Journal of Orthopaedic Surgery and Research

RESEARCH ARTICLE

Open Access

Analysis of the predictive value of microRNA-199b-5p combined with nitric oxide for venous thrombosis in patients undergoing total knee arthroplasty

Shen Zhou[1†], Wenhan Ma[2†], Yuan Li[3], Li Liu[3*] and Shenyi Lu[4*]

Abstract

Background Deep vein thrombosis (DVT) of lower extremity is a common complications after total knee arthroplasty (TKA). The purpose of this study was to evaluate the risk factors for DVT after TKA and analyze the expression of miR-199b-5p and nitric oxide (NO) before and after TKA, as well as their predictive value for DVT.

Methods Basic clinical information of 121 patients with TKA was analyzed retrospectively. RT-qPCR was used to detect the relative expression level of miR-199b-5p in patients before and after TKA treatment. Based on the occurrence of DVT, patients were divided into DVT and non-DVT groups. Logistic regression analysis evaluated the risk factors of DVT. The receiver operating characteristic (ROC) curve assessed the predictive value of postoperative miR-199b-5p level, preoperative NO level, and their combination in DVT. The target genes of miR-199b-5p and their functions were predicted and annotated using bioinformatics analysis.

Results The level of miR-199b-5p after TKA was upregulated compared with that before TKA ($P < 0.001$). DVT occurred in 20 of 121 patients after TKA, with an incidence of 16.53%. Multivariate analysis showed that age, family history of DVT, decrease of NO and increase of miR-199b-5p were risk factors for DVT after TKA ($P < 0.05$). The ROC curve showed that both miR-199b-5p and NO had certain diagnostic value for DVT, but the combination of miR-199b-5p and NO had the highest diagnostic accuracy ($P < 0.001$).

Conclusion This study showed that the expression of miR-199b-5p was up-regulated after TKA, and miR-199b-5p levels were higher in DVT patients than in non-DVT patients. miR-199b-5p combined with NO is of great value in the diagnosis of DVT after TKA.

Keywords MiR-199b-5p, Nitric oxide, Total joint arthroplasty, Blood coagulation, Thrombus

[†]Shen Zhou and Wenhan Ma contributed equally to this work.

*Correspondence:
Li Liu
liulipzh1@163.com
Shenyi Lu
drlushenyi@163.com
[1]Department of Articular Surgery, XuZhou Renci Hospital, Xuzhou 221400, China
[2]Department of Cardiology, the Second Hospital of Shandong University, Jinan 250022, China
[3]Department of General Practice, Affiliated Hospital of Panzhihua University, 27 Taoyuan Street, Panzhihua 617200, Sichuan, China
[4]Department of Rehabilitation, The Affiliated Hospital of Youjiang Medical University for Nationalities, No.18, Zhongshan 2nd Road, Youjiang District, Baise 533000, Guangxi, China

Introduction

Rheumatoid arthritis as an inflammatory disease has been plagued by the vast number of people about the treatment of such diseases, mainly drug therapy and surgical treatment. Recent studies have found that small interfering RNA (siRNA) can be used to identify molecular targets and play a therapeutic effect on arthritis [1, 2]. Total joint arthroplasty (TJA) is an effective treatment for joint diseases, especially total knee arthroplasty (TKA) and total hip arthroplasty (THA) [3]. Deep vein thrombosis (DVT) is one of the most serious complications after TKA, often occurring in the lower leg. Some patients have symptoms such as lower limb swelling and tenderness, while most patients are asymptomatic [4]. TKA is a powerful thrombosis stimulator because involves three thrombotic factors: venous stasis, blood hypercoagulability, and vascular endothelial injury. These factors interact together to promote thrombosis [5, 6].

