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Original Research

Platelet-derived miR-142-3p induces apoptosis of endothelial cells in hypertension

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Abstract: The dysfunction of endothelial cells (ECs) plays crucial roles in vascular remodeling during hypertension. Researches suggested that ECs are regulated by the circulating platelets in vivo, which may participate in abnormal EC apoptosis in hypertension. However the molecular mechanism in this process is still unclear. Here we focused on the microRNAs (miRs) in platelets, and detected the potential role and delivery mechanism of platelet-derived miRs in ECs. Using microarray, the differentially expressed profile of miRs between platelets and ECs was detected. The results revealed that compared with ECs, 67 miRs highly expressed in platelets including the most significant one- miR-142-3p. Since platelets are activated by thrombin in hypertension, we detected the miR-142-3p transferring mechanism of activated platelet, and proved that platelet-derived microparticles (PMPs), but not platelets directly, delivered miR-142-3p into ECs via cellular adherent. Furthermore, BCL2L1, an important molecule in cell apoptosis, was predicted to be a putative target of miR-142-3p by multiple algorithms. Dual luciferase reporter assays, as well as miR-142-3p mimics treatment were used to confirm the interplay between miR-142-3p and BCL2L1. Meanwhile, using in vivo hypertensive rat model, our results showed that the expression of platelet-derived miR-142-3p and the apoptosis were both significantly increased in ECs during hypertension. The present results suggested that platelet-derived miR-142-3p is delivered into ECs via PMPs, and may modulate the expression of target molecule- BCL2L1, which may subsequently display a negative function by modulating EC apoptosis in hypertension.

Key words: Endothelial cells; Apoptosis; Platelet-derived microparticles; microRNA-142-3p; Hypertension.

Introduction

Endothelial cells (ECs), located at the intima, are important cellular components of the vessel wall. It had been widely proved that normal functions of ECs are critical for vascular homeostasis, while various pathological factors such as smoking, high cholesterol, high homocysteine and hypertension (1), induces the dysfunctions of ECs which participates in the subsequent vascular remodeling (2). In hypertension, ECs in artery undergoes disorganization and dysfunctions, which involves the pathologically increased apoptosis and the loss of microvascular rarefaction (3).

Growing researches had proved that ECs are exposed to complex physical and biochemical situations *in vivo* during hypertension. As the interface between the blood flow and the vessel wall, ECs are modulated by both the neighboring VSMCs and the circulating components (4, 5). In the recent years, the role of circulating platelets in EC functions gained more and more attention. Platelets had been widely reported to play crucial roles in thrombosis (6). Whereas, recent researches also proved that platelets participate in physiological hemostasis, as well as pathological dysfunctions of ECs under different conditions. In hypertension, the plasma concentrations of many inflammatory factors, such as thrombin (7), 5-HT (8), and ox-LDL (9), are significantly increased which abnormally activated circulating platelets. The enhanced production of thrombin in hypertension induces platelets abnormal activation through targeting on protease-activated receptor-1 (PAR1) (10) and other receptors (11). It had also been proved that activated platelets trigger various inflammatory reactions and apoptosis of ECs (12). Although studies had suggested that activated platelets induce EC dysfunctions, which may be an important risk factor of vascular remodeling during hypertension, the molecular mechanism in this process is still unclear.

Interestingly, research revealed that activated platelets release a membrane-contained vesicles- the platelet-derived microparticles (PMPs), and the amount of circulating PMPs is markedly increased in hypertension (13). PMPs are the most abundant subtype of microparticles in the circulation, which are small extracellular vesicles ranging from 0.1 to 1 μm in diameter (5). The significance of PMPs lies in their capability of delivering their contents to the recipient cell, including leukocytes and ECs, and thereby modulates cellular functions (14). In the past decade, abundant proteins and microRNAs (miRs) were proved to be the important contents of PMPs. Recent research showed that PMPs deliver miR-223 to ECs and repressed the expression of target mRNA, i.e. FBXW7 and EFNA1 (5). However, the roles of miRs from platelets in EC function during hypertension are not clarified.

