Int J Clin Exp Med 2018;11(5):5375-5382
www.ijcem.com /ISSN:1940-5901/IJCEM0074238

Original Article

microRNA-194 in the development of aortic aneurysms and dissection

Jian Li[1], Dayong Liu[2]

Departments of [1]Cardiology, [2]Cardiothoracic Surgery, Da Qing Long Nan Hospital, Daqing, Heilongjiang Province, China

Received February 8, 2018; Accepted March 22, 2018; Epub May 15, 2018; Published May 30, 2018

Abstract: Objective: Our aim was to investigate the role and mechanism of microRNA-194 in aortic aneurysms and dissection. Methods: Fifteen patients with aortic aneurysms and dissection undergoing aortic valve replacement and thirteen normal donors without aortic lesions were selected to analyze expression levels of microRNA-194 in aortic tissue. The effect of microRNA-194 knockdown on expression of α-SMA, extracellular matrix secretory function (collagen III and osteopontin), in human smooth muscle cells was also investigated. Additionally, expression of Smad1 and Smad4, key molecules in TGF-β1 signaling pathway, was analyzed by qRT-PCR and Western blot. Results: Compared with normal aorta, microRNA-194 was significantly downregulated in aorta with aneurysms and dissection (P=0.019). Knockdown of microRNA-194 expression promoted contractile-to-synthetic conversion of human vascular smooth muscle cells (P=0.005), increased expression of collagen III and osteopontin (P<0.001, P=0.022), and promoted expression of Smad1 and Smad4 mRNA (P=0.016, P=0.001) and protein (P<0.001, P<0.001). Conclusion: Inhibition of microRNA-194 expression can activate TGF-β1 signaling pathways and affect the phenotype and function of vascular smooth muscle cells, possibly relating to development of aortic dissection.

Keywords: microRNA-194, aortic dissection, aortic aneurysm, vascular smooth muscle cells, transforming growth factor-β1 (TGF-β1)

Introduction

Aortic aneurysm is a local expansion of the blood vessel wall because of the patient's aortic wall atherosclerosis, decreased elasticity, and inability to withstand hemodynamic impact [1]. In the case of aortic aneurysms and other underlying lesions, due to the impact of blood flow, aortic dissection can lead to progressive intimal tear and the formation of true and false lumens of arterial lumen with high risk of major bleeding. The majority of patients die because of failure to get timely surgical treatment. It is a common critical illness in cardiovascular disease [2, 3]. Currently, the formation mechanism of aortic aneurysms and dissection is thought to be mainly related with changes of the structure and function of aortic tunica media, vascular smooth muscle cells, and extracellular matrix, the main components of tunica media [4]. Vascular smooth muscle cells include both contractile and synthetic forms. Contractile smooth muscle cells play a major role in elasticity and tensile strength of the vascular wall. Synthetic smooth muscle cells play an important role in cell proliferation and migration. When the aortic vessel wall is damaged in aortic aneurysms and dissection, smooth muscle cells can be converted from contractile to synthetic cells, resulting in promotion of development of aortic dissection [5].

microRNA, a highly conserved non-coding RNA containing about 22 nucleotides, can negatively regulate target gene expression through specific binding to the 3'untranslated region of mRNA. It plays an important role in aortic atherosclerosis, inflammation, cancer, and other diseases. It has been reported in the literature that some microRNAs play a key role in the pathogenesis of aortic aneurysms and dissection. For example, aorta microRNA-4787-5p and microRNA-4306 were found to be significantly increased in aortic dissection patients by high-throughput microRNA microarray analysis. These two microRNAs might participate in the

pathogenesis of aortic dissection by regulating signaling pathway of Polycystin-1 (PKD1) and transforming growth factor-β1 (TGF-β1) [6]. In addition, another study found that microRNA-15a and microRNA-23a have a clinical diagnostic value of aortic dissection by performing microRNA screening of 37 patients with acute aortic dissection, 26 patients with chronic aortic dissection, and 40 controls [7]. Therefore, study of the role and mechanism of microRNA in aortic dissection has important clinical value for aortic dissection diagnosis, treatment, and prognosis.

microRNA-194 is an important regulator of cell proliferation, differentiation, and migration and is widely involved in development of melanoma, degenerative disc disease, psoriasis, diabetes, influenza, pneumonia, and other diseases [8-13]. However, the role of microRNA-194 in aortic aneurysms and dissection remains unclear. Therefore, in this study, we analyzed expression of microRNA-194 in the aortic wall by selecting aortic dissection patients and normal control subjects as research objects. We studied the function of microRNA-194 in cultured vascular smooth muscle cells.

