Abstract. Atherosclerotic plaque growth requires angiogenesis, and acute coronary syndrome (ACS) is usually triggered by the rupture of unstable atherosclerotic plaques. Previous studies have identified typically circulating microRNA (miRNA/miR) profiles in patients with ACS. miRNAs serve important roles in the pathophysiology of atherosclerotic plaque progression. The present study aimed to investigate the potential role and mechanism of miR-19b in plaque stability. miRNA array data indicated that 28 miRNAs were differentially expressed in the plasma of patients with unstable angina (UA; n=12) compared with control individuals (n=12), and miR-19b exhibited the most marked upregulation. Circulating miR-19b levels were further validated in another independent cohort, which consisted of 34 patients with UA and 24 controls, by quantitative polymerase chain reaction. Gene Ontology annotations of the predicted target genes of miR-19b suggested that miR-19b may be involved in endothelial cell (EC) proliferation, migration and angiogenesis, which was confirmed by Cell Counting kit-8, wound healing and tube formation assays in the present study. Finally, the present study indicated that miR-19b may suppress signal transducer and activator of transcription 3 (STAT3) tyrosine phosphorylation and transcriptional activity in ECs, as determined by western blot analysis and luciferase reporter assay. In conclusion, the present study revealed that increased miR-19b expression may delay unstable plaque progression in patients with UA by inhibiting EC proliferation, migration and angiogenesis via the suppression of STAT3 transcriptional activity.

Introduction

Coronary artery disease (CAD) and its major complication, acute coronary syndrome (ACS), remain the most common causes of morbidity and mortality worldwide (1). ACS comprises two main entities: Unstable angina (UA) and acute myocardial infarction (AMI), which is usually triggered by the rupture of unstable atherosclerotic plaques (2). Vulnerable plaques, also known as unstable plaques, are typically characterized by a thin fibrous cap, large lipid core (>40% of the plaque), inflammatory infiltration, angiogenesis and intraplaque hemorrhage (3). The risk factors of CAD include hypertension, diabetes mellitus, elevated low-density lipoprotein cholesterol levels and cigarette smoke, all of which may lead to endothelial injury. Impaired vascular endothelium has an essential role in the initiation of atherosclerosis and in ultimate formation of unstable plaques (4). Dysfunctional endothelial cells (ECs) exhibit increased proliferation and migration, eventually resulting in re-endothelization and angiogenesis of the atherosclerotic plaque (5); the latter is a major step in promoting plaque destabilization, rupture and thrombus formation (6).

MicroRNAs (miRNAs/miRs) are small non-coding RNA molecules that negatively regulate gene expression, predominantly at the post-transcriptional level. Altered miRNA expression has been identified in the circulation of patients with ACS (7). Furthermore, it is now recognized that miRNAs are involved in almost all steps of atherogenesis, including endothelial damage and dysfunction, monocyte invasion and activation, lipoprotein formation and deposition, and vascular smooth muscle cell and platelet dysfunction, where they exert either beneficial or harmful effects (8). miR-126 is the most abundant miRNA in ECs, which may prompt EC repair by inhibiting inflammation (9). In addition, miR-181b and miR-146a are able to alleviate EC inflammation by regulating the nuclear factor-κB signaling pathway (10-12). Our previous study revealed that miR-19b may function as an antithrombotic protective miRNA in UA by targeting tissue factor in ECs (13). However, it remains unclear as to whether there are other protective roles for miR-19b in UA. It has previously
been suggested that miR-19b may be closely associated with atherosclerosis. miR-19b is downregulated in the aortic walls of apolipoprotein E (apoE) knockout mice, and suppressor of cytokine signaling 3 is a potential target of this miRNA (14). Signal transducer and activator of transcription 3 (STAT3) is an important nuclear transcription factor. The promoters of numerous proliferation, migration and angiogenesis-associated genes, including vascular endothelial growth factor, placental growth factor and SRY-box 18, contain STAT3 consensus sequences (15-17). Therefore, STAT3 activation may induce the expression of these genes and regulate cell proliferation, migration and angiogenesis.