In the present study, we demonstrated the profile of miRs expressed in platelet, and revealed specific miRs in platelets compared with ECs. Focused on the most differentially expressed miR-142-3p, we further detected its possible role in apoptosis of ECs in hypertension. The study may give a new insight into understanding the mechanism of EC dysfunction during hypertension, and may provide novel targets on maintaining EC hemostasis.

Materials and Methods

Hypertensive rat model

The animal care and experimental protocols were in accordance with the Animal Management Rules of China (Documentation 55, 2001, Ministry of Health, China), and the study was approved by the Animal Research Committee of Shanghai Jiao Tong University.

The hypertensive rat model was established by abdominal aortic coarctation (15). Male Sprague-Dawley (SD) rats weighing 200 ± 15 g were anesthetized with isoflurane inhalation. The abdominal aorta was exposed at 5 mm above artery bifurcation to the left kidney. A needle with an outer diameter of 0.9 mm was placed in parallel to the aortic segment and surgical silk suture (3-0) was used to surround the aorta. After tying the needle and aortic segment together with the suture, the needle was retrieved which leaves a lumen with a diameter of 0.9 mm at the constriction site. Sham-operated rats were treated with the same procedure except there was no constriction of the aorta. Blood pressure (BP) was measured through a catheter introduced into one of the carotid arteries (16).

Cell culture and platelet preparation

Primary rat aortic ECs were obtained by the digestive method (14). ECs were cultured in EGM™-2 Endothelial Cell Growth Medium-2 (Lonza) and characterized by immunohistochemical staining for the EC marker, von Willebrand factor (DAKO). ECs with passages 2-4 and cell populations with more than 95% purity were used.

Blood was collected from the abdominal aorta of isofluorane-anesthetized rat, and transferred into syringes containing 100 μL/mL white anticoagulant (2.94% sodium citrate, 136 mM glucose [pH 6.4]), 0.1g/mL PGE1, and 1 U/mL apyrase). Washed platelets were prepared by centrifugation of the platelet-rich plasma (PRP) containing 5 mM EDTA at 1,100 g for 10 minutes. Platelets were resuspended in modified Tyrode solution (12 mM $NaHCO_3$, 138 mM NaCl, 5.5 mM glucose, 2.9 mM KCl, 2 mM $MgCl_2$, 0.42 mM NaH_2PO_4, 10 mM HEPES, pH 7.4) after 2,100 g for 10 minutes.

Production of PMPs and ECs-PMP coincubation assays

Platelets were activated with 0.1 U/ml thrombin for 1 hour, while platelets treated with same volume of HEPES-Tyrode buffer were used as control. PMPs in the supernatant were obtained by centrifugation at 20,500 g for 90 minutes, and resuspended in HEPES-Tyrode buffer.

ECs were seeded on 6-well plates at a density of 2×10^5 cells per well. PMPs (2×10^9 per well) were added into the medium and incubated for 1 or 6 hours at 37 ºC under 5% CO_2 in a humidified atmosphere.

To confirm the transfer of miR, 1 U / ml RNase, which degrade miRs, was used to pretreat platelets or PMPs for 1 hour at 37 ºC before stimulating ECs.

To analyze the adhesion of PMPs to ECs, PMPs were labeled with PKH67 Fluorescent Cell Linker Kits (Sigma-Aldrich). The adhesion and internalization was evaluated by confocal microscopy (Olympus FV1000, Japan) analysis.

MiRNAs microarray analysis

Total RNA was harvested using TRIzol (Invitrogen) and miRNeasy mini kit (QIAGEN) according to manufacturer's instructions. After having passed RNA quantity measurement using the NanoDrop 1000, the samples were labeled using the miRCURY™ Hy3™/Hy5™ Power labeling kit and hybridized on the miRCURY™ LNA Array (v.18.0). Following the washing steps, the slides were scanned using the Axon GenePix 4000B microarray scanner.

Scanned images were then imported into GenePix Pro 6.0 software (Axon) for grid alignment and data extraction. Replicated miRs were averaged and miRs that intensities >=30 in all samples were chosen for calculating normalization factor. Expressed data were normalized using the Median normalization. Volcano Plot filtering showed the significant differentially expressed miRs with both fold chang >=2 and P-value <0.05 between EC and platelets. Finally, hierarchical clustering was performed using MEV software (v4.6, TIGR) to show distinguishable miRNA expression profiling among samples.

