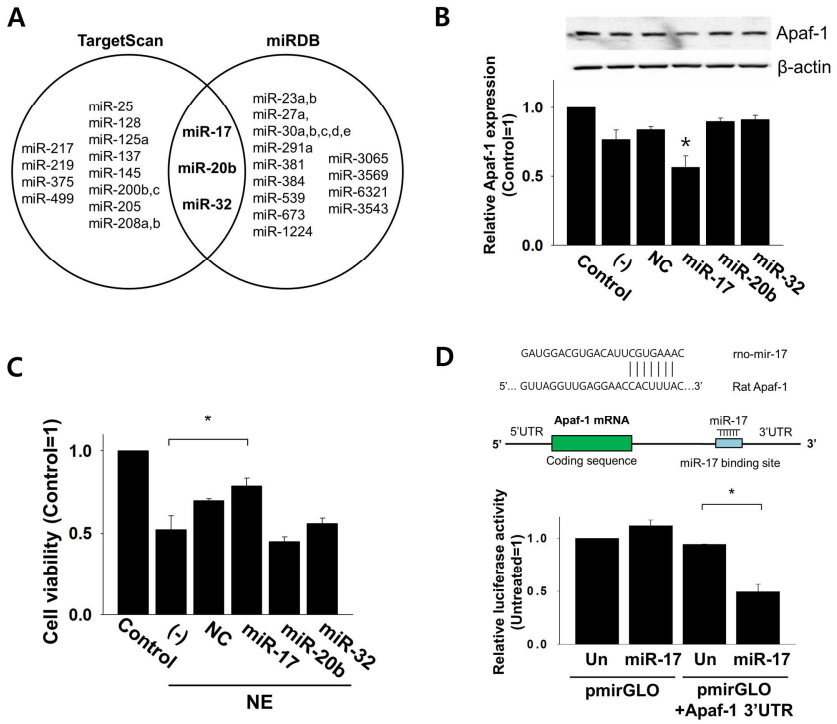

Figure.2 Screening of candidate miRNAs targeting Apaf-1. A) Rat Apaf-1 targeting miRNAs were 1st screened by two miRNA-target prediction databases: TargetScan and miRDB. miRNAs predicted by both databases were subjected to 2nd empirical validation. B) Cardiomyocytes were transfected with candidate miRNAs and the expression of Apaf-1 was detected by Western blotting. C) Rat cardiomyocytes were transfected with candidate miRNAs, and day after the transfection, the cells were treated with NE. Cell viability was measured using a cell counting kit. D) upper panel: miR-17 binding site in the 3'UTR of Apaf-1. bottom panel: luciferase assay was performed using pmirGLO vector containing the 3'UTR of Apaf-1. pmirGLO served as control. *$p<0.05$ compared to control. (-) indicates transfection reagent only. NC: negative control miRNA. All the quantitative data represent the means ± S.E.M of at least three independent experiments.

To find candidate miRNAs that target Apaf-1, two miRNA-target prediction databases were used (TargetScan: www.targetscan.org and miRDB: mirdb.org/miRDB). Three miRNAs (miR-17, miR-20b and miR-32) were predicted by both databases (Fig. 2A). Among the three miRNAs, miR-17 most significantly reduced Apaf-1 expression (Fig. 2B). Furthermore, miR-17

