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Effect of miR-223 on thrombophlebitis rats through regulating Toll-like receptor signaling pathway

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Abstract. – OBJECTIVE: To explore the effect of the micro ribonucleic acid (miR)-223 on the thrombophlebitis rats by regulating the Toll-like receptor (TLR) signaling pathway.

MATERIALS AND METHODS: The rat model of thrombophlebitis was established, and miR-223 was silenced or overexpressed through lentiviral transfection. The rats were divided into miR-223 inhibitors group (Inhibitors group), miR-223 mimics group (Mimics group), and normal group (Control group). The transfection efficiency of miR-223 in venous tissues was detected via Reverse Transcription-Polymerase Chain Reaction (RT-PCR), the hemorheological indexes plasma viscosity (PV) and hematocrit (HCT) were observed, and the content of the serum inflammatory factors interleukin-6 (IL-6) and tumor necrosis factor-β (TNF-β) were detected via enzyme-linked immunosorbent assay (ELISA). Moreover, the fibrinolytic indexes plasminogen activator inhibitor (PAI) and the tissue-type plasminogen activator (t-PA) were detected, the morphological changes in the venous tissues were observed via hematoxylin-eosin (HE) staining, and the gene and protein expressions of the TLR signaling pathway were detected via RT-PCR and Western blotting.

RESULTS: The expression of miR-223 was significantly increased in the Mimics group ($p<0.05$) and significantly decreased in the Inhibitors group ($p<0.05$). The high-shear and low-shear whole blood viscosity and HCT in the Inhibitors group were significantly higher than those in the Mimics group ($p<0.05$). The levels of serum IL-6, IL-1β, and TNF-β in the Inhibitors group were remarkably higher than those in the Mimics group ($p<0.05$). The Inhibitors group had a remarkably lower level of t-PA ($p<0.05$) and a remarkably higher level of PAI than the Mimics group ($p<0.05$). Besides, the inferior vena cava wall shed and disappeared due to complete necrosis in the Inhibitors group. In the Mimics group, the vascular lumen was slightly expanded, and the vascular wall had intact contour. It was found in the gene detection that the mRNA levels of TLR2, myeloid differential protein-88 (MyD88) and c-Jun N-terminal kinase (JNK) were evidently increased in the Inhibitors group, and the significant increases in the protein levels of TLR2 and MyD88 were also observed in the protein detection.

CONCLUSIONS: The overexpression of miR-223 can inhibit the TLR signaling pathway, thereby promoting the recovery of thrombophlebitis rats.

Key Words:
MiR-223, Toll-like receptor signaling pathway, Thrombophlebitis, Rats.

Introduction

The morbidity rate of varicosity can reach 77% sometimes in different populations, seriously affecting the patients' quality of life[1,2]. Varicosity is also associated with many complications, including thrombophlebitis with an increasing morbidity rate year by year. Superficial thrombophlebitis leads to 3-fold increased risk of deep vein thrombosis (DVT) in varicosity patients[3]. Venography was a golden standard several decades ago among the research methods for the lower extremity vein system. Therefore, increasingly more cases of asymptomatic DVT have been confirmed via examination of the venous system using venography. However, currently, the main cause of thrombophlebitis has not been fully clarified[4]. Thrombophlebitis is often secondary to epifascial varicosity in acute thrombosis, whose clinical manifestation is superficial vein accompanied by induration. Moreover, thrombophlebitis may be associated with skin fever, chromatosis, swelling, and other adverse reactions in some patients, and such systemic symptoms as fever and headache will occur at the same time, seriously affecting the work and life of patients[5,6]. Thrombophlebitis has not attracted enough attention. As described above, thrombophlebitis is often secondary to epifascial varicosity[7,8], in which special treatment is required, including the antibiotics, anticoagulants, and often thrombolysis, anti-platelet aggregation, and anti-infective therapy in modern clinical medicine, but the long-term application

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of these drugs will lead to bleeding and other side effects[9]. Therefore, a deeply understanding of its molecular regulatory network is essential for the treatment of thrombophlebitis; moreover, the design of new target drugs will provide new ideas for accelerating the treatment of thrombophlebitis. Therefore, searching for new targets for thrombophlebitis has become a problem urgently to be solved.