Ingenuity Pathway Analysis

The relevant biology function with significant P values ($P < 0.05$) were generated by the Ingenuity Pathway Analysis (IPA) server (http://www.ingenuity.com/products/ipa), which integrated more than 5 million manually curated biological findings and 1.5 million interaction relationships derived from the Ingenuity Pathways Knowledge Database Literature (17).

MiRs transfection

rno-miR-142-3p mimics as well as corresponding negative control (NC) were transfected into ECs for miR-142-3p overexpression by using Lipofectamine™ 2000 (Invitrogen) according to the manufacturer's instructions. After 48-hour incubation, cells were harvested for further research. The sequence of rno-miR-142-3p mimics was 5'- UGUA GUGU UUUC CUAC UUUA UGGA -3' (forward) and 5'- CAUA AAGU AGGA AAAC ACUA CAUU-3' (reverse). Non-silencing siRNA with no known homology to rat genes was synthesized as a negative control (NC). The sequence of NC was 5'- UUCU CCGA ACGU GUCA CGUT T -3' (forward) and 5'- ACGU GACA CGUU CGGA GAAT T -3' (reverse).

qPCR

Total RNA was isolated using TRIzol reagent (Invitrogen). For the isolation of RNAs from PMPs, Caenorhabditis elegans miR-39 (cel-miR-39) was added to each sample as a spike-in control. Isolated RNAs were reversely transcribed into complementary DNA with the M-MLV RT system using the RT primer for the respective miRs (GenePharma). qPCR was performed with the SYBR Green Supermix (TaKaRa), and the levels of precursor and mature miRs in ECs were normalized against the control U6 snRNA in ECs or cel-miR-39 in PMSs by the $2^{-\Delta\Delta CT}$ method.

Western blotting

ECs were gently washed with cold PBS and were lysed at 4°C for 5 min with lysis buffer (0.15 M Tris, pH

6.8, 1.2% SDS, 15% mercaptoethanol). Lysates were subjected to electrophoretic separation by 10% SDS-PAGE and transferred to nitrocellulose membranes (Hybond, Amersham). Western blots were performed using antibodies directed against BCL2L1 (1:500, Cell Signaling Technologies) and β-tubulin (1:1000, Cell Signaling Technologies). After incubation with alkaline phosphatase-conjugated secondary antibodies (Jackson Immunoresearch), the signals were visualized by nitro-blue tetrazolium-bromochloroindolyl phosphate (Bio Basic, Inc.), and quantified with Quantity One software (Bio-Rad).

Dual luciferase reporter assay

The conserved miR-142-3p-binding sequences in 3' untranslated region (UTR) of BCL2L1 and the mutation on target site were both obtained by gene synthesis, and then inserted into the downstream of the luciferase reporter gene (psiCHECK-2, Promega) respectively. To determine suppression efficiency of miR-142-3p, human HEK-293T cells were transfected with the reporter plasmid or the mutated vectors together with miR-142-3p mimics and NC respectively. Twenty-four hours or 48 hours later, firefly and Renilla luciferase activities were measured consecutively by using the dual luciferase reporter assay system (Promega).

Apoptosis Assay

For in vivo assay, Cleaved Caspase-3 ELISA kit (Cell Signaling Technology, USA) was used to assess apoptosis of ECs in rat model according to the manufacturer's instructions. Vascular ECs were treated with lysis buffer (with 1 mM PMSF freshly added) on ice. Lysates were transfer to appropriate tubes and then centrifuge for 10 minutes at 14,000 rpm, 4°C. The absorbance at 450 nm was measured in an ELISA plate reader (Bio-Rad 680).

Statistical analysis

Each experiment was performed at least four times, and all values are expressed as the mean ± SD. Student's t test was used to compare the results between two groups. Values of $P < 0.05$ were considered statistically significant.

Results

Platelets contain abundant differentially expressed miRs in comparison with ECs

To demonstrate the potential roles of platelet-derived miRs on ECs, the miRs significantly and highly expressed in platelets were firstly detected by using miR microarray.