Figure. 3

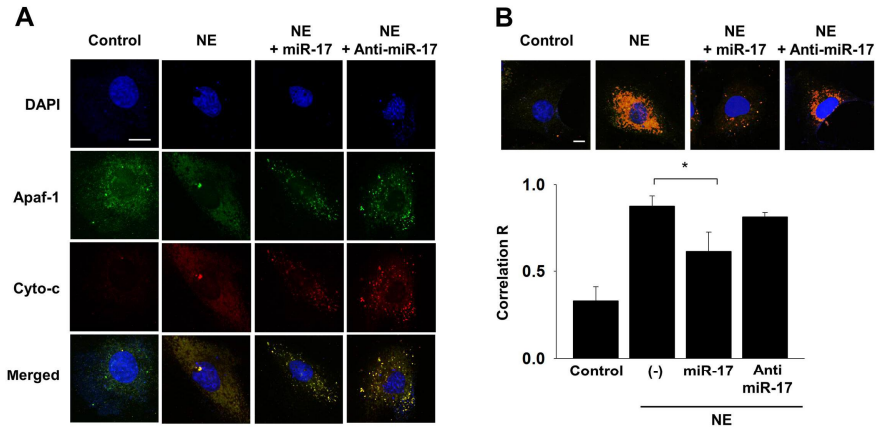

Fig.3 miR-17 suppresses NE-induced apoptosome formation. A) Rat cardiomyocytes were transfected with either miR-17 or anti-miR-17 prior to NE treatment. Immunocytochemistry using anti-Apaf-1 and anti-cytochrome c (Cyto-c) antibodies was performed. Scale bar = 20 μm. B) Co-localization of Apaf-1 and cytochrom-c (shown as orange) calculated. Scale bar = 10 μm. *p<0.05 compared to control. All the quantitative data represent the means ± S.E.M of at least three independent experiments.

significantly increased the cell viability of NE-treated cardiomyocytes, whereas the other candidate miRNAs did not (Fig. 2C). Sequence analysis of the 3'UTR of Apaf-1 indicated that it contains a putative miR-17 binding site (Fig. 2D, upper panel). The result of the dual-luciferase activity assay using the 3'UTR of Apaf-1 also indicated that miR-17 significantly decreased the expression of luciferase, demonstrating that miR-17 targets the 3'UTR of Apaf-1 (Fig. 2D, bottom panel).

3. miR-17 suppresses apoptosome formation under apoptotic conditions

NE increased co-localization of Apaf-1 and Cyto-c in the cytosol. However, miR-17 transfection prior to NE treatment suppressed such increased co-localization of Apaf-1 and Cyto-c (Fig. 3A). Additionally, to determine

Figure. 4

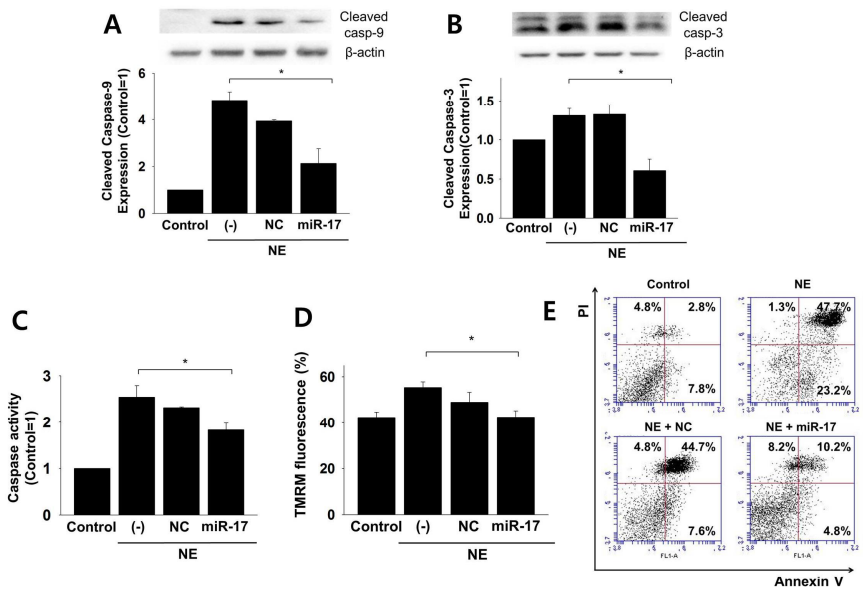

Fig. 4 Anti-apoptotic effect of miR-17 on NE-treated cardiomyocytes. A) Rat cardiomyocytes were transfected with or without miR-17 prior to NE treatment. Activated (cleaved) casepase-9 was examined by Western blotting. β-actin was used to normalize. B) Activated (cleaved) caspase-3 was detected by Western blotting. C) Caspase 3/7 activity assay was performed. D) Evaluation of mitochondria membrane permeability using TMRM. E) Flow cytometry using Annexin V and PI to for detecting cellular apoptosis. *p<0.05 compared to control. (-) indicates transfection reagent only. NC: negative control miRNA. All the quantitative data represent the means ± S.E.M of at least three independent experiments.