Micro ribonucleic acids (miRNAs) are non-coding RNAs involved in the specific regulation of protein-coding and non-coding genes. Increasingly more studies have indicated that one-third of the human genes may be regulated by miRNAs[10]. MiRNAs can also be involved in the regulation of the cell cycle, metabolism, and various immune responses[11]. In particular, Carleton et al[12] have shown that the role of miRNAs in the pathogenesis of different diseases has been fully explored, as it plays important roles in physiology and various diseases. Therefore, miRNAs have become an important gene expression regulator in many diseases, and their regulatory network has attracted much attention in recent years[13]. MiR-223 was previously observed in the medullary system, especially neutrophils, which is associated with many inflammatory diseases, infection, and cancer[14]. However, its specific role in thrombophlebitis remains unclear. The Toll-like receptors (TLRs) are a kind of cell surface sensor, and the interaction among them triggers the activation of the downstream pathways, leading to phosphorylation and translocation of the downstream factors[15]. After downstream, TLR2 activates the myeloid differential protein-88 (MyD88), while the MyD88-mediated signals promote the early activation of the nuclear factor-κB (NF-κB), responsible for the production of many pro-inflammatory cytokines, resulting in cell adhesion, proliferation, angiogenesis, and apoptosis[16]. The MyD88 independent pathway involves c-Jun N-terminal kinase (JNK), which leads to the late activation of NF-κB and induces interferon regulating factor 3 (IRF3), thereby increasing the production of interferon (INF) and INF-induced gene products[17]. However, the specific mechanism of the effect of TLR signaling pathway on thrombophlebitis is still unclear, and the regulatory role of miR-223 in thrombophlebitis rats through TLR needs further research. In this experiment, therefore, the effect of miR-223 on thrombophlebitis through the TLR signaling pathway was verified using various molecular techniques, hoping to provide an experimental and theoretical basis for the prevention and treatment of thrombophlebitis through the TLR signaling pathway.

In the present study, the rat model of thrombophlebitis was established, miR-223 was overexpressed and silenced, and the effect of miR-223 on thrombophlebitis in rats was clarified using *in vivo* experiments and various molecular biological techniques. Finally, the specific molecular mechanism of such an effect was explored. To sum up, the experimental results enrich and improve the theoretical basis of the effects of miR-223 on thrombophlebitis in rats and TLR signaling pathway, as a consequence, the protective effect of miR-223 in thrombophlebitis in rats was revealed.

Materials and Methods

Reagents and Consumables

The main reagents were: tissue homogenizer (Haimen Aiband Laboratory Equipment Co., Ltd., Haimen, China), electrophoresis apparatus (EPS300, Bio-Rad, Hercules, CA, USA), microplate reader (Multiskan MK3, Thermo, Waltham, MA, USA), 2500 gel imager (Bio-Rad, Hercules, CA, USA), quantitative polymerase chain reaction (qPCR) instrument (7900 Fast, Applied Biosystems, Thermo, Waltham, MA, USA), TRIzol (Invitrogen, Carlsbad, CA, USA) reagent, diethyl pyrocarbonate (DEPC)-treated water (Beyotime, Shanghai, China), ultrapure agarose, SuperScript III reverse transcription kit and SYBR qPCR Mix (Thermo, Waltham, MA, USA), pipettor (Eppendorf, Hamburg, Germany), interleukin-1 (IL-1) and IL-6 enzyme-linked immunosorbent assay (ELISA) kits (Novus, Littleton, CO, USA), RIPA lysis buffer (Beyotime, Shanghai, China), dodecyl sulfate, sodium salt-polyacrylamide gel electrophoresis (SDS-PAGE) buffer, protease inhibitor and bicinchoninic acid (BCA) protein concentration assay kit (Biosharp, Hefei, China), β-actin and secondary antibodies (Beijing Ray Antibody Biotech, Beijing, China), and primary antibodies (Cell Signaling Technology, Danvers, MA, USA).

Animal Modeling and Grouping

After fasting for solids and liquids for 12 h before the operation, the male Wistar rats were anesthetized via intraperitoneal injection of pentobarbital sodium and fixed on an operating table in a supine position. The skin was cut along the median abdominal line, and the inferior vena cava was separated and ligated using the thick silk be-

low the left renal vein to establish the rat model of thrombophlebitis. In the Control group, the inferior vena cava was not ligated, and the other operations were the same as above. Based on the model of thrombophlebitis, miR-223 was silenced and overexpressed, followed by amplification using the gene specific primers. After purification and recycling, the reaction products and vector fragments were ligated using T4 DNA, and the ligation product was transferred into the competent cells. Then, miR-223 was transferred into adenovirus vectors and transfected into rats. The rats were divided into miR-223 inhibitors group (Inhibitors group, n=20), miR-223 mimics group (Mimics group, n=20), and normal group (Control group, n=20). After reaction for 24 h, subsequent experiments were performed. All experiments were approved by the Animal Ethics Committee of the hospital.