In Fig. 1A, volcano plot showed that compared with ECs, 67 miRs (red dots) were highly expressed (more than 2-fold) in platelets. Whereas, there were 111 miRs (green dots in Fig. 1A) highly expressed in ECs compared with that in platelets. Fig. 1B showed the top 10 miRs that enriched in platelets, and miR-142-3p was the most significant one.

These results suggested that platelets contain abundant miRs and some of them are ECs depleted.

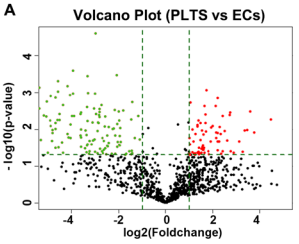
IPA predicates the biological functions of platelet-derived miRs

Use of IPA software, the relevant diseases, functions and potential regulated molecules of the top ten enriched miRs in platelets were analyzed. Fig. 2A revealed the potential diseases and functions in which the platelet-derived miRs may be involved in, which include the cancer, cardiovascular disease, cell circle, cell death and survival, cell morphology, and et al. Meanwhile, the targeting molecules modulated by platelet-derived miRs were classified into transcription factor, enzyme, kinase, transmembrane receptor, peptidase, and et al (Fig. 2B).

To detect whether platelet-derived miRs participate in modulating EC functions, miR-142-3p the most enriched miRs in platelet was selected for further research.

PMPs effectively deliver miR-142-3p to ECs

To determine the process and potential biological functions of the miRs-containing platelets, activated platelets were used to stimuli ECs in order to detect whether platelets deliver containing miRs directly into ECs or not. qPCR assay revealed that, after platelet-incubation for 12 hours, the expression of miR-142-3p and the positive control miR-223-3p were not significantly



Top 10 differentially expressed miRNAs between PLT and EC (ordered highest to lowest and P value significance)

miRs	fold changes	p-value	signal tensity(PLT/ECs)
miR-142-3p	1531.1116	0.0496	52283.25/35.5
miR-199a-5p	1128.3942	N/A	2410/-0.5
miR-199a-3p	755.6676	0.0179	4169.25/5.75
miR-142-5p	592.2267	0.0014	11011/18.75
miR-26b-5p	160.6981	0.0281	22501/141.25
miR-223-3p	156.7053	0.0365	14210.75/85.75
miR-146b-5p	140.3709	0.0002	5566/42.5
miR-26a-5p	134.7264	0.0197	7940.25/61
miR-146a-5p	114.8137	0.0119	4990/46
miR-20b-5p	64.2724	0.0001	3836.25/58.5

Figure 1. Microarray revealed the miRs differentially expressed between platelets (PLT) and ECs. **(A)** Volcano plot shows that 67 miRs were higher in platelets than that in ECs (more than 2-fold, red dots), and 111 miRs were lower in platelets than that in ECs (more than 2-fold, green dots). **(B)** The top 10 miRs that highly expressed and enriched in platelets with respective fold change, p-value and signal tensity compared to ECs.

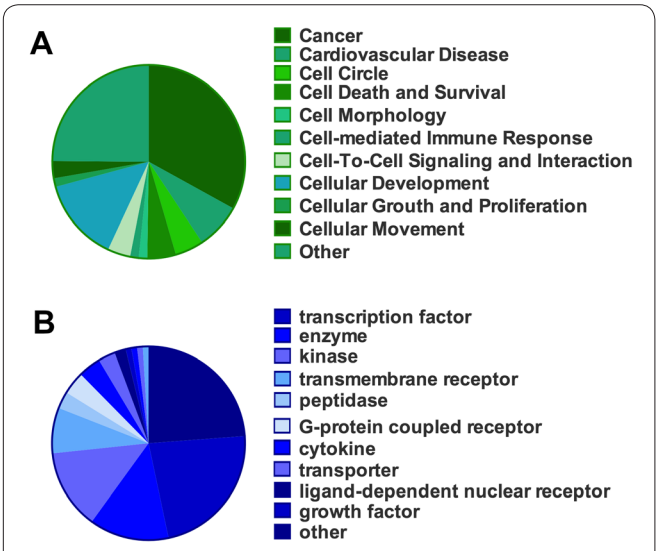


Figure 2. The involved functions and targeting molecules of the top 10 platelet-derived miRs showed in Fig. 1B use ingenuity pathway analysis (IPA). **(A)** The diseases and functions in which platelet-derived miRs were involved. **(B)** The classification of molecules related with platelet-derived miRs.