The aim of the study was to evaluate the potential role and mechanism of miR-19b in plaque stability. The present study demonstrated that miR-19b was markedly upregulated in the plasma of patients with UA, and was predicted to be involved in the regulation of cell proliferation, migration and angiogenesis by bioinformatics analysis. Finally, the present study indicated that miR-19b may inhibit EC proliferation, migration and tube formation in vitro by suppressing STAT3 transcriptional activity.

Materials and methods

Study population. The present study was performed in accordance with the Helsinki declaration and was approved by the ethics review board of Peking University People's Hospital (Beijing, China). All individuals recruited to the present study provided written informed consent. Patients who were suspected of CAD with negative angiography were enrolled in the control group (n=36). Patients with typical UA that were angiographically documented as having CAD were enrolled in the UA group (n=46). All individuals, recruited to the present study between April 2012 to November 2013, provided written informed consent. The criteria for the diagnosis of UA were based on the American College of Cardiology Foundation/American Heart Association 2010 guidelines for the management of patients with UA/non-ST-segment-elevation MI (18). Patients presenting elevated troponin I (≥0.04 ng/ml) and/or creatine kinase-MB (≥5 ng/ml) levels, myocarditis, cardiogenic shock, a history of severe hepatic or renal dysfunction, leukemia, leukopenia and ongoing inflammatory malignant disease were excluded from the present study.

Blood collection and RNA extraction. Blood was collected from patients via arterial puncture into tubes containing EDTA (BD Biosciences, Franklin Lakes, NJ, USA) prior to coronary angiography, and was processed for isolation of plasma within 4 h. Blood was centrifuged at 1,300 x g for 10 min at 4°C. miRNAeasy mini kit (Qiagen, Inc., Valencia, CA, USA) was used for RNA extraction according to the manufacturer's protocol.

miRNA TaqMan low density array (TLDA). TLDA was used to detect differentially expressed miRNAs in the plasma of patients with UA (n=12) and controls (n=12). Total RNA (~15 ng) was reverse transcribed using the TaqMan miRNA reverse transcription (RT) kit and TaqMan miRNA Multiplex RT assays (human pool) (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA). The RT products were preamplified with TaqMan PreAmp kit (Applied Biosystems; Thermo Fisher Scientific, Inc.), after which preamplification reaction products were amplified using Human MicroRNA TLDA card A+B version 3.0 (Applied Biosystems; Thermo Fisher Scientific, Inc.), which could detect 754 miRNAs simultaneously.

All steps were performed using a 7900HT Fast real-time polymerase chain reaction (PCR) system (Applied Biosystems; Thermo Fisher Scientific, Inc.). Results were expressed as Cq. Raw data were analyzed using Data Assist software for TaqMan gene expression assays version 3.0 (Applied Biosystems; Thermo Fisher Scientific, Inc.) for miRNAs quantification according to the manufacturer's protocol. Real-time PCR reactions were performed on an Applied Biosystem ViiA™ 7 Real-Time PCR system with the following program: 10 min pre-incubation at 95°C, 40 cycles of 15 sec denaturation at 95°C and 60 sec of elongation at 60°C. Values were expressed as 2-ΔΔCq (19). miR-19b levels in the plasma were normalized to a spiked-in control, synthetic Caenorhabditis elegans miR-39 (10 fmol/sample; Qiagen, Inc.).

Cell culture and transfection. Cell culture and transfection were performed as previously described (11). Briefly, EA.hy926 cells (fusion cell line derived from human umbilical vein ECs and lung carcinoma cells), obtained from Shanghai Institutes for Biological Sciences (Chinese Academy of Sciences, Shanghai China), were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) (both from Gibco, Grand Island, NY, USA) at 37°C in a humidified atmosphere containing 5% CO2, Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) was used for transfection at a final concentration of 3 μg/ml according to the manufacturer's protocol. miR-19b mimic (cat. no. MC10629) or negative control (NC) mimic (cat. no. 4464058) (Applied Biosystems; Thermo Fisher Scientific, Inc.) were transfected into EA.hy926 cells (70-80% confluence) at a final concentration of 30 pmol/ml for 24 or 48 h.