Transfection Efficiency of MiR-223 in Each Group

To deeply explore the role of miR-223 in thrombophlebitis in rats, miR-223 was transfected into rats using the adenovirus, and the transfection efficiency of miR-223 in venous tissues was detected via Real Time-Polymerase Chain Reaction (RT-PCR), so as to prepare for the subsequent study of the molecular mechanism of the miR-223 protective effect on thrombophlebitis.

Detection of Plasma Viscosity (PV) and Hematocrit (HCT)

The low-shear and high-shear whole blood viscosity and HCT were measured using a blood viscometer. 4 mL of blood was aseptically drawn from the caudal vein, part of the whole blood was centrifuged at 3000 rpm for 30 min, and HCT was read. Then, the remaining blood was centrifuged at 3000 rpm for 5 min, and the upper-layer plasma was aspirated to detect PV according to the instructions of the instrument.

Detection of Fibrinolytic Indexes Plasminogen Activator Inhibitor (PAI) and Tissue-Type Plasminogen Activator (t-PA)

After that 4 mL of blood was aseptically drawn from the caudal vein and centrifuged at 3000 rpm for 10 min under low temperature, the supernatant was collected, subpackaged in 200 μL centrifuge tubes, added with an equal volume of acidizing fluid and mixed evenly. Then, 100 μL of the mixture was aspirated into micro-wells containing standards and plasma to be tested, followed by incubation at 37°C for 120 min. The reaction was terminated using 30 μL of stop buffer, the wells were washed, and the changes in each index were detected using the kits according to the actual conditions and the instructions. Finally, the absorbance of indexes in each group was detected using a microplate reader.

Determination of Inflammatory Factors via ELISA

The content of the serum inflammatory factors was detected via ELISA. After that 4 mL of blood was aseptically drawn from the caudal vein and centrifuged at 3000 rpm for 10 min under low temperature, the supernatant was collected and sub-packaged in 200 μL centrifuge tubes, followed by incubation at 37°C for 60 min and washing. The changes in each index were detected using the kits according to the actual conditions and the instructions. Finally, the absorbance of the inflammatory factors in each group was detected using the microplate reader.

Observation of Changes in Tissues via Hematoxylin-Eosin (HE) Staining

After anesthesia with pentobarbital sodium, the rats in each group were aseptically sacrificed, and the venous tissues were separated. Then, the dissected venous tissues were soaked in formalin, washed with running water for 24 h, transparentized, and embedded in paraffin. The paraffin block was prepared into pathological sections (about 5 μm in thickness), stained with hematoxylin for 15 min, washed with water and counterstained with eosin for 5 min, followed by dehydration with alcohol, transparentization, and sealing with neutral resins. Finally, the tissues were observed under a light microscope.

Detection of Expressions of Related Genes via RT-PCR

After anesthesia with the pentobarbital sodium, the rats in each group were aseptically sacrificed, and the venous tissues were separated. About 100 mg of sterile venous tissues were accurately taken under low temperature and ground with liquid nitrogen, followed by homogenization with lysis buffer under low temperature at 2200 rpm for 15 s to extract the total RNA, and the RNA concentration was detected qualified. Then, mRNA was reversely transcribed into cDNA and stored for later use. The primer amplifica-

tion was performed using 20 μL of system (2 μL of cDNA, 10 μL of mix, 2 μL of primers, and 6 μL of ddH_2O, for a total of 40 cycles). The PCR amplification was performed: pre-denaturation at 95°C for 2 min, 94°C for 20 s, 60°C for 20 s and 72°C for 30 s, for a total of 40 cycles. The primer sequences of the target genes and the internal reference glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were designed according to those in the GenBank (Table I). The expression levels of the target genes were detected via RT-PCR, and the relative expression levels of the related genes in venous tissues in each group were calculated using the $2^{-\Delta\Delta Ct}$ method.

Western Blotting

After anesthesia with pentobarbital sodium, the rats in each group were aseptically sacrificed, and the venous tissues were separated. About 150 mg of sterile venous tissues were accurately taken into the 10 mL Eppendorf (EP) tube, grounded under low temperature, and quickly smashed using a homogenizer under low temperature, followed by centrifugation. The supernatant was collected and placed into the EP tube, followed by the detection of protein concentration according to the instructions of the BCA kit. Then, the Western blotting was performed as follows: the total protein was extracted from the venous tissues, subjected to water bath for 8 min, centrifuged at 1000 g for 5 min and subjected to electrophoresis. The protein was transferred onto a membrane and incubated with the primary and secondary antibodies. The protein band was scanned and quantified using the scanner (Bio-Rad, Hercules, CA, USA), and the level of the protein to be detected was corrected using GAPDH. Finally, the gray value of the protein band was detected using Image Lab software.