whether anti-miR-17 affected apoptosome formation, we transfected the cells with anti-miR-17 prior to NE treatment, and there was no significant increase of Apaf-1 or Cyto-c staining compared with the NE-only treated group. When the co-localization of Apaf-1 and Cyto-c was quantified, NE-induced an increase of Apaf-1/Cyto-c co-localization was significantly suppressed by miR-17 transfection performed prior to NE exposure (Fig. 3B). Again, no significant effect of anti-miR-17 on the co-localization of Apaf-1/Cyto-c was observed,

suggesting that the endogenous level of miR-17 in NE-treated cardiomyocytes may be negligible.

4. Anti-apoptotic effect of miR-17on NE-treated cardiomyocytes

NE significantly increased the amount of activated (cleaved) caspase-9, but this increase was significantly suppressed by miR-17 pretreatment (Fig. 4A). Additionally, the expression of activated (cleaved) caspase-3, which is produced by activated caspase-9, was significantly suppressed by miR-17 pretreatment (Fig. 4B). In agreement with these data, the NE-induced caspase activity was significantly suppressed by miR-17 pretreatment (Fig. 4C). In addition, we measured permeability changes of the mitochondria membrane using TMRM. NE increased the permeability of the mitochondrial membrane compared with the untreated controls, but this NE-induced increase of the permeability of the mitochondrial membrane was significantly suppressed by pretreatment with miR-17 (Fig. 3D). Lastly, the results of flow cytometry using Annexin V/propidium iodide (PI) to evaluate apoptosis showed that NE increased the number of Annexin V/PI double positive cells, indicating that NE induced the apoptosis of cardiomyocytes. However, the NE-induced apoptosis of cardiomyocytes was suppressed by miR-17 pretreatment, suggesting that miR-17 effectively prevented the NE-induced apoptosis of cardiomyocytes (Fig. 4E).

IV. DISCUSSION

Because the formation of the apoptosome facilitates the propagation of apoptotic signaling cascades, a therapeutic approach to down-regulate the formation of apoptosomes may prevent the apoptosis of cardiomyocytes in pathologic conditions of the heart. Here, we report that exogenous miR-17 delivery attenuated the NE-induced apoptosis of cardiomyocytes by targeting Apaf-1, one of the major components of the apoptosome complex.

The initiation of apoptosome formation is triggered by the binding of mitochondria-released Cyto-c to Apaf-1 [6]. Our data suggest that although miR-17 did not completely inhibit the expression of Apaf-1, miR-17 significantly decreased the formation of apoptosomes. However, there was one interesting result of the IPs. Because the amount of Apaf-1 in the input did not show any significant difference between the control and NE-treated groups, the decrease of Apaf-1 in the NE-treated group compared with control after the IP was perplexing. There are two possibilities to account for this phenomenon. First is the size of the apoptosome. There have been reported to be two main sizes of the apoptosome complex, ~700 kDa and ~1.4 MDa [19]. Because these complexes are too large to be resolved by common SDS page gel (8~12%), incomplete denaturation of those large complexes during the IP could have caused a decrease in the Apaf-1 detected by common western blotting. Another possibility is the cleavage of Apaf-1 by caspase-3 during apoptosis [20]. Nevertheless, the amount of activated (cleaved) caspase-9 in the NE-treated

group increased, which clearly indicated NE-induced apoptosome formation, validating the adequacy of the *in vitro* NE-mediated apoptosis system utilized in the present study.