Western blot analysis. Western blot analysis was performed as previously described (20). The following antibodies were used in the present study: Anti-STAT3 (cat. no. 9132S; 1:1,000 diluted) and anti-phosphorylated-STAT3 (Tyr 705) (cat. no. 9131S; 1:1,000 diluted; both from Cell Signaling Technology, Inc., Beverly, MA, USA). GAPDH (cat. no. SC-32233; 1:3,000 diluted; Santa Cruz Biotechnology, Santa Cruz, CA, USA) as a loading control. HRP-conjugated goat-anti-rabbit (cat. no. sc-2004) mouse (cat. no. sc-2005) IgG secondary antibodies (both from Santa Cruz Biotechnology).

Luciferase reporter assay. EA.hy926 cells plated in a 24-well plate at 70-80% confluence were cotransfected with NC or miR-19b mimic (final concentration, 60 pmol/ml) and
STAT3-driven promoter (2xAPRE) firefly luciferase reporter plasmid (final concentration, 300 ng/ml), as well as the internal control Renilla luciferase reporter plasmid (final concentration, 10 ng/ml; phRL-TK; Promega Corporation, Madison, WI, USA) using Lipofectamine 2000 (final concentration, 4 µg/ml). After 24 h, cell extracts were prepared and assessed according to the manufacturer’s protocol (Dual Luciferase assay system; Promega Corporation). Firefly luciferase activity was normalized to Renilla luciferase activity in the same well. To construct the STAT3-driven promoter, the 2xAPRE sequence was cloned into the multiple cloning site of pGL3-TATA plasmid, which encodes the firefly luciferase gene containing an upstream TATA element (21).

Cell proliferation assay. Cell proliferation activity was measured using Cell Counting kit-8 (CCK-8; Dojindo Molecular Technologies, Inc., Kumamoto, Japan). EA.hy926 cells in 96-well plates were transfected with miR-19b or NC mimic for 24 or 48 h. Subsequently, 20 µl CCK-8 solution was added to each well with 200 µl culture medium and the plates were incubated for 1 h at 37°C. The absorbance was measured at 450 nm using a microplate reader.

Wound healing migration assay. EA.hy926 cells in 24-well plates were transfected with miR-19b or NC mimic for 24 h, and were then serum-starved in DMEM containing 1% FBS overnight. Subsequently, the cell monolayer was scratched with a sterile 10-µl pipette tip. The cells were gently washed with DMEM to remove detached cells and were incubated with DMEM containing 1% FBS for 24 h at 37°C. Images of four injured fields in each well were captured (Olympus IX70; Olympus Corporation, Tokyo, Japan). Cell migration was semi-quantified by measuring the recovered area using ImageJ software (version 1.38; National Institutes of Health, Bethesda, MD, USA) (22).

Tube formation assay. Melted Matrigel (150 µl; BD Biosciences) was applied to 48-well plates and was
incubated at 37°C for 30 min. EA.hy926 cells were transfected with miR-19b mimic or NC mimic for 24 h. Transfected cells (4x10⁴/well) were seeded in the gel and incubated at 37°C for 16 h to allow tube formation. The effects of miR-19b on EA.hy926 cell differentiation stimulated by EC growth supplement (contained within Matrigel) at 37°C were observed under a microscope (Olympus IX70) and images of four representative fields were captured. Average total tube numbers in four representative fields were determined.

Table II. miRNA expression profiles in the plasma of patients with UA compared with controls.