Table I. Primer sequences in RT-PCR.

Target gene	Primer sequences
TLR2	F: 5'-TGAACCAGGGCATACCT-3' R: 5'-AGAAGTCCATGTCCGCAT-3'
MyD88	F: 5'-GCTGGAGCAGACGGAGTG-3' R: 5'-AGGCTGAGAGCAAACTTGC-3'
JNK	F: 5'-TTCATTGTGGGTAGGTGG-3' R: 5'-CTACAGCTTCCGCTTCAG-3'
miR-223	F: 5'-GTATCCAGTGCAGGGTCCA-3' R: 5'-TCGCACTGGATACGACCCC-3'
GAPDH	F: 5'-GACTTCAACAGCGACACCC-3' R: 5'-ACCCTGTTGCTGTAGCCAA-3'

Statistical Analysis

The Statistical Product and Service Solutions (SPSS) 21.0 (IBM Corp., Armonk, NY, USA) software was used to process the raw experimental data, and the multiple comparisons were performed for the data. The experimental results obtained were expressed as mean ± standard deviation ($\bar{\chi}\pm SD$), and $p<0.05$ suggested the statistically significant difference. The bar graph was plotted using the GraphPad Prism 8.0 (La Jolla, CA, USA).

Results

Transfection Results of MiR-223 in Each Group

To observe the transfection efficiency of miR-223 in each group, the gene expression level was detected. As shown in Figure 1, the expression of miR-223 was significantly increased in the Mimics group ($p<0.05$) and significantly decreased in the Inhibitors group ($p<0.05$), indicating that the transfection effect is so evident that the subsequent experiments can be performed.

PV and HCT in Each Group

As shown in Table II, the high-shear and low-shear whole blood viscosity and HCT in the Inhibitors group were significantly higher than those in the Control group ($p<0.05$), while they were remarkably lower in the Mimics group than those in the Inhibitors group ($p<0.05$).

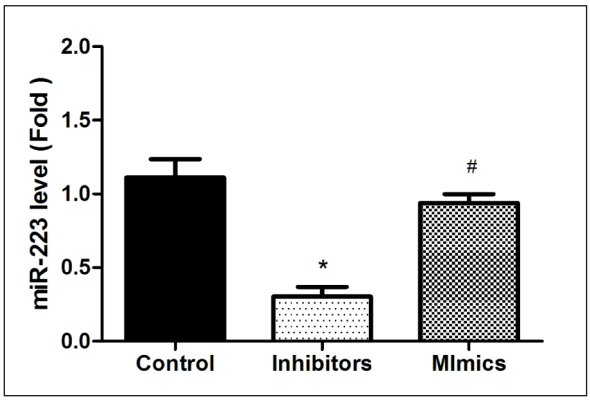

Figure 1. The transfection effect of miR-223. The expression of miR-223 is increased in the Mimics group ($p<0.05$) and decreased in the Inhibitors group ($p<0.05$). *$p<0.05$ *vs.* Control group, #$p<0.05$ *vs.* Inhibitors group.

Table II. PV and HCT.

Group	High-shear whole blood viscosity (mPa.s)	Low-shear whole blood viscosity (mPa.s)	HCT (%)
Control	3.3 ± 0.2	7.1 ± 0.5	40.8 ± 1.5
Inhibitors	6.0 ± 0.4*	14.2 ± 0.3*	50.7 ± 1.6*
Mimics	4.6 ± 0.7#	9.4 ± 0.5#	42.9 ± 1.2#

Note: The high-shear and low-shear whole blood viscosity and HCT in the Inhibitors group are significantly higher than those in the Control group ($p<0.05$), while they are remarkably lower in the Mimics group than those in the Inhibitors group ($p<0.05$). *$p<0.05$ *vs.* Control group, #$p<0.05$ *vs.* Inhibitors group.

Detection Results of Fibrinolytic Indexes PAI and t-PA

The inhibitors group had a remarkably lower level of t-PA ($p<0.05$) and a remarkably higher level of PAI than the Control group ($p<0.05$), while the Mimics group had the opposite expression trends of the Inhibitors group ($p<0.05$) (Table III).