Endothelial cells exist in the inner walls of blood vessels and play a crucial role in maintaining vascular structure, regulating vessel tension, and ensuring normal blood flow [7]. Studies show that evaluating vascular endothelial injury indicators can help judge the occurrence and progression of DVT [8]. Nitric oxide (NO) is a small molecular produced by endothelial cells that functions in vasodilation, reducing blood pressure, and inhibiting platelet aggregation [9]. Under physiological conditions, NO helps maintain the function of vascular endothelial cells and the structural integrity of vascular walls. It is also a common measure for clinically observing vascular endothelial function. A decrease in NO levels reduces vasodilation and antiplatelet effect, leading to vessel contraction and platelet adhesion, which promote venous thrombosis formation [10].

As an emerging biomarker, circulating microRNA (miRNA) plays an important role in joint disease such as osteoarthritis and tendon injury [11–13]. For example, Studies have shown significant changes in the plasma of specific miRNAs in patients with DVT, such as increased expression of miR-582 and miR-96, and comprehensive analysis of the combination of miR-96 and D-dimer can help predict the occurrence of DVT [14]. MiR-199b-5p is a non-coding RNA associated with human malignancy and plays a crucial regulatory role in the progression of breast and gastric cancer [15, 16]. A growing number of studies have shown that miR-199b-5p is dysregulated in inflammatory diseases like osteoarthritis [17]. A recent study reported that miR-199b-5p is associated with the occurrence of DVT [18]. However, there is currently no evidence to confirm the trend of miR-199b-5p changes before and after TKA.

In this study, patients with TKA were selected to analyze the correlation between miR-199b-5p and the occurrence of DVT, as well as its predictive value for DVT.

Materials and methods

Study populations and clinical information

This study retrospectively analyzed the clinical data of 121 patients who received TKA from December 2020 to March 2023 in the Affiliated Hospital of Panzhihua University. Inclusion criteria: 1) Patients with knee osteoarthritis or rheumatoid knee arthritis with increased knee pain, obvious deformity (knee valgus or knee varus, severely limited flexion and extension activities, making it difficult for the knee to meet the needs of daily activities, and have failed conservative treatment; 2) Patients whose surgery was performed by the same physician and team; 3) Patients with unilateral or bilateral simultaneous TKA; 4) Patients using the same surgical protocol; 5) Patients with complete clinical data. Exclusion criteria: (1) Patients with unilateral or bilateral lower extremity DVT present before TKA; (2) Patients with a history of cerebral hemorrhage and/or gastrointestinal hemorrhage within 6 months before surgery; (3) Patients had a history of myocardial infarction within 6 months before surgery; (4) Patients with liver and kidney failure, blood system diseases, or malignant tumors; (5) Patients who were bedridden or wheelchair-assisted for a long time before TKA. This study has been approved by the Ethics Committee of Affiliated Hospital of Panzhihua University Hospital, and informed consent has been obtained from the participants involved.

General data of all subjects were collected, including gender, age, body mass index (BMI), complications, smoking and drinking history. Clinical data were collected, including the type and the duration of surgery, intraoperative blood loss, and other indicators.

Plasma sample collection and detection

Venous blood from all patients was collected before and 24 h after TKA, and the relative expression level of miR-199b-5p was detected by reverse transcription-quantitative PCR (RT-qPCR). An enzyme-linked immunosorbent assay (ELISA) kit was used to detect the endothelial injury marker NO in all patients before TKA. The C-reactive protein (CRP), fibrinogen (Fib) and prothrombin time (PT) in blood samples before TKA were determined by automatic biochemical analyzer.

Prediction and functional analysis of miR-199b-5p target genes

TargetScan, ENCORI, EVmiRNA, miRPathDB and MiRDB databases were used to predict the downstream target genes of miR-199b-5p. A Venn diagram was created for the predicted target genes. Subsequently, the cellular components, biological processes, and molecular functions associated with these target genes were identified using Gene Ontology (GO) analysis. The Kyoto Encyclopedia of Genes and Genomes (KEGG) database was

used to analyze the functions and pathways of these target genes in genetic information processing and human diseases. Protein-protein interactions (PPI) networks were constructed using the Search tool for the retrieval of interacting genes (STRING) database to obtain the interaction diagram of interacting genes.