| No. | Gene ID      | Score (d) | Fold change (UA/controls) | q-value |
|-----|--------------|-----------|---------------------------|---------|
| 1   | hsa-miR-19b  | 3.37      | 17.03                     | <0.0001 |
| 2   | hsa-miR-106a | 3.09      | 11.04                     | <0.0001 |
| 3   | hsa-miR-20a  | 3.04      | 21.22                     | <0.0001 |
| 4   | hsa-miR-16   | 3.00      | 9.18                      | <0.0001 |
| 5   | hsa-miR-17   | 2.97      | 9.07                      | <0.0001 |
| 6   | hsa-miR-451  | 2.78      | 28.65                     | <0.0001 |
| 7   | hsa-miR-24   | 2.72      | 7.67                      | <0.0001 |
| 8   | hsa-miR-223  | 2.58      | 10.13                     | <0.0001 |
| 9   | hsa-miR-92a  | 2.53      | 15.46                     | <0.0001 |
| 10  | hsa-miR-146a | 2.23      | 9.01                      | <0.0001 |
| 11  | hsa-miR-320  | 2.05      | 7.01                      | <0.0001 |
| 12  | hsa-miR-19a  | 1.99      | 4.84                      | <0.0001 |
| 13  | hsa-miR-30a-5p| 1.98     | 37.19                     | <0.0001 |
| 14  | hsa-miR-126  | 1.97      | 7.16                      | <0.0001 |
| 15  | hsa-miR-126* | 1.95      | 18.13                     | <0.0001 |
| 16  | hsa-miR-720  | 1.94      | 6.82                      | <0.0001 |
| 17  | hsa-miR-26a  | 1.91      | 22.25                     | <0.0001 |
| 18  | hsa-miR-26b  | 1.88      | 20.72                     | <0.0001 |
| 19  | hsa-miR-191  | 1.80      | 4.58                      | <0.0001 |
| 20  | hsa-miR-195  | 1.79      | 11.81                     | <0.0001 |
| 21  | hsa-miR-20b  | 1.78      | 23.13                     | <0.0001 |
| 22  | hsa-miR-30c  | 1.77      | 23.08                     | <0.0001 |
| 23  | hsa-miR-1274b| 1.76      | 16.24                     | <0.0001 |
| 24  | hsa-miR-30b  | 1.71      | 28.93                     | <0.0001 |
| 25  | hsa-miR-93   | 1.54      | 35.92                     | <0.0001 |
| 26  | hsa-miR-484  | 1.53      | 4.89                      | <0.0001 |
| 27  | hsa-miR-197  | 1.52      | 2.52                      | <0.0001 |
| 28  | hsa-miR-222  | 1.39      | 3.52                      | <0.0001 |

Circulating miRNAs exhibited significant alterations in patients with UA (n=12) compared with controls (n=12). Only miRNAs with >2-fold change and false discovery rate <0.0001% are presented. miR/miRNA, microRNA; UA, unstable angina.

miR-19b levels were increased in plasma samples from patients with UA. The present study initially detected the miRNA expression profiles in plasma samples from patients with UA (n=34) and controls (n=24). miR-19b levels were normalized to the spiked-in Caenorhabditis elegans miRNA, cel-miR-39. Data are presented as the mean ± standard error of the mean. **P<0.01 compared with the control group. miR, microRNA; UA, unstable angina.

Statistical analysis. Quantitative data are presented as the mean ± standard deviation or standard error of the mean. For continuous variables, statistical significance was calculated using one-way analysis of variance followed by the Tukey multiple comparisons test. For comparisons between two groups, Student's t-test was conducted. For categorical variables, statistical significance was calculated using the χ² test for the comparison of two groups. SPSS 17.0 (SPSS, Inc., Chicago, IL, USA) was used for all statistical analyses. P<0.05 was considered to indicate a statistically significant difference.

Results

The present study further validated circulating miR-19b levels in another independent cohort (Table I), which comprised 34 patients with UA and 24 controls, by RT-qPCR. The results indicated that the expression levels of miR-19b were increased in the UA group compared with in the control group (P<0.01; Fig. 1); this finding was consistent with the miRNA array data.
Functional enrichment analysis of miR-19b. Dysfunctional endothelium is an important pathological basis of unstable plaque formation, which triggers the clinical symptoms of UA. Therefore, the present study performed a functional enrichment analysis for miR-19b in ECs, in order to reveal the potential role of miR-19b in patients with UA. GO annotations of 276 predicted target genes in ECs demonstrated that miR-19b may be involved in regulating cell proliferation, migration and angiogenesis, which are closely associated with the formation of unstable plaques (Table III).

Effects of miR-19b on EC proliferation, migration and angiogenesis. To determine the effects of miR-19b on EC proliferation, miR-19b mimic was transfected into EA.hy926 cells for 24 or 48 h. The degree of miR-19b overexpression was detected 24 h (fold change, 653±90; P<0.001; Fig. 2A) and 48 h (fold change, 704±40; P<0.001; Fig. 2B) post-transfection compared with the NC group. Furthermore, the results of a CCK-8 assay demonstrated that miR-19b significantly inhibited cell proliferation at the two time points (P<0.001; Fig. 3).

Wound healing assay was conducted to investigate the effects of miR-19b on EA.hy926 cell migration. As presented in Fig. 4, EA.hy926 cell migration was markedly inhibited by 50% post-transfection with miR-19b mimic for 24 h compared with in the NC group (P<0.001).