Detection Results of Cytokines in Each Group

The levels of the serum inflammatory factors TNF-β, IL-6, and IL-1β were evidently higher in the Inhibitors group than those in the other two groups, while they evidently declined in the Mimics group ($p<0.05$) (Table IV), suggesting that the overexpression of miR-223 can inhibit the production of the inflammatory factors.

Table III. Fibrinolytic indexes PAI and t-PA (IU/mL).

Group	PAI	t-PA
Control	0.5 ± 0.1	0.4 ± 0.4
Inhibitors	1.5 ± 0.2*	0.1 ± 0.1*
Mimics	0.8 ± 0.3#	0.3 ± 0.5#

Note: The Inhibitors group has a remarkably lower level of t-PA ($p<0.05$) and a remarkably higher level of PAI than the Control group ($p<0.05$), while the Mimics group has the opposite expression trends of the Inhibitors group ($p<0.05$). *p<0.05 vs. Control group, #$p<0.05$ *vs.* Inhibitors group.

HE Staining Results

In the Control group, the vascular structure was normal. The inferior vena cava wall shed and disappeared due to complete necrosis in the Inhibitors group. In the Mimics group, the vascular lumen was slightly expanded, and the vascular wall had an intact contour (Figure 2).

Gene Detection Results

It was found in the gene detection that the mRNA levels of TLR2, MyD88, and JNK were evidently increased in the Inhibitors group, while they were evidently decreased in the Mimics group (Figure 3).

Protein Detection Results

The significant increases in the protein levels of TLR2 and MyD88 were also observed in the protein detection in the Inhibitors group, while the Mimics group had the opposite trends (Figure 4).

Discussion

Thrombophlebitis will cause a variety of secondary reactive diseases in the severe stage, so the early accurate diagnosis is the key to a successful treatment and improvement of prognosis. MiR-223 can be involved in the regulation of thrombophlebitis, and its main role is to negatively regulate the inflammatory response. However, the regulatory effect of miR-233 on thrombo-

Table IV. Cytokines.

Group	IL-1β (mg/L)	TNF-β (fmol/mL)	IL-6 (mg/L)
Control	55.38 ± 4.01	36.10 ± 5.00	71.14 ± 3.47
Inhibitors	131.40 ± 3.07*	80.99 ± 4.41*	135.14 ± 3.21*
Mimics	70.08 ± 2.01#	46.88 ± 4.84#	82.88 ± 3.91#

Note: The levels of IL-6, IL-1β, and TNF-β are significantly increased in the Inhibitors group and remarkably decreased in the Mimics group ($p<0.05$). *$p<0.05$ *vs.* Control group, #$p<0.05$ *vs.* Inhibitors group.

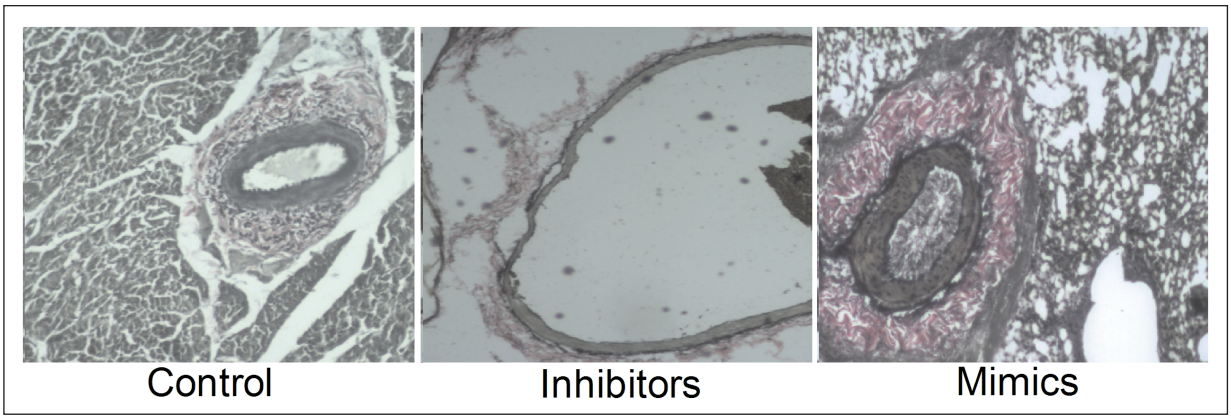

Figure 2. HE staining results. In the Control group, the vascular structure is normal (×200). The inferior vena cava wall sheds and disappears due to complete necrosis in the Inhibitors group (×200). In the Mimics group, the vascular lumen is slightly expanded, and the vascular wall has intact contour (×200).