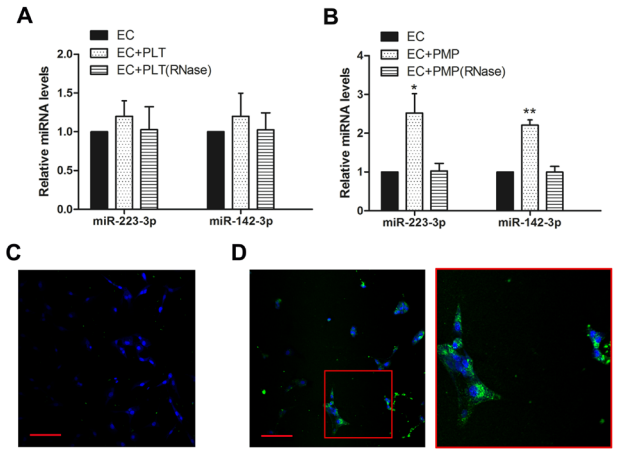


Figure 3. Delivery of platelet-derived miR-142-3p into ECs by PMPs. **(A)** After incubated with activated platelet (PLT) (with or without 1U/ml RNase) for 12 hours, the expression of miR-142-3p and miR-223-3p in ECs were not significantly changed. The experiment has been performed in quadruplicate and the data are shown as mean ± *SD*. * $P < 0.05$ *v.s.* control. **(B)** After incubated with platelet-derived microparticles (PMPs) (with or without 1U/ml RNase) for 12 hours, the expression of miR-142-3p and miRNA-223-3p in ECs were significantly increased which could be reversed by RNase pretreatment. The experiment has been performed in quadruplicate and the data are shown as mean ± *SD*. *$P < 0.05$ *v.s.* control. **(C)** PMPs incubated with ECs for 15 minutes, and there was not observed adhesion of PMPs to ECs. Green fluorescence is the labeled PMPs using PKH67 Fluorescent cell linker; blue fluorescence is the labeled EC nuclei using DAPI. Scale bar = 100 μm. **(D)** PMPs incubated with ECs for 1 hour, and PMPs obviously enriched in the cytoplasm of ECs. The right image is the magnified part squared in the left image. Green fluorescence is the labeled PMPs using PKH67 Fluorescent cell linker; blue fluorescence is the labeled EC nuclei using DAPI. Scale bar = 100 μm.

changed in ECs (Fig. 3A), which suggested that platelet cannot deliver the containing miRs into ECs directly.

Researches had reported that activated platelets secrete PMPs which deliver growth factors and miRs into reception cells, and modulate the genes expression and cellular functions (5). Hence, we detected whether PMPs participate in the miRs transfer between platelets and ECs. qPCR showed that, after incubated with PMPs for 12 hours, the expression of miR-142-3p and miR-223-3p (positive control) in ECs were markedly increased in ECs (Fig. 3B). Furthermore, this effect of PMPs could be reversed by the pretreatment of RNase (1U/ ml) (Fig. 3B).

Taken together, these results implicated that PMPs, but not platelets, deliver platelet-derived miRs, including miR-142-3p, into ECs.

The adhesion and internalization of PMPs to ECs

To confirm the communicational role of PMPs between ECs and platelets, PMPs were incubated with ECs for 15 minutes (Fig. 3C) and 1 hour respectively (Fig. 3D). PKH67 Fluorescent cell linker, the living cell membrane labeling reagent, was used to trace PMPs.

As shown in Fig. 3C and Fig. 3D, fluorescent PMPs did not adhere to ECs after 15-minute incubation, while after 1-hour incubation, PMPs obviously enriched in the cytoplasm of ECs. The results suggested that PMPs could adhere to ECs and may trigger the PMP internalization of ECs or membrane fusion between ECs and PMPs, which may participate in the delivery of platelets-derived miRs into ECs.