Tube formation assay was performed to determine the role of miR-19b in angiogenesis. The results demonstrated that compared with in the NC group, EA.hy926 cells tube formation was decreased by ~70% in the miR-19b mimic group (P<0.001; Fig. 5).

All together, these findings indicated that miR-19b may act as an inhibitor of unstable plaque formation by negatively regulating EC proliferation, migration and angiogenesis.

Effects of miR-19b on STAT3 transcriptional activity. STAT3 is an important nuclear transcription factor and a major regulator of cell proliferation, migration and angiogenesis. In order to investigate whether the inhibitory role of miR-19b in ECs was mediated by STAT3, EA.hy926 cells were transfected with miR-19b mimic for 24 h and STAT3 tyrosine phosphorylation was detected by western blot analysis. As shown in Fig. 6A,
STAT3 phosphorylation was markedly decreased by ~40% in the miR-19b mimic group compared with the NC group (P<0.01).

STAT3 transcriptional activity was also examined by transfecting STAT3-driven reporter luciferase plasmid into EA.hy926 cells. Consistent with the results of STAT3 tyrosine phosphorylation analysis, miR-19b significantly inhibited the transcriptional activity of STAT3 by ~48% compared with the NC group (P<0.05, Fig. 6B).

These results suggested that the negative regulation of miR-19b in vulnerable plaque formation may be mediated by inhibiting STAT3 signaling.

**Discussion**

Angiogenesis serves a critical role in the development and ultimate rupture of unstable plaques, which is considered to be responsible for the majority of acute coronary artery events (25). Previous studies have suggested that miRNAs have an important role in regulating atherosclerotic diseases (26), and some miRNAs have been reported to regulate the angiogenic response to various pathological stimuli (27). The present study demonstrated that miR-19b may serve as a regulator of unstable plaque formation in patients with UA by inhibiting EC proliferation, migration and angiogenesis.

There are specific circulating miRNA profiles associated with various diseases. The present study revealed that circulating miR-19b was significantly upregulated in patients with UA compared with in controls (Fig. 1); this finding was consistent with our previous study (13). Zeller et al also reported that miR-19b levels were increased in plasma samples from patients with UA (28). Furthermore, a recent study observed that the levels of plasma miR-19b were significantly increased in early stage AMI (29). Our previous study indicated that miR-19b acted
as a potential antithrombotic miRNA in patients with UA by targeting tissue factor in ECs (13). A single miRNA may serve various biological roles by targeting numerous genes; therefore, the present study aimed to explore the possible effects of miR-19b upregulation on patients with UA. By bioinformatics analysis, the present study demonstrated that miR-19b may be involved in EC proliferation, migration and angiogenesis (Table III), which is closely associated with unstable atherosclerotic plaque formation. Subsequently, numerous assays were conducted to determine the effects of miR-19b on ECs. Consistent with the bioinformatics prediction, the experimental results indicated that miR-19b could inhibit EC proliferation, migration and tube formation in vitro (Figs. 3-5). In addition, Han et al reported that miR-19b was downregulated in aortic tissues of apoE gene knockout mice compared with in healthy C57BL/6 (B6) mice (14). Tang et al suggested that miR-19b alleviated EC apoptosis, which was also predicted in the functional enrichment analysis of the present study (Table III) (30). These results suggested that atherosclerosis may be delayed by supplementing miR-19b mimic. However, miR-19b has also been reported to

Figure 5. miR-19b inhibits proangiogenic functions of EA.hy926 cells. (A) EA.hy926 cells transfected with miR-19b mimic or NC mimic (final concentration, 30 pmol/ml) for 24 h were subjected to tube formation assay in Matrigel. (B) Number of tube-like networks were calculated (n=5). Data are presented as the mean ± standard error of the mean. ""P<0.001 compared with the NC mimic group. miR-19b, microRNA-19b; NC, negative control.