Materials and methods

Aortic dissection tissue collection

This study was reviewed and approved by the Ethics Committee and informed consent was obtained. Aortic tissue samples were taken from 15 aortic dissection patients, undergoing aortic valve replacement in our Department of Thoracic Surgery, and 13 normal donors without aortic lesions from May 2014 to December 2017. Inclusion criteria for aortic dissection patients was Stanford Type A thoracic aortic dissection with ascending aortic dissection determined by aortic arch digital subtraction angiography. Exclusion criteria included genetic factors (like familial aortic aneurysm), trauma, infections (such as syphilis), connective tissue disease (such as Marfan syndrome), and other causes of aortic lesions.

Construction of lentiviral vector for downregulation of microRNA-194

After the complementary sequence (TCCACATGGAGTTGCTGTTACA) and nonsense sequence (TTCTCCGAACGTGTCACGT) of synthetic microRNA-194 were synthesized and annealed to double-stranded sequence, it was inserted into a lentiviral vector Plkd-CMV-G & PR (Shanghai Heyuan) by molecular biology methods such as restriction endonuclease, ligation, etc. [14, 15]. Then, the constructed lentiviral vectors were divided into two groups: lentiviral vector constructed by microRNA-194 complementary sequence as microRNA-194 intervention group (Anti-miR194 group) and lentiviral vector constructed by nonsense sequence as microRNA-194 control Group (Scramb-Anti-miR194 group).

Culture and treatment of human aortic smooth muscle cells

Human aortic wall smooth muscle cells were purchased from ATCC cell bank. Cells were first incubated with high glucose DMEM medium containing 10% FBS, 1% penicillin, and streptomycin at a 37°C cells incubator with 5% CO_2. Cells were inoculated into 10 cm petri dishes and cultured until the logarithmic growth phase. The medium was discarded and 15 mL of Opti-MEM medium was added. Transfection solution (25 μg of plasmids constructed above and 25 μL Obio transfection solution were respectively dissolved in 800 μL of Opti-MEM medium) was also added. After culturing for 6 hours, the medium was discarded. Culturing continued for 48 hours with high glucose DMEM medium containing 10% FBS. Subsequently, the cells were stimulated with TGF-β1 (PeproTech) at a final concentration of 10 ng/mL for 12 hours before collection for qRT-PCR and Western blot [16].

Detection of mRNA expression by fluorescence quantitative PCR

Expression of microRNA-194 mRNA in aortic tissue and expression of α-smooth muscle actin (α-SMA), collagen III, Osteopontin, Smad1 and Smad4 mRNA in human aortic smooth muscle cells was detected. Specifically, RNA extraction was performed using TRIzol one-step method. RNA was reverse transcribed into cDNA using miScript RT Kit (QIAGEN) and fluorescence quantitative PCR amplification was performed by miScript SYBR Green PCR Kit. Reaction conditions: 94°C 15 s, 55°C 30 s, 70°C 30 s, a total of 30 cycles. Primers were synthesized in Shanghai Biological Engineering

Table 1. Primer sequence of fluorescent quantitative PCR detection

Gene	Upstream sequence	Downstream sequence
microRNA-194	5'CATGATCAGCTGGGCCAAGATCCACATGGAGT3'	5'TGTAACAGCAACTCCA3'
α-SMA	5'TGGCTACTCCTTCGTGACCA3'	5'GCCGACTCCATACCGATGAA3'
Collagen III	5'AAGAGCGGAGAATACTGGG3'	5'CAATGTCATAGGGTGCGATA3'
Osteopontin	5'TGATGCTACAGACGAGGAC3'	5'ACTATCAATCACATCGGAAT3'
Smad1	5'ACAGTCTGTGAACCATGGATTTGA3'	5'TGAGGTGAACCCATTTGAGTAAGAA3'
Smad4	5'GCGACGAAGGTCATCAACAC3'	5'TCATCGACAGATGCAGCAGC3'
GAPDH	5'CCTCTGACTTCAACAGCGACA3'	5'TGGTCCAGGGGTCTTACTCC3'

Note: α-SMA, α-smooth muscle actin.