The concept of preventing apoptosis by inhibiting apoptosome formation has been experimentally verified, and various approaches such as using the short isoform of caspase-3 [21], amyloid [22], and taurine [12] have been utilized to inhibit apoptosome formation. Among the individual components of apoptosomes, Apaf-1 is one of the most frequently studied for suppressing apoptosome formation and subsequent apoptosis. The lack of Apaf-1 has been reported to prevent the ultraviolet-induced apoptosis of fibroblasts [23], with the inhibition of Apaf-1 by chemical inhibitors producing cytoprotective effects [24,25]. These studies suggest that down-regulating Apaf-1 to prevent apoptosis is a well-grounded therapeutic approach.

Employing miRNAs is one of the most recent approaches to down-regulate the expression of Apaf-1. miRNA-24a has been reported to negatively regulate caspase-9 and Apaf-1 during the normal development of neural retina, suppressing apoptosis [16]. Additionally, miR-23a/b and -27a/b have been reported to target Apaf-1 and repress hypoxia-induced neuronal apoptosis [17], and these miRNAs constitute the miR-23/24/27 family with miR-24, which is known to be involved in the regulation of angiogenesis and apoptosis during cardiac ischemia [26]. Nevertheless, the role of the miR-23/24/27 family in regulating apoptosis is difficult to generalize because this family of miRNAs has also been

reported to possess pro-apoptotic effects [27]. These observations suggest that the effect of miRNAs, even in a specific pathologic condition, can vary depending on the context in which they operate, and thus the use of miRNAs for therapeutic applications should be meticulously designed to produce the desired effects.

V. CONCLUSION

In the present study, we have demonstrated that miR-17 attenuated the NE-induced formation of apoptosomes by targeting Apaf-1, thus providing evidence that the delivery of exogenous miR-17 may be an effective anti-apoptotic therapy.

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ABSTRACT(IN KOREAN)

마이크로 RNA-17에 의한 Apaf-1 발현억제를 통한 심근세포에서의 apoptosome 형성억제 및 세포고사 억제

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심근경색과 같은 심장질환은 개별적인 심근세포의 손상을 유발하여 결국 세포사멸 신호를 활성화 시킬 수 있다. 가장 많이 연구가 된 세포사멸 기전으로는 세포고사 (apoptosis)가 있으며 이러한 세포고사의 과정에서 일어나는 중요한 이벤트 중 하나는 세포고사 소체 (apoptosome) 의 형성이다. 이러한 세포고사 소체의 주요 구성 요소단백 중 하나로는 Apaf-1 (apoptotic protease activating factor 1) 이 있으며 이 단백질은 cytochrome c 및 dATP 와 함께 세포고사 소체를 구성하게 된다. 따라서 마이크로 RNA를 이용하여 Apaf-1의 발현을 억제하면 세포고사 소체의 형성을 저해하여 결과적으로 세포고사 신호전달을 억제할 수 있을 것으로 기대된다. 이러한 가설을 검증하기 위해 본 연구에서는 마이크로RNA의 표적 단백을 예측해주는 데이터베이스를 이용하여 Apaf-1을 표적 할 것으로 예측되는 후보 마이크로 RNA를 우선 선정하였으며 추가적인 실제 실험을 통해 miR-17을 Apaf-1을 효과적으로 억제하는 마이크로 RNA로 선별하였다. 본 연구 결과에 따르면 외부에서 miR-17을 전달한 경우 Apaf-1의 발현이 억제되었으며 결과적으로 세포고사 소체의 형성이 억제되었다. 추가적으로 miR-17의 전달이 세포고사 신호 전달에서 중요한 역할을 하는 caspase-9의 전구 물질인 procaspase-9의 활성화를 억제하였고 그 하위 중요 인자인 caspase-3의 활성 역시 억제하는 것을 확인하였다. 세포생존도 측정 결과 역시 miR-17 전처리를 통해 노르에피네프린 (norepinephrine)에 의해 유도된 심근세포의 세포고사를 억제할 수 있음을 보여주었다. 본 연구의 결과는 miR-17이 효과적인 세포고사 억제제가 될 수 있음을 보여주었으며 이러한 효과의 생체 내 검증을 위한 동물 실험이 향후 진행되어야 할 것으로 생각된다.

핵심되는 말 : Apaf-1, cardiomyocytes, apoptosome, miR-17

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