Platelet-derived miR-142-3p targets on endothelial BCL2L1

To determine whether platelet-derived miR-142-3p delivered by PMPs has a biological function in ECs, we analyzed the potential target genes of miR-142-3p using three computer-aided algorithms, i.e. TargetScan (http://www.targetscan.org/vert_71/), miRanda (http://www.microrna.org/microrna/home.do), and PicTar (http://pictar.mdc-berlin.de/). Basing on potential genes predicted by all these three algorithms, BCL2L1 were predicted as a target gene of platelet-derived miR-142-3p (Fig. 4A).

To confirm whether miR-142-3p bines with the 3' UTR of BCL2L1 and leads to translational repression of the molecules, the dual luciferase reporter assay was performed to validate the results of algorithm prediction. Segment encompassing the proposed miR-142-3p target site, the mutation for target site (Fig. 4A) and the positive control were inserted into the downstream of the luciferase reporter gene respectively. There were no obvious changes of luciferase activity in HEK-293T cells co-transfected with NC and BCL2L1-3' UTR,

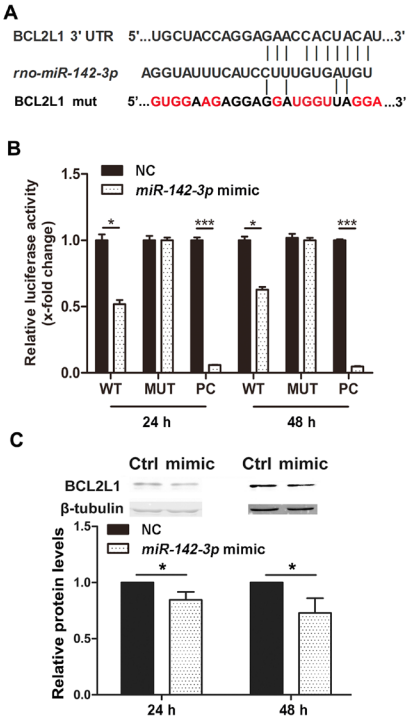


Figure 4. miR-142-3p targets BCL2L1. **(A)** Sequence alignment between rno-miR-142-3p and its putative binding sites in the 3'-UTR of rat BCL2L1 mRNA. The mutations on the binding sites (BCL2L1 mut) were marked with red color. **(B)** Luciferase activity in wild-type BCL2L1 3'-UTRs (WT), mutant BCL2L1 3'-UTRs (MUT) and positive control (PC), which were cotransfected with negative control (NC) and miR-142-3p mimics in HEK-293T cells. miR-142-3p significantly reduced luciferase levels of the BCL2L1-3'UTR luciferase reporter. The experiment has been performed in triplicate and the data are shown as mean ± *SD*. *$P < 0.05$ *v.s.* control. **(C)** Western blot indicated that in ECs, miR-42-3p mimics greatly reduced protein expression of BCL2L1. The experiment has been performed in quadruplicate and the data are shown as mean ± *SD*. *$P < 0.05$ *v.s.* control.

BCL2L1-3' UTR mutation, NC and BCL2L1-3' UTR mutation compared respectively with that transfected with BCL2L1-3' UTR alone (Fig. 4B).

More than 40% reduction of luciferase level was observed in HEK-293T cells co-transfected with miR-142-3p mimics and BCL2L1-3' UTR compared with that co-transfected with NC and BCL2L1-3' UTR. Besides, luciferase level was restored in HEK-293T cells co-transfected with miR-142-3p mimics and BCL2L1-3' UTR mutation compared with that co-transfected with mimics and BCL2L1-3' UTR (Fig. 4B).

Furthermore, to detect the potential effect of platelet-derived miR-142-3p on ECs, miR-142-3p mimics were transfected to mimic the delivery from PMPs. It revealed that miR-142-3p specific mimics significantly reduced the expression of BCL2L1 in ECs (Fig. 4C).

These results confirmed the negative regulation of miR-142-3p on BCL2L1 expression which suggested that platelet-derived miR-142-3p may induce EC functions via the target gene BCL2L1.