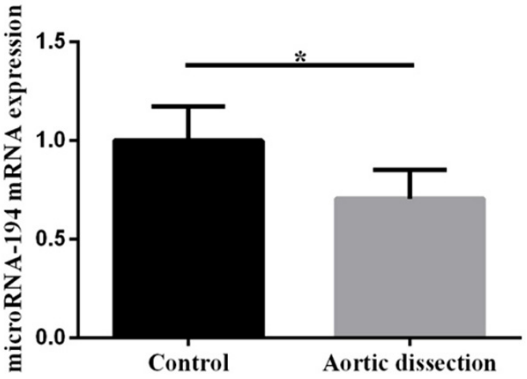

Figure 1. Expression of microRNA-194 mRNA in aortic dissection. AD group compared with the normal group, *P<0.05.

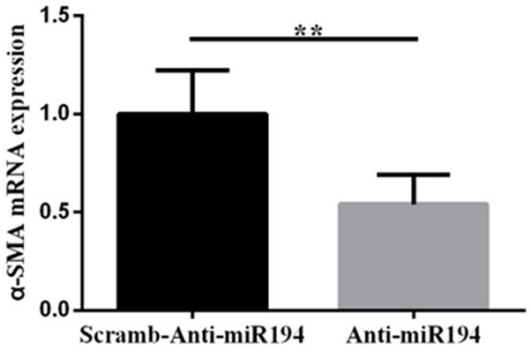

Figure 2. Downregulation of microRNA-194 expression can promote the phenotype of human vascular smooth muscle cells indicated by increased α-SMA expression. Anti-miR194 group compared with the Scramb-Anti-miR194 group, **P<0.01.

Co., Ltd. [14, 17]. Specific sequences are provided in **Table 1**.

Smad1 and Smad4 protein expression detected by Western blotting

After collecting the cells, cell lysis and BCA (bicinchoninic acid) protein quantification were performed sequentially [18]. Western blotting was performed at the same total protein concentration. Samples were subjected to electrophoresis in a 10% polyacrylamide gel and then protein was electrotransferred to a polyvinylidene difluoride membrane. 3% bovine serum albumin was used for block at room temperature for 1 hour. Anti-Smad1 (1:500), anti-Smad4 (1:500), and anti-GAPDH (1:1,000) (Abcam company) diluted with 3% bovine serum albumin were added for overnight incubation at 4°C. After washing with PBST buffer three times, horseradish peroxidase-labeled goat anti-rabbit secondary antibody (BOSTER) was added for 1 hour at room temperature. After washing with PBST buffer three times, membranes were covered with ECL liquid (Beyotime) and finally photographed in the gel imaging system.

Statistical analysis

All measurement data are expressed as mean ± standard deviation. SPSS 20.0 was used for data analysis. Measurement data were subjected to normal distribution test and compared using independent t-test. P<0.05 means the difference is statistically significant.

Results

microRNA-194 mRNA is downregulated in the aorta with dissection

To analyze expression of microRNA-194 in aortic dissection, we first performed microRNA-194 fluorescence quantitative PCR detection on tissues from aortic dissection patients and control participants. Results in **Figure 1** show that microRNA-194 mRNA expression was significantly decreased in patients with

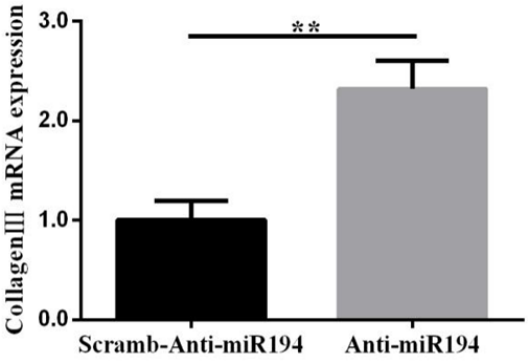

Figure 3. Downregulating expression of microRNA-194 can promote expression of collagen III mRNA. Anti-miR194 group compared with the Scramb-Anti-miR194 group, **P<0.01.