phlebitis in rats through TLR still needs further research. In the present study, the rat model of thrombophlebitis was established, miR-223 was silenced and overexpressed, and whether miR-223 plays a role in thrombophlebitis was further observed, so as to find potential therapeutic methods. This model has a similar physiological process of thrombophlebitis to human, so it can be well used as the object of study. To observe the transfection efficiency of miR-223 in each group, the gene expression level was detected. The results showed that the expression of miR-223 was significantly increased in the Mimics group, indicating that the transfection effect is so evident that the subsequent experiments can be performed. In addition, it was found in the HE staining that the inferior vena cava wall shed and disappeared due to complete necrosis in the Inhibitors group, the vascular lumen was slightly expanded, and the vascular wall had intact contour in the Mimics group, which is consistent with the study of Li et al[18]. There are often changes in hemorheology in thrombophlebitis, and the blood viscosity under different shear rates can reflect the hemorheological features. T-PA is the major activator of the fibrinolytic system, and PAI is a specific inhibitor of t-PA[19,20]. In this study, the high-shear and low-shear whole blood viscosity and HCT in the Inhibitors group were higher than those in the Control group, while the Inhibitors group had a remarkably lower level of t-PA and a remarkably higher level of PAI than the Control group, in line with the above studies.

Studies have demonstrated that miR-223 plays an important role in inflammation. MiR-223 was first identified as an important regulator of formation and homeostasis of mature neutrophils; later it was found that miR-223 is an important anti-inflammatory mediator, and the up-regulation of miR-223 in neutrophils produces a potent

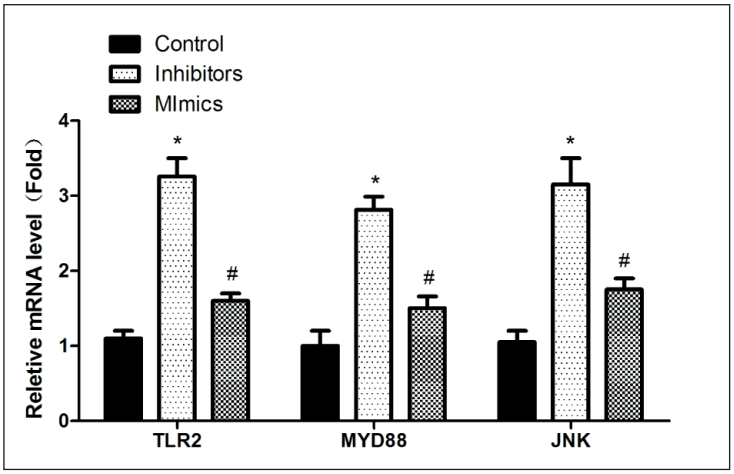

Figure 3. Gene detection results. The mRNA levels of TLR2, MyD88 and JNK, are increased in the Inhibitors group, while they are decreased in the Mimics group. *$p<0.05$ vs. Control group, #$p<0.05$ *vs.* Inhibitors group.

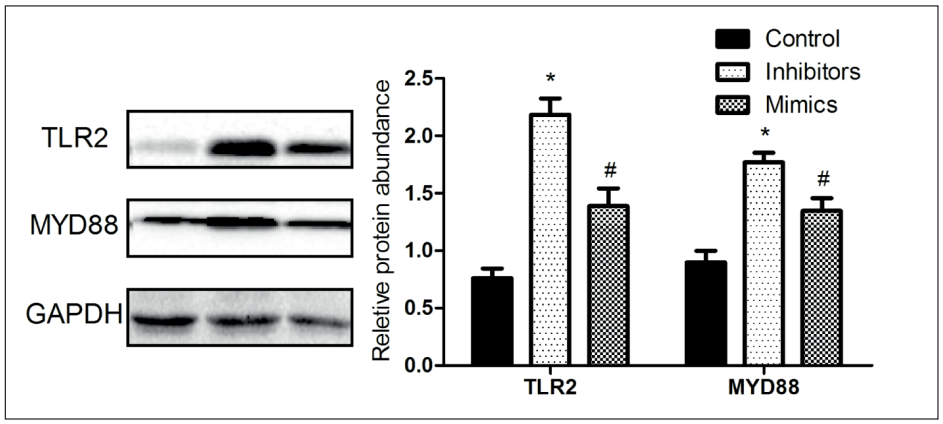

Figure 4. Protein detection results. The significant increases in the protein levels of TLR2 and MyD88 are also observed in the Inhibitors group, while the Mimics group has the opposite trends. $^{*}p<0.05$ *vs.* Control group, $^{\#}p<0.05$ *vs.* Inhibitors group.