Data analysis

SPSS 19.0 and GraphPad Prism 7.0 software were used for data analysis and image rendering. Measurement data is expressed as mean±standard deviation (SD), and counting data is expressed as n (%). Independent sample t-tests and Chi-square tests were used to compare the two groups. Pearson correlation analysis evaluated the correlation between NO and miR-199b-5p. Receiver operating characteristic(ROC)curves assessed the diagnostic value of DVT. Binary logistic regression was used to examine the relationship between each indicator and the occurrence of DVT. The indicators included in the univariate analysis are defined as follows: Sex (male=1, female=0), age (>65=1, ≤65=0), BMI (>23.68=1, ≤23.68=0), smoking, drinking, Bilateral TKA, diabetes, hypertension, family history of venous thromboembolism (VTE) (yes=1, no=0), operation duration (>157=1, ≤157=0), intraoperative bleeding loss (>141=1, ≤141=0), CRP (>17.6=1, ≤17.6=0), PT (>11.4=1, ≤11.4=0), Fib (>3.88=1, ≤3.88=0), NO (>35.8=1, ≤35.8=0), miR-199b-5p (>1.42=1, ≤1.42=0). Candidate variables with a p value<0.1 on univariate analysis were included in multivariable regression analysis. $P<0.05$ indicates a significant difference.

Results

Screening and expression level analysis of miR-199b-5p

Figure 1A showed the analysis of miRNA differentially expressed in the serum of patients with DVT and