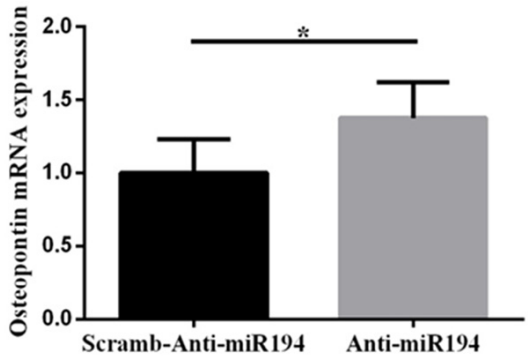

Figure 4. Downregulating expression of microRNA-194 can promote expression of osteopontin mRNA. Anti-miR194 group compared with the Scramb-Anti-miR194 group, *P<0.05.

aortic dissection (P=0.019), suggesting that microRNA-194 may play a role in the pathogenesis of aortic dissection.

microRNA-194 knockdown promotes phenotypic transformation of human vascular smooth muscle cells

In order to further study the mechanism of decreased expression of microRNA-194 in aortic dissection, we constructed a lentiviral vector which could downregulate expression of microRNA-194 and transfected it into human vascular smooth muscle cells to observe the effects of microRNA-194 on human vascular smooth muscle cells. Results in **Figure 2** show that expression of α-SMA, a specific molecule of contracted vascular smooth muscle cells [19], decreased significantly by downregulation of microRNA-194 expression in human vascular smooth muscle cells (P=0.005).

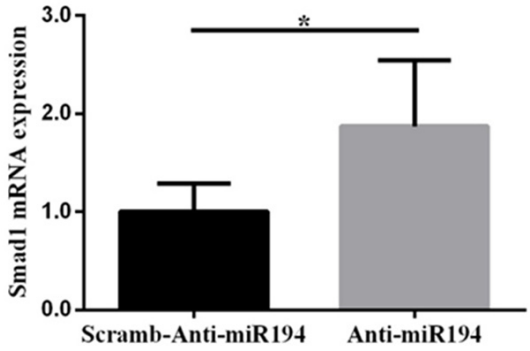

Figure 5. Downregulating expression of microRNA-194 can promote expression of Smad1 mRNA. Anti-miR194 group compared with the Scramb-Anti-miR194 group, *P<0.05.

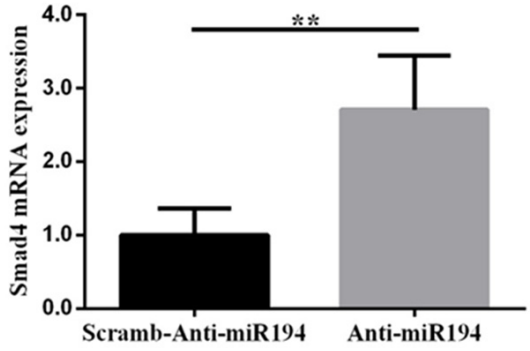

Figure 6. Downregulating expression of microRNA-194 can promote expression of Smad4 mRNA. Anti-miR194 group compared with the Scramb-Anti-miR194 group, **P<0.01.

microRNA-194 knockdown promotes expression of collagen III and osteopontin

To further investigate whether intervention of microRNA-194 can further affect the function of smooth muscle cells to promote progression of aortic dissection, we selected collagen III and osteopontin as the indicator [20-22]. Results in **Figures 3** and **4** show that downregulating expression of microRNA-194 can promote expression of collagen III and osteopontin (P<0.001, P=0.022). Collagen III and osteopontin are components of the vascular wall but do not have the function of maintaining elasticity of the vessel wall. Excessive levels of collagen III and osteopontin can cause stiffness and lack of elasticity of the vessel wall, which is associated with remodeling of the vessel wall [17, 23].

These results imply that downregulation of microRNA-194 expression can promote the