inhibitory pathway[21]. According to He et al[22], the specific overexpression of miR-223 may alleviate the inflammatory response in neutrophils, and also inhibit the production of IL-1β. Moreover, miR-223 regulates leukocyte chemotaxis through chemotactic agents, which is essential for controlling the chronic inflammatory diseases. In addition, miR-223 can suppress the activation of macrophages[23]. In the present investigation, the levels of the serum inflammatory factors TNF-β, IL-6, and IL-1β were remarkably higher in the Inhibitors group than those in the other two groups, while they significantly declined in the Mimics group, suggesting that the overexpression of miR-223 can inhibit the production of the inflammatory factors. TLRs are the transmembrane proteins in the mammalian cells which are mainly involved in the identification of the bacterial and viral components[24]. There are increasingly more studies that TLRs participate in the immune responses[25]. The long-term tissue damage and production of endogenous TLR2 ligands can activate TLR2 signals, leading to phosphorylation and translocation of the downstream factors, mainly including MyD88 and JNK. The MyD88-mediated signals will promote the early activation of NF-κB, responsible for the production of many pro-inflammatory cytokines, resulting in cell adhesion, proliferation, and angiogenesis[26]. In this study, it was found in the gene detection that the mRNA levels of TLR2, MyD88, and JNK were increased in the Inhibitors group, while they were decreased in the Mimics group. The significant increases in the protein levels of TLR2 and MyD88 were also observed in the protein detection in the Inhibitors group, in line with the research results of Zhou et al[27] and Picardi et al[28]. This work demonstrates that the up-regulation of miR-223 expression can inhibit the secretion of the inflammatory factors, and suppress the expression of the TLR signaling pathway, thereby promoting the recovery of thrombophlebitis. However, there are also some deficiencies in this research. For example, only *in vivo* experiments were performed, and there was no cell culture *in vitro* to confirm the effect of miR-223 on thrombophlebitis and its influence on the TLR signaling pathway.

Conclusions

A series of pathological changes, such as inflammation and abnormalities in the hemorheological indexes, will occur in thrombophlebitis, but miR-223 can inhibit the occurrence of the diseases through the TLR signaling pathway, so miR-223 and TLR can serve as new targets for the treatment of thrombophlebitis. To sum up, the present study provides a theoretical basis for the prevention and treatment of thrombophlebitis and offers new ideas and an experimental basis for further research.

Conflict of Interests

The authors declared that they have no conflict of interests.

References

1) Guex JJ, Myon E, Didier L, Nguyen LC, Taieb C. Chronic venous disease: health status of a population and care impact on this health status through quality of life questionnaires. Int Angiol 2005; 24: 258-264.