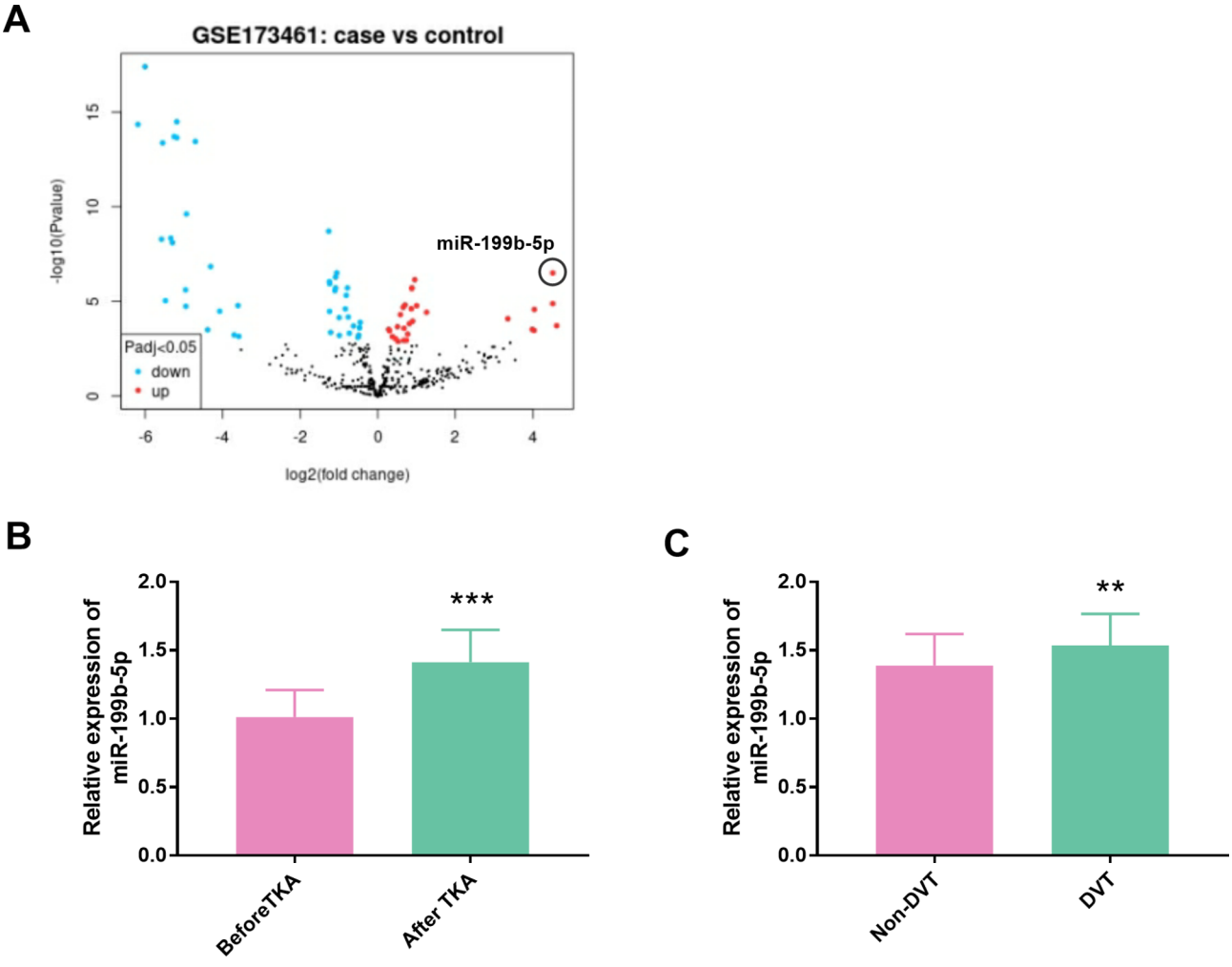

Fig. 1 Screening and expression detection of miR-199b-5p. (**A**) Volcano plot of abnormally expressed miRNAs in DVT patients in the GSE173461 dataset. (**B**) Expression level of miR-199b-5p in serum of patients before and after TKA. (**C**) miR-199b-5p expression levels in serum of DVT patients and non-DVT patients. $^{**}P<0.01$, $^{***}P<0.001$

the control group in the GSE173461 dataset. Based on this, miR-199b-5p, which is abnormally elevated in patients with DVT, was selected as the research index for this study. The level of miR-199b-5p was detected in 121 patients undergoing TKA, revealing a significant increase in serum miR-199b-5p expression 24 h after TKA (Fig. 1B, $P<0.001$). All patients were divided into the DVT group ($n=20$) and the non-DVT group ($n=101$) based on the results of color Doppler ultrasound detection of lower extremity veins on the 5th day after TKA. The incidence of DVT after TKA was 16.53%. In addition, RT-qPCR results showed that the expression level of serum miR-199b-5p in the DVT group was higher than that in the non-DVT group (Fig. 1C, $P<0.01$).

Basic information and clinical data of between DVT and non-DVT groups

As shown in Table 1, there were no significant differences between the two groups in gender, smoking and drinking history, diabetes, hypertension, disease course, Fib ($P>0.05$). Additionally, it was also observed that the levels of age, bilateral TKA patients, family history of DVT, operation time, intraoperative blood loss, CRP, and PT in the DVT group were higher than those in the non-DVT group, while the levels of NO were lower than those in the non-DVT group ($P<0.05$).

Table 1 Comparison of preoperative clinical information between DVT patients and non-DVT patients

Items	Non-DVT group ($n=101$)	DVT group ($n=20$)	*P*
Sex (male/female)	51/50	8/12	0.467
Age (years)	**65.48 ± 7.59**	**70.55 ± 7.24**	**0.005**
BMI (kg/m^2)	23.62 ± 3.18	24.41 ± 3.08	0.119
Smoking history (n, %)	36 (35.6%)	9 (45.0%)	0.456
Drinking history (n, %)	26 (25.7%)	7 (35.0%)	0.417
Bilateral TKA (n, %)	**35 (34.7%)**	**12 (60.0%)**	**0.045**
Diabetes (n, %)	15 (14.9%)	6 (30.0%)	0.114
Hypertension (n, %)	20 (19.8%)	5 (25.0%)	0.787
Family history of VTE (n, %)	**2 (2.0%)**	**4 (20.0%)**	**0.007**
Disease duration (years)	5.30 ± 2.86	6.63 ± 2.34	0.055
Operation duration (min)	**150.81 ± 50.75**	**190.22 ± 47.73**	**0.002**
Intraoperative bleeding loss (mL)	**135.66 ± 55.93**	**169.60 ± 39.18**	**0.011**
CRP (mg/L)	**16.82 ± 8.93**	**21.32 ± 7.59**	**0.037**
PT (s)	**11.30 ± 0.77**	**11.83 ± 1.11**	**0.010**
Fib (g/L)	3.82 ± 1.78	4.22 ± 1.46	0.347
NO (μmol/L)	**36.91 ± 12.59**	**30.40 ± 12.12**	**0.036**