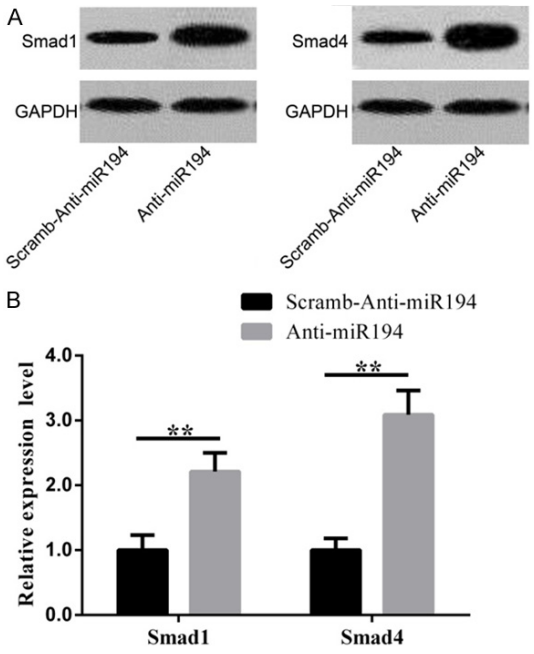

Figure 7. A: Downregulation of microRNA-194 expression can promote expression of Smad1 and Smad4 protein, B: Western blot analysis of Smad1 and Smad4 protein expression. GAPDH is an internal reference protein, Anti-miR194 group compared with the Scramb-Anti-miR194 group, $^{**}P<0.01$.

transformation of human vascular smooth muscle cells from contractile to synthetic. Therefore, downregulating expression of microRNA-194 can further affect function of smooth muscle cells and play an important role in the pathological progress of aortic dissection.

microRNA-194 downregulation is associated with TGF-β1 signaling activation

In order to further explore specific mechanisms of phenotypic transformation and functional changes by *microRNA-194* in smooth muscle cells, we analyzed the TGF-β1 signaling pathway. **Figures 5** and **6** show that downregulation of microRNA-194 could promote expression of Smad1 and Smad4 mRNA (P=0.016, P=0.001). We also conducted Western blot to detect protein levels. Results in **Figure 7A** show that Smad1 and Smad4 proteins were significantly increased when microRNA-194 was downregulated. **Figure 7B** shows that Smad1 and Smad4 proteins were significantly increased after microRNA-194 knockdown (P<0.001, P<0.001). As key molecules in TGF-β1 signaling pathway, significant increase in mRNA and protein expression of Smad1 and Smad4 indicate the activation of TGF-β1 signaling pathway.

Discussion

Aortic dissection is a vascular system disease with high mortality and high disability. The main pathological changes are changes of extracellular matrix such as apoptosis, degeneration, phenotypic transformation, and elastic fibers and collagen fibers in the aortic smooth muscle cells but the specific pathogenesis remains unclear and there remains a lack of corresponding prevention and curative measures [4]. Aortic dissection is often accompanied by changes in expression of microRNAs. microRNAs play an important role in the pathological progression of aortic dissection by regulating key cell and signaling pathways [24, 25]. microRNA-194 is widely involved in regulation of biological processes such as cell proliferation, differentiation, and apoptosis. It has been found that microRNA-194 plays an important regulatory role in cancer, infectious diseases, and metabolic diseases. However, little research has been done on aortic dissection. Therefore, we investigated expression levels of microRNA-194 in aortic dissection and studied the specific mechanism of microRNA-194 *in vitro* using vascular smooth muscle cells.

We first performed microRNA-194 fluorescence quantitative PCR on aortic dissection and normal aorta tissues. We found that expression of microRNA-194 mRNA was significantly decreased in patients with aortic dissection, suggesting that microRNA-194 may play a role in the pathogenesis of aortic dissection (P<0.05). Then, by constructing a lentiviral vector to interfere with expression of microRNA-194 in human vascular smooth muscle cells, we found that downregulation of microRNA-194 can promote transformation of vascular smooth muscle cells from contractile to synthetic and increase expression of collagen III and osteopontin. Previous studies have found that microRNA-194 could regulate differentiation of hepatocytes, osteoblasts, neurons, epithelial cells, and mesenchymal stem cells [26-28]. Overexpression of microRNA-194 can promote differentiation of mesenchymal stem cells into osteoblasts [29]. However, there have not been any reports on the role of microRNA-194 in vascular smooth muscle cells. Here, we report for the first time that downregulation of microRNA-194 plays an important role in development of aortic dissection by promoting transformation from contractile to synthetic phenotypes of vascular smooth muscle cells.