Hypertension increased the expression of platelet-derived miR-142-3p and increased the apoptosis of ECs

The hypertensive rat model was used to detect platelet-derived miRs expression and the apoptosis of ECs in vivo. Aorta and blood were isolated after 1-week coarctation. The blood pressure of each individual was detected, and the mean arterial pressure of hypertensive rat was significantly higher than that of the sham control (Fig. 5A). Detecting with cleaved-Caspase3 ELISA assay, EC apoptosis was markedly increased by hypertension (Fig. 5B). Compared with the sham control, the expression of miR-142-3p in circulating PMPs isolated from hypertensive rats was significantly increased (Fig. 5C). The basic expression of PMP-derived miR-142-3p in normal ECs was very low, and the expression of PMP-derived miR-142-3p in hypertensive rats was also significantly increased in ECs compared with that in the sham control (Fig. 5D).

These results indicate that during hypertension miR-142-3p is enriched in circulating PMPs and is delivered into ECs, which may subsequently affect ECs apoptosis.

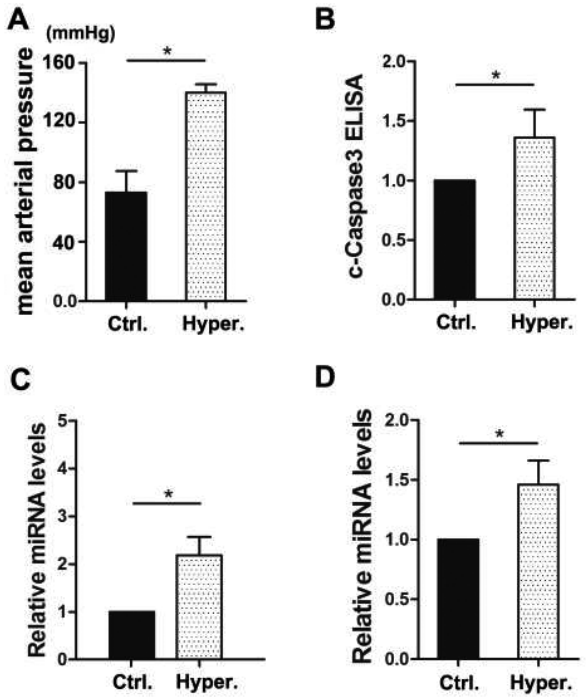


Figure 5. Hypertension increased apoptosis and miRNA-142-3p expression in ECs expression. (A) The mean arterial pressure of hypertensive rat (Hyper) was significantly higher than that of the sham control (Ctrl). **(B)** Compared with the sham control, the apoptosis of ECs in thoracic aorta of hypertensive rat was significantly increased. **(C)** Compared with the sham control, the expression of miRNA-142-3p in circulating PMPs isolated from hypertensive rats was significantly increased. **(D)** Compared with the sham control, the expression of miRNA-142-3p in ECs in thoracic aorta of hypertensive rat was significantly increased. The experiment has been performed at least in quadruplicate and the data are shown as mean ± *SD*. **P* < 0.05 *v.s.* control.

Discussion

Platelets are small (1.5 -3μm), anucleate cell fragments released from bone marrow precursor megakaryocytes (18). Increasing evidences proved that platelets have central roles in the development of acute thrombotic events during several cardiovascular diseases, such as acute coronary syndrome and stroke (19). Researches also revealed that in hypertension platelets are activated by thrombin and the activated platelets furthermore release many kinds of cytokines and signaling molecules, which subsequently trigger a complex intercellular communication and induce dysfunction of other circulating cells and vascular cells (19). Andreassen et al reported that activated platelets release solute ligand that interacts with CD40, which is constitutively expressed on B cells, macrophages, vascular smooth muscle cells, and ECs, and results in various inflammatory responses (20). In pulmonary hypertension, platelets adhere to the sub-endothelial basement membrane at sites of blood vessel injury, and are activated to secret vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF) to modulate EC functions (21). Interestingly, besides the cytokines and growth factors, the other kind of components, miRs were also detected in platelets.

miRs belong to a class of small, endogenous non-coding RNA molecules that negatively regulate gene expression by targeting specific messenger RNAs. miRs repress the degradation or translational of targeting mRNAs, and then participate in the modulating of numerous cellular processes, such as organism growth, development, homeostasis, and disease (22). Our present study revealed 178 kinds of miRs that are differentially expressed between platelets and ECs in which 67 miRs are highly expressed in platelets. Whereas, further present study showed that it is PMPs, but not platelets, which deliver the platelet-derived miRs into ECs.