Figure 6. miR-19b inhibits STAT3 transcriptional activity in EA.hy926 cells. (A) p-STAT3 levels were detected in EA.hy926 cells transfected with miR-19b mimic or NC mimic (final concentration, 30 pmol/ml) for 24 h by western blot analysis. Densitometry was performed and levels were normalized to t-STAT3 expression (n=3). (B) EA.hy926 cells were transfected with STAT3-driven promoter (2xAPRE) firefly luciferase reporter plasmid, alongside miR-19b mimic or NC mimic. Luciferase activities were normalized to Renilla activities (n=4). Data are presented as the mean ± standard error of the mean. *P<0.05 and **P<0.01 compared with the NC mimic group. miR-19b, microRNA-19b; NC, negative control; p-, phosphorylated; STAT3, signal transducer and activator of transcription 3; t-, total.
prompt macrophage cholesterol accumulation and aortic atherosclerosis by targeting ATP-binding cassette transporter A1, whereas diosgenin could inhibit atherosclerosis via suppressing the effects of miR-19b (31,33). Therefore, it is unfeasible to prevent the development of atherosclerosis by delivering a miR-19b mimic in a systemic manner. Previous studies have demonstrated success at penetrating the vascular endothelium of the vessel wall and peripheral blood mononuclear cells (10,11). Delivery of a miRNA mimic (or inhibitor) in a targeted cell- or tissue-specific manner may represent a novel strategy to suppress the progression of atherosclerosis.

STAT3 inhibitor could suppress STAT3-mediated human EC proliferation, migration and tube formation (17). However, it remains unclear as to whether the inhibitory effect of miR-19b on EC proliferation, migration and tube formation is mediated by STAT3. In the present study, overexpression of miR-19b in ECs markedly decreased STAT3 tyrosine phosphorylation (Fig. 6A). The inhibitory effects of miR-19b on STAT3 transcriptional activity were further confirmed by STAT3-driven promoter luciferase assay (Fig. 6B). These results suggested that the role of miR-19b in regulating EC proliferation, migration and tube formation may be mediated by the STAT3 signaling pathway. Furthermore, miR-19b has also been reported to inhibit the migration and angiogenesis of human umbilical vein ECs by targeting the proangiogenic protein, fibroblast growth factor receptor 2, and by suppressing the expression of cyclin D1. However, this previous study failed to observe the inhibitory role of miR-19b on cell proliferation (33), which may be due to differences in cell culture.

In patients with UA, differentially expressed circulating miRNAs may serve opposite effects in regulating angiogenesis. miR-19b belongs to the miRNA-17-92 cluster, which comprises miR-17-5p, miR-18a, miR-19a, miR-20a, miR-19b and miR-92a. This miRNA cluster is highly expressed in human ECs and is upregulated by ischemia (34). In the present study, miR-17-5p, miR-19a, miR-20a, miR-19b and miR-92a were all upregulated in patients with UA compared with in controls. It has also been reported that members of the miRNA-17-92 cluster exhibit an antiangiogenic effect in ECs (34). Upregulated miR-106a, miR-222, miR-320 and miR-451 were also observed to inhibit angiogenic activity in ECs (35-42). Conversely, miR-30a/b, miR-93, miR-126, miR-146a and miR-24 may promote proangiogenic activity (43-47). These circulating miRNAs may influence vulnerable plaque formation by forming complex regulatory networks in patients with UA. Our future studies aim to determine the key miRNAs in the network using systems biology, in order to identify effective therapeutic targets.

In conclusion, the present findings suggested that miR-19b may exert protective effects on plaque stability by modulating the STAT3-mediated signaling pathway. These findings may provide information regarding vulnerable plaque formation intervention in patients with UA.

Acknowledgements

The present study was supported by the Beijing Science and Technology Major Project (grant no. D14110003014002) and the National Natural Science Foundation of China (grant nos. 81270274, 81470473, 81400265, 81400264 and 81600340). The present study was presented at the ESC Congress 2017, August 26-30, 2017, in Barcelona, Spain and published as abstract no. P675 in Atherosclerosis 38 (Suppl 1): 2017.