Abbreviations DVT, deep venous thrombosis; BMI, body mass index; TKA, total knee arthroplasty; VTE: venous thromboembolism; CRP, C-reactive protein; PT, prothrombin time; Fib, fibrinogen; NO, nitric oxide. $P<0.05$ means significant difference

Logistic regression analysis of the risk factors of DVT after TKA

The assessment of the risk factors of DVT were analyzed by univariate and multivariate Logistic regression. As shown in Table 2, univariate logistic regression showed that age, BMI, bilateral TKA, family history of DVT, duration of operation, CRP, NO and miR-199b-5p were risk factors for DVT after TKA ($P<0.05$). Besides, after adjusting for confounders, multivariate logistic regression proved that increased age, family history of DVT, decreased NO and increased miR-199b-5p were the independent risk factors for DVT ($P<0.05$).

Correlation between miR-199b-5p and NO

The correlation between miR-199b-5p after TKA and preoperative NO levels was evaluated using the Pearson correlation coefficient method. As shown in Fig. 2, the level of NO in patients showed a significant negative correlation with the level of miR-199b-5p ($P<0.001$).

ROC analysis

The diagnostic value of miR-199b-5p, NO, and the combination of miR-199b-5p and NO for DVT was determined using ROC curve. The ROC curve for miR-199b-5p showed an area under the curve (AUC) value of 0.766, with a sensitivity of 75.0%, and a specificity was 71.3% (see Fig. 3A). For NO, the ROC curve showed an AUC of 0.730, and the sensitivity and specificity were 62.4% and 65.1%, respectively (Fig. 3B). Additionally, the ROC curve for the combination of miR-199b-5p and NO had an AUC value of 0.847, with a sensitivity of 85.0% and a specificity of 76.2% (Fig. 3C). These results indicate that preoperative NO levels and the postoperative miR-199b-5p levels have high diagnostic value in distinguishing between DVT and non-DVT patients, with their combination providing the highest diagnostic value in patients with DVT.

Bioinformatics analysis of miR-199b-5p

TargetScan predicted 634 target genes, ENCORI predicted 1582 target genes, EVmiRNA predicted 811 target genes, miRPathDB predicted 7551 target genes, and miRDB predicted 556 target genes. The Venn diagram was constructed using the target genes predicted by these five databases, and the results were shown in Fig. 4A. At least 85 target genes of miR-199b-5p were predicted jointly by the five databases (shown in Table 3). GO analysis of the 85 predicted target genes showed that these target genes were mainly enriched in cytoplasmic ribonucleoprotein particles. They are involved in various biological processes such as embryonic development, adipocyte differentiation, transcription regulation. Additionally, they have molecular functions such as DNA binding, transcription factor binding, nuclear receptor activity,

Table 2 Logistic regression analysis of clinical indicators related to DVT after TKA