PMPs are small extracellular vesicles ranging from 0.1 to 1 μm in diameter shed from the cytoplasmic membrane. PMPs are originally referred to be the "platelet dust". However, after several decades' investigation, PMPs had been widely proved to be released from activated platelet under different conditions (23), and based on the abundant amount in circulation, PMPs cause extensive concern on both the disease biomarker and the information carrier (24). The amount of cir-

culating PMPs are significantly increased which may contribute to inflammatory diseases, such as arthritis (25) and atherosclerosis (26). In addition to sharing the surface markers of platelets, PMPs transfer a broad variety of cytoplasmic components, including platelet-specific proteins, lipids and nucleic acids to target cells (27). These small lipid vesicles may act, therefore, as intercellular carriers and deliver bioactive molecules, including small mRNA regulatory miRs, to the recipient cells (28). Except for microparticles, recent studies also showed that Argonaute2 could bind with miRs and contribute to the transfer of miRs to another cell directly (29). Further researches are still needed to demonstrate the mechanism in miRs intercellular transference between different kinds of cells.

Our array data revealed that except for miR-142-3p, there are many other kinds of platelet-specific miRs, including miR-199a-5p, miR-199a-3p, miR-142-5p, miR-26b-5p, miR-223-3p, and et al. miR-223 is a wide reported platelet-specific miR (5). Study revealed that uptake of PMP-derived miR-223 by ECs *in vitro*, repressed the target gene expression of miR-223, i.e. FBXW7 and EFNA1, at the mRNA level in recipient ECs. For miR-142-3p, our result showed its negative regulation on the expression of target gene- BCL2L1, which is the crucial regular of cell apoptosis.

Mitochondrial outer membrane permeabilization is essential for the regulation of the apoptotic process through the release of killing effectors from mitochondria to cytosol (cytochrome c, Smac/Diablo, Omi/Htra2, et al) (30). The Bcl-2 family are the main regulators of this deadly switch and the understanding of their function has been the focus of intensive studies for more than 20 years. BCL2L1 (also named BCL-xL) has been proposed to inhibit Bax binding to mitochondria by acting as a dominant negative Bax and anti-apoptosis (31). In ECs, the down-regulation of BCL-2 family is one of the most significant factors in apoptosis. In the course of psoriasis treatment, a significant reduction in BCL2L1 expression induces the apoptosis of ECs (32). The protective effect BCL-xL overexpression was also reported against oxidative stress-induced vascular endothelial cell injury and apoptosis (33).

Although our result suggested the negative effect of miR-142-3p on BCL2L1 expression, the *in vitro* mimics and inhibitor of miR-142-3p both revealed a repressed effect on EC apoptosis (data not shown). These interesting and confused results may be explained by the multi-target effect of miRs. Using IPA software, several other apoptosis related molecules including PRKCA, BCLAF1, AGO2, ADCY9 and LIFR are also predicted to be the downstream of miR-142-3p (Supplementary Fig. S1.). On the other hand, there may have some other miRs that participated in regulation of BCL2L1 expression. For example, miR-665 was also reported to target on BCL2L1 and elevate expression of cleaved caspase-3 in immature astrocytes *in vitro* (34). The effect of multi-miRs and multi-targets warrants further studies in the future.

In addition, the molecular mechanism of hypertension is also very complex. At the molecular level, natriuretic peptides (35), renin-angiotensin-aldosterone system (36), kinin-kallikrein system (37), and adrenergic receptor system (38) are all involved in the pathogenesis of hypertension. The pathogenesis role of platelet-dervied miRs in hypertension needs future research as well.

In summary, the current study demonstrated the effect of PMPs, released by active platelets, on ECs, as well as revealed a potential mechanism on which PMPs affect EC apoptosis during hypertension. PMPs deliver platelet-specific miR-142-3p into ECs which may target on BCL2L1 and induce EC apoptosis. These results provide a possible mechanism by which activated platelets regulate the function of ECs in hypertension, and suggest a potential new therapeutic approach in EC dysfunctions based on circulating PMPs.

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