Vascular smooth muscle cells are important cells that maintain the structure and function of blood vessels. When they undergo apoptosis, denaturation, and phenotypic transformation by external stimuli, they can cause structural remodeling of blood vessels and reduction of elasticity [30]. In a resting state, smooth muscle cells show contractile type. After stimulation, they can be converted to synthetic type, which can enhance cell proliferation with elasticity significantly reduced, causing pathological changes such as vascular remodeling [31-33]. Pei et al. found that expression of collagen III and osteopontin in the aortic wall was significantly increased in patients with aortic aneurysm. Moreover, contractile-specific α-SMA of vascular smooth muscle cells was significantly decreased *in vitro* cell experiments, while synthesis of collagen III and osteopontin was significantly increased. Their results indicated that phenotypic transformation of vascular smooth muscle cells is involved in development of aortic aneurysms pathogenesis [17]. Therefore, expression of α-SMA, collagen III, and osteopontin in human vascular smooth muscle cells was also detected in our study. It was found that downregulation of microRNA-194 could significantly reduce α-SMA, whereas osteopontin, a marker of synthetic vascular smooth muscle cells, was significantly increased [19, 20]. These results suggest that microRNA-194 is involved in phenotypic modulation of vascular smooth muscle cells. Furthermore, elastic fibers and collagen fibers constitute the main matrix components of blood vessels and their imbalance in proportion is the structural basis for reduction of blood vessel elasticity. Wang et al. found that connective tissue growth factor can promote expression of collagen III in the aortic wall and play a key role in the pathogenesis of aortic dissection [23]. We also found that downregulation of microRNA-194 can significantly increase expression of collagen III, suggesting that microRNA-194 plays an important role in synthesis and regulation of extracellular matrix.

TGF-β1 can promote transformation of vascular smooth muscle cells from contraction to synthetic, with biological functions of promoting cell migration and secretion, playing a key role in vascular remodeling. According to the literature, expression of TGF-β1 in aortic dissection is significantly increased, with the most prominent in the intima and media of vessels [34]. Therefore, in order to further explore the mechanism by which microRNA-194 induces vascular smooth muscle cell phenotype transformation and extracellular matrix regulation, we detected Smad1 and Smad4, key molecules of TGF-β1 signaling pathway. We found that Smad1 and Smad4 were significantly increased at mRNA and protein levels. These results suggest that inhibition of microRNA-194 may play a role in the pathological progress of aortic dissection by activating the TGF-β1 signaling pathway to promote vascular smooth muscle cell phenotype transformation and extracellular matrix regulation.

We found that inhibition of microRNA-194 expression can activate TGF-β1 signaling pathway and affect the phenotype and function of vascular smooth muscle cells, thereby promoting development of aortic dissection. There were still many shortcomings: 1) The effect of microRNA-194 overexpression on the phenotype and function of vascular smooth muscle cells was not studied; 2) Vascular endothelial cells are also important cells in the aortic wall, whether microRNA-194 can participate in development of aortic dissection by regulating function of vascular endothelial cells was not determined; and 3) Most of the experimental results were obtained by cell experiments *in vitro* and were not verified in animal experiments. Therefore, we plan to further carry out microRNA-194 overexpression in human vascular smooth muscle cells and cultivate vascular endothelial cells *in vitro* to investigate whether intervention of microRNA-194 influences the biological function of vascular endothelial cells. We plan to construct an animal model of aortic dissection and conduct *in vivo* validation of the roles of microRNA-194.

In this study, we analyzed expression of microRNA-194 in the aortic walls of 15 aortic dissection patients undergoing aortic valve replacement and 13 normal donors without aortic lesion. We found that inhibition of microRNA-194 expression may be involved in the pathogenesis of aortic dissection. Subsequently, through downregulating expression of microRNA-194 in human vascular smooth muscle cells by lentiviral transfection, it was found that downregulation of microRNA-194 can promote transformation of vascular smooth muscle cells from contractile to synthetic and increase expressions of collagen III and osteo-

pontin. In addition, activation of TGF-β1 signaling pathway plays an important role. Therefore, inhibition of microRNA-194 expression can activate TGF-β1 signaling pathways and affect the phenotype and function of vascular smooth muscle cells, resulting in development of aortic dissection.

Disclosure of conflict of interest

None.