2) Naoum JJ, Hunter GC, Woodside KJ, Chen C. Current advances in the pathogenesis of varicose veins. J Surg Res 2007; 141: 311-316.
3) Guex JJ. Thrombotic complications of varicose veins. A literature review of the role of superficial venous thrombosis. Dermatol Surg 1996; 22: 378-382.
4) Wichers IM, Di Nisio M, Buller HR, Middeldorp S. Treatment of superficial vein thrombosis to prevent deep vein thrombosis and pulmonary embolism: a systematic review. Haematologica 2005; 90: 672-677.
5) Samuelson B, Go AS, Sung SH, Fan D, Fang MC. Initial management and outcomes after superficial thrombophlebitis: The Cardiovascular Research Network Venous Thromboembolism study. J Hosp Med 2016; 11: 432-434.
6) Cascella M, Viscardi D, Bifulco F, Cuomo A. Postoperative massive pulmonary embolism due to superficial vein thrombosis of the upper limb. J Clin Med Res 2016; 8: 338-341.
7) Bagot CN, Arya R. Virchow and his triad: a question of attribution. Br J Haematol 2008; 143: 180-190.
8) Paker M, Cohen JT, Moed N, Shleizerman L, Masalha M, Ashkenazi D, Mazzawi S. Facial vein thrombophlebitis: a case report and literature review. Int J Pediatr Otorhinolaryngol 2018; 113: 298-301.
9) Maddox RP, Seupaul RA. What is the most effective treatment of superficial thrombophlebitis? Ann Emerg Med 2016; 67: 671-672.
10) Nagao Y, Hisaoka M, Matsuyama A, Kanemitsu S, Hamada T, Fukuyama T, Nakano R, Uchiyama A, Kawamoto M, Yamaguchi K, Hashimoto H. Association of microRNA-21 expression with its targets, PDCD4 and TIMP3, in pancreatic ductal adenocarcinoma. Mod Pathol 2012; 25: 112-121.
11) Wang L, Song G, Liu M, Chen B, Chen Y, Shen Y, Zhu J, Zhou X. MicroRNA-375 overexpression influences P19 cell proliferation, apoptosis and differentiation through the Notch signaling pathway. Int J Mol Med 2016; 37: 47-55.
12) Carleton M, Cleary MA, Linsley PS. MicroRNAs and cell cycle regulation. Cell Cycle 2007; 6: 2127-2132.
13) Wang S, He W, Wang C. MiR-23a regulates the vasculogenesis of coronary artery disease by targeting epidermal growth factor receptor. Cardiovasc Ther 2016; 34: 199-208.
14) Haneklaus M, Gerlic M, O'Neill LA, Masters SL. MiR-223: infection, inflammation and cancer. J Intern Med 2013; 274: 215-226.
15) Latz E. The inflammasomes: mechanisms of activation and function. Curr Opin Immunol 2010; 22: 28-33.
16) Beinke S, Ley SC. Functions of NF-kappaB1 and NF-kappaB2 in immune cell biology. Biochem J 2004; 382: 393-409.
17) French SW, Oliva J, French BA, Li J, Bardag-Gorce F. Alcohol, nutrition and liver cancer: role of Toll-like receptor signaling. World J Gastroenterol 2010; 16: 1344-1348.
18) Li G, Litscher G, Pang H, Yang B, Litscher D, Wang L. Experimental study of phlebitis ointment administration in acute superficial thrombophlebitis. Evid Based Complement Alternat Med 2018; 2018: 2983195.
19) Lee MC, Absalom AR, Menon DK, Smith HL. Awake insertion of the laryngeal mask airway using topical lidocaine and intravenous remifentanil. Anaesthesia 2006; 61: 32-35.
20) Sardzhveladze NM, Kakabadze Z, Tvaladze MG. [Treatment of thrombophlebitis by the method of the transplantation of the endothelial cells]. Georgian Med News 2006; (136): 91-95.
21) Johnnidis JB, Harris MH, Wheeler RT, Stehling-Sun S, Lam MH, Kirak O, Brummelkamp TR, Fleming MD, Camargo FD. Regulation of progenitor cell proliferation and granulocyte function by microRNA-223. Nature 2008; 451: 1125-1129.
22) He Y, Feng D, Li M, Gao Y, Ramirez T, Cao H, Kim SJ, Yang Y, Cai Y, Ju C, Wang H, Li J, Gao B. Hepatic mitochondrial DNA/Toll-like receptor 9/MicroRNA-223 forms a negative feedback loop to limit neutrophil overactivation and acetaminophen hepatotoxicity in mice. Hepatology 2017; 66: 220-234.
23) Wang Q, Zhao DY, Xu H, Zhou H, Yang QY, Liu F, Zhou GP. Down-regulation of microRNA-223 promotes degranulation via the PI3K/Akt pathway by targeting IGF-1R in mast cells. PLoS One 2015; 10: e0123575.
24) Brown J, Wang H, Hajishengallis GN, Martin M. TLR-signaling networks: an integration of adaptor molecules, kinases, and cross-talk. J Dent Res 2011; 90: 417-427.
25) Olivieri F, Rippo MR, Prattichizzo F, Babini L, Graciotti L, Recchioni R, Procopio AD. Toll like receptor signaling in "inflammaging": microRNA as new players. Immun Ageing 2013; 10: 11.
26) Mahanonda R, Pichyangkul S. Toll-like receptors and their role in periodontal health and disease. Periodontol 2000 2007; 43: 41-55.
27) Zhou W, Pal AS, Hsu AY, Gurol T, Zhu X, Wirbisky-Hershberger SE, Freeman JL, Kasinski AL, Deng Q. MicroRNA-223 suppresses the canonical NF-kappaB pathway in basal keratinocytes to dampen neutrophilic inflammation. Cell Rep 2018; 22: 1810-1823.
28) Picardi M, Pagliuca S, Chiurazzi F, Iula D, Catania M, Rossano F, Pane F. Early ultrasonographic finding of septic thrombophlebitis is the main indicator of central venous catheter removal to reduce infection-related mortality in neutropenic patients with bloodstream infection. Ann Oncol 2012; 23: 2122-2128.