References

1. GBD 2013 Mortality and Causes of Death Collaborators: Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: A systematic analysis for the Global Burden of Disease Study 2013. Lancet 385: 117-171, 2015.
2. Vester F, Stein B, Ambrose JA, Badimon L, Badimon JJ and Chesebro JH: Atherosclerotic plaque rupture and thrombosis. Evolving concepts. Circulation 82 (Suppl 3): I47-I59, 1990.
3. Hellings WE, Peeters W, Moll FL and Pasterkamp G: From vulnerable plaque to vulnerable patient: The search for biomarkers of plaque destabilization. Trends Cardiovasc Med 17: 162-171, 2007.
4. Hopkins PN: Molecular biology of atherosclerosis. Physiol Rev 93: 1317-1542, 2013.
5. Shi L, Fisslthaler B, Zippel N, Frömel T, Hu J, Elgezhanawy A, Heide H, Popp R and Fleming I: MicroRNA-223 antagonizes angiogenesis by targeting β1 integrin and preventing growth factor signaling in endothelial cells. Circ Res 113: 1320-1330, 2013.
6. Haver VG, Slart RH, Zeebregts CJ, Peppelebosch MP and Tio RA: Rupture of vulnerable atherosclerotic plaques: microRNAs conducting the orchestra? Trends Cardiovasc Med 20: 65-71, 2010.
7. Okamura K, Ishizuka A, Siomi H and Siomi MC: Distinct roles for Argonauta proteins in small RNA-directed RNA cleavage pathways. Genes Dev 18: 1655-1666, 2004.
8. Feinberg MW and Moore KJ: MicroRNA Regulation of Atherosclerosis. Circ Res 118: 703-720, 2016.
9. Harris TA, Yamakuchi M, Ferlito M, Mendell JT and Lowenstein CJ: MicroRNA-126 regulates endothelial expression of vascular cell adhesion molecule 1. Proc Natl Acad Sci USA 105: 1516-1521, 2008.
10. Sun X, Icli B, Wara AK, Belkin N, He S, Kozbik L, Hunninghake GM, Vera MP, Blackwell TS, Baron RM, et al.; MICU Registry: MicroRNA-181b regulates NF-kB-mediated vascular inflammation. J Clin Invest 122: 1973-1990, 2012.
11. Sun X, He S, Wara AK, Icli B, Shvartz E, Tesmenitsky Y, Belkin N, Li D, Blackwell TS, Sukhova GK, et al.: Systemic delivery of microRNA-181b inhibits nuclear factor-kB activation, vascular inflammation, and atherosclerosis in apolipoprotein E-deficient mice. Circ Res 114: 32-40, 2014.
12. Chen HS, Sivachandran N, Lau A, Boudreau E, Zhao JL, Baltimore D, Delgado-Olguin P, Cybulsky MI and Fish JE: MicroRNA-146 represses endothelial activation by inhibiting pro-inflammatory pathways. EMBO Mol Med 5: 1071-1034, 2013.
13. Li S, Ren J, Xu N, Zhang J, Geng Q, Cao C, Lee C, Song J, Li J and Chen H: MicroRNA-19b functions as potential anti-thrombotic protector in patients with unstable angina by targeting tissue factor. J Mol Cell Cardiol 75: 49-57, 2014.
14. Han H, Wang YH, Gu GJ, Sun TT, Li FQ, Jiang W and Luo SS: Differentiated miRNA expression and validation of signaling pathways in apoE gene knockout mice by cross-verification microarray platform. Exp Mol Med 45: e13, 2013.
15. Wegryn J, Potla R, Chwae YJ, Sepuri NB, Zhang Q, Koeck T, Derecka M, Szczepanek K, Szelag M, Gornicka A, et al.: Function of mitochondrial Stat3 in cellular respiration. Science 323: 793-797, 2009.
16. Folkman J: Fundamental concepts of the angiogenic process. Curr Mol Med 3: 643-651, 2003.
17. Mehta JL, Mercanti C, Stone A, Wang X, Ding Z, Romeo F and Chesebro JH: Atherosclerotic plaque rupture and thrombosis. Circ Res 118: 703-720, 2016.
18. Anderson JL, Adams CD, Antman EM, Bridges CR, Califf RM, Casey DE Jr, Chavey WE II, Fesmire FM, Hochman JS, Levin TN, et al.; 2011 WRITING GROUP MEMBERS; ACCF/AHA TASK FORCE MEMBERS: 2011 ACCF/AHAFocused Update Incorporated Into the ACC/AHA 2007 Guidelines for the Management of Patients With Unstable Angina/Non-ST-Elevation Myocardial Infarction: A report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Circulation 125: e426-e579, 2011.