Address correspondence to: Dayong Liu, Department of Cardiothoracic Surgery, Da Qing Long Nan Hospital, No.35 Aiguo Road, Ranghulu District, Daqing 163001, Heilongjiang Province, China. Tel: +86-0459-5910211; E-mail: liudayong96@163.com

References

[1] Strayer RJ. Thoracic aortic syndromes. Emerg Med Clin North Am 2017; 35: 713-725.
[2] Siddiqi HK, Bossone E, Pyeritz RE and Eagle KA. Chronobiology of acute aortic syndromes. Heart Fail Clin 2017; 13: 697-701.
[3] Kuo EC and Han SM. Treatment of complex thoracoabdominal aortic disease. Cardiol Clin 2017; 35: 411-429.
[4] Nienaber CA, Clough RE, Sakalihasan N, Suzuki T, Gibbs R, Mussa F, Jenkins MP, Thompson MM, Evangelista A, Yeh JS, Cheshire N, Rosendahl U and Pepper J. Aortic dissection. Nat Rev Dis Primers 2016; 2: 16071.
[5] Peterss S, Mansour AM, Ross JA, Vaitkeviciute I, Charilaou P, Dumfarth J, Fang H, Ziganshin BA, Rizzo JA, Adeniran AJ and Elefteriades JA. Changing pathology of the thoracic aorta from acute to chronic dissection: literature review and insights. J Am Coll Cardiol 2016; 68: 1054-1065.
[6] Wang L, Zhang S, Xu Z, Zhang J, Li L and Zhao G. The diagnostic value of microRNA-4787-5p and microRNA-4306 in patients with acute aortic dissection. Am J Transl Res 2017; 9: 5138-5149.
[7] Dong J, Bao J, Feng R, Zhao Z, Lu Q, Wang G, Li H, Su D, Zhou J, Jing Q and Jing Z. Circulating microRNAs: a novel potential biomarker for diagnosing acute aortic dissection. Sci Rep 2017; 7: 12784.
[8] Bai M, Zhang M, Long F, Yu N, Zeng A and Zhao R. Circulating microRNA-194 regulates human melanoma cells via PI3K/AKT/FoxO3a and p53/p21 signaling pathway. Oncol Rep 2017; 37: 2702-2710.
[9] Hu B, Xu C, Tian Y, Shi C, Zhang Y, Deng L, Zhou H, Cao P, Chen H and Yuan W. Inflammatory microRNA-194 and -515 attenuate the biosynthesis of chondroitin sulfate during human intervertebral disc degeneration. Oncotarget 2017; 8: 49303-49317.
[10] Yu X, An J, Hua Y, Li Z, Yan N, Fan W and Su C. MicroRNA-194 regulates keratinocyte proliferation and differentiation by targeting Grainyhead-like 2 in psoriasis. Pathol Res Pract 2017; 213: 89-97.
[11] Latouche C, Natoli A, Reddy-Luthmoodoo M, Heywood SE, Armitage JA and Kingwell BA. MicroRNA-194 modulates glucose metabolism and its skeletal muscle expression is reduced in diabetes. PLoS One 2016; 11: e0155108.
[12] Wang K, Lai C, Gu H, Zhao L, Xia M, Yang P and Wang X. miR-194 Inhibits innate antiviral immunity by targeting FGF2 in influenza H1N1 virus infection. Front Microbiol 2017; 8: 2187.
[13] Xie F, Yang L, Han L and Yue B. MicroRNA-194 regulates lipopolysaccharide-induced cell viability by inactivation of nuclear factor-kappa B pathway. Cell Physiol Biochem 2017; 43: 2470-2478.
[14] Couto LB and High KA. Viral vector-mediated RNA interference. Curr Opin Pharmacol 2010; 10: 534-542.
[15] Han K, Zhao T, Chen X, Bian N, Yang T, Ma Q, Cai C, Fan Q, Zhou Y and Ma B. microRNA-194 suppresses osteosarcoma cell proliferation and metastasis in vitro and in vivo by targeting CDH2 and IGF1R. Int J Oncol 2014; 45: 1437-1449.
[16] Shi N and Chen SY. Mechanisms simultaneously regulate smooth muscle proliferation and differentiation. J Biomed Res 2014; 28: 40-46.
[17] Pei H, Tian C, Sun X, Qian X, Liu P, Liu W and Chang Q. Overexpression of MicroRNA-145 promotes ascending aortic aneurysm media remodeling through TGF-beta1. Eur J Vasc Endovasc Surg 2015; 49: 52-59.
[18] Li JL, Liu N, Chen XH, Sun M and Wang CB. Inhibition of UVA-induced apoptotic signaling pathway by polypeptide from Chlamys farreri in human HaCaT keratinocytes. Radiat Environ Biophys 2007; 46: 263-268.
[19] Mack CP. Signaling mechanisms that regulate smooth muscle cell differentiation. Arterioscler Thromb Vasc Biol 2011; 31: 1495-1505.
[20] Yuan SM, Wang J, Huang HR and Jing H. Osteopontin expression and its possible functions in the aortic disorders and coronary artery disease. Rev Bras Cir Cardiovasc 2011; 26: 173-182.
[21] Wilson E, Parrish AR, Bral CM, Williams ES and Ramos KS. Collagen suppresses the proliferative phenotype of allylamine-injured vascular smooth muscle cells. Atherosclerosis 2002; 162: 289-297.

[22] Parrish AR and Ramos KS. Differential processing of osteopontin characterizes the proliferative vascular smooth muscle cell phenotype induced by allylamine. J Cell Biochem 1997; 65: 267-275.
[23] Wang X, LeMaire SA, Chen L, Shen YH, Gan Y, Bartsch H, Carter SA, Utama B, Ou H, Coselli JS and Wang XL. Increased collagen deposition and elevated expression of connective tissue growth factor in human thoracic aortic dissection. Circulation 2006; 114: I200-205.
[24] Leeper NJ and Maegdefessel L. Non-coding RNAs: key regulators of smooth muscle cell fate in vascular disease. Cardiovasc Res 2018; 114: 611-621.
[25] Wang XJ, Huang B, Yang YM, Zhang L, Su WJ, Tian L, Lu TY, Zhang S, Fan XH and Hui RT. Differential expression of microRNAs in aortic tissue and plasma in patients with acute aortic dissection. J Geriatr Cardiol 2015; 12: 655-661.
[26] Zhuang H, Zhang R, Zhang S, Shu Q, Zhang D and Xu G. Altered expression of microRNAs in the neuronal differentiation of human Wharton's Jelly mesenchymal stem cells. Neurosci Lett 2015; 600: 69-74.
[27] Jung KH, McCarthy RL, Zhou C, Uprety N, Barton MC and Beretta L. MicroRNA regulates hepatocytic differentiation of progenitor cells by targeting YAP1. Stem Cells 2016; 34: 1284-1296.
[28] Hino K, Tsuchiya K, Fukao T, Kiga K, Okamoto R, Kanai T and Watanabe M. Inducible expression of microRNA-194 is regulated by HNF-1alpha during intestinal epithelial cell differentiation. Rna 2008; 14: 1433-1442.
[29] Li J, He X, Wei W and Zhou X. MicroRNA-194 promotes osteoblast differentiation via downregulating STAT1. Biochem Biophys Res Commun 2015; 460: 482-488.
[30] Al-Huseini I, Ashida N and Kimura T. Deletion of IkappaB-Kinase beta in smooth muscle cells Induces vascular calcification through beta-catenin-runt-related transcription factor 2 signaling. J Am Heart Assoc 2018; 7.
[31] Leeper NJ, Raiesdana A, Kojima Y, Chun HJ, Azuma J, Maegdefessel L, Kundu RK, Quertermous T, Tsao PS and Spin JM. MicroRNA-26a is a novel regulator of vascular smooth muscle cell function. J Cell Physiol 2011; 226: 1035-1043.
[32] Maegdefessel L, Azuma J, Toh R, Merk DR, Deng A, Chin JT, Raaz U, Schoelmerich AM, Raiesdana A, Leeper NJ, McConnell MV, Dalman RL, Spin JM and Tsao PS. Inhibition of microRNA-29b reduces murine abdominal aortic aneurysm development. J Clin Invest 2012; 122: 497-506.
[33] Yu Y, Shi E, Gu T, Tang R, Gao S, Wang Y and Liu H. Overexpression of microRNA-30a contributes to the development of aortic dissection by targeting lysyl oxidase. J Thorac Cardiovasc Surg 2017; 154: 1862-1869.
[34] Tang Y, Yu S, Liu Y, Zhang J, Han L and Xu Z. MicroRNA-124 controls human vascular smooth muscle cell phenotypic switch via Sp1. Am J Physiol Heart Circ Physiol 2017; 313: H641-h649.