Factors	Univariate		Multivariate	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Sex (male/female)	1.530 (0.577–4.060)	0.393	/	/
Age (years)	3.447 (1.165–10.201)	0.025	4.476 (1.136–17.633)	0.032
BMI (kg/m^2)	2.904 (1.033–8.164)	0.043	3.156 (0.798–12.486)	0.101
Smoking history (n, %)	0.677 (0.256–1.787)	0.431		
Drinking history (n, %)	1.040 (0.344–3.143)	0.945	/	/
Bilateral TKA (n, %)	2.829 (1.057–7.567)	0.038	2.662 (0.734–9.368)	0.138
Diabetes (n, %)	2.457 (0.816–7.440)	0.110	/	/
Hypertension (n, %)	1.012 (0.305–3.362)	0.984	/	/
Family history of VTE (n, %)	12.375 (2.092–73.209)	0.006	16.854 (1.681-168.963)	0.016
Disease duration (years)	2.790 (0.993–7.841)	0.052	3.016 (0.816–11.150)	0.098
Operation duration (min)	3.447 (1.165–10.201)	0.025	2.028(0.532–7.728)	0.301
Intraoperative bleeding loss (mL)	2.051 (0.756–5.565)	0.159	/	/
CRP (mg/L)	3.886 (1.312–11.511)	0.014	3.673 (1.002–13.459)	0.050
PT (s)	2.134 (0.786–5.792)	0.137	/	/
Fib (g/L)	1.061 (0.407–2.770)	0.903	/	/
NO (μmol/L)	0.290 (0.098–0.859)	0.028	0.227 (0.057–0.895)	0.034
miR-199b-5p	3.868 (1.371–10.919)	0.011	5.054 (1.339–19.078)	0.019

Abbreviations DVT: deep vein thrombosis; TKA: total knee arthroplasty; OR: odds ratio; BMI: body mass index; CRP: C-reactive protein; PT: prothrombin time; Fib: fibrinogen; NO: nitric oxide. $P<0.05$ indicates a significant difference

and corticosteroid receptor binding (Fig. 4B-D). KEGG pathway enrichment analysis indicated that miR-199b-5p target genes were primarily enriched in TGF-β, MAPK, TNF, estrogen, and Ras signaling pathways (Fig. 4E). The PPI network was constructed with 102 target genes, revealing that the hub protein coding genes most interacting with other proteins were SOX9, HIF1A, COL2A1, JAG1, SNAI1, SERPINH1 and ZEB1 (Fig. 4F).

Discussion

In this study, it was observed that the expression of miR-199b-5p in the serum of patients after TKA was significantly higher than before TKA. The incidence of DVT after TKA was 16.53%. Multi-factor analysis showed that increased age, family history of DVT, decreased NO, and increased miR-199b-5p were risk factors for postoperative DVT. Additionally, the study found that the level of NO before TKA was negatively correlated with the expression of miR-199b-5p after TKA. Furthermore, it evaluated the predictive value of miR-199b-5p and NO for DVT and found that their combination of miR-199b-5p and NO had the highest diagnostic accuracy for DVT.

For a long time, the academic community has generally believed that age is one of the risk factors for DVT after orthopedic lower extremity surgery. In this study, multivariate logistic regression showed that increasing age is an independent risk factor for DVT. China's thrombosis prevention guidelines pointed out that the older the patient, the greater the risk of DVT. Lee et al. found that the incidence of lower extremity DVT increased by about 5 times in people aged 50–59 years compared to those younger than 49 years [19]. Aging may increase the risk of DVT due to decreased elasticity of blood vessel walls, venous valve atrophy, and increased blood viscosity [20]. Additionally, the increase of oxygen free radicals and reactive oxygen species further damages the blood vessel intima, activates the coagulation system, and further stimulates the occurrence of thrombosis [21]. Therefore, with increasing age, the risk of DVT after TKA rises, necessitating stronger DVT prevention measures for the elderly. In this study, it was observed that the proportion of patients with a family history of DVT was significantly higher in the DVT group than in the non-DVT group. Some studies have shown that a family history of VTE is also a risk factor for DVT [22]. Vascular endothelium is a highly differentiated squamous cell type that can change vascular permeability, mediate vascular inflammation, and inhibit platelet aggregation and adhesion through endocrine and autocrine pathways [23]. Vascular endothelial injury is a major cause of thrombosis, so the detection of vascular endothelial injury markers has important clinical significance. Currently, the commonly used clinical markers include ET-1, vWF, PECAM-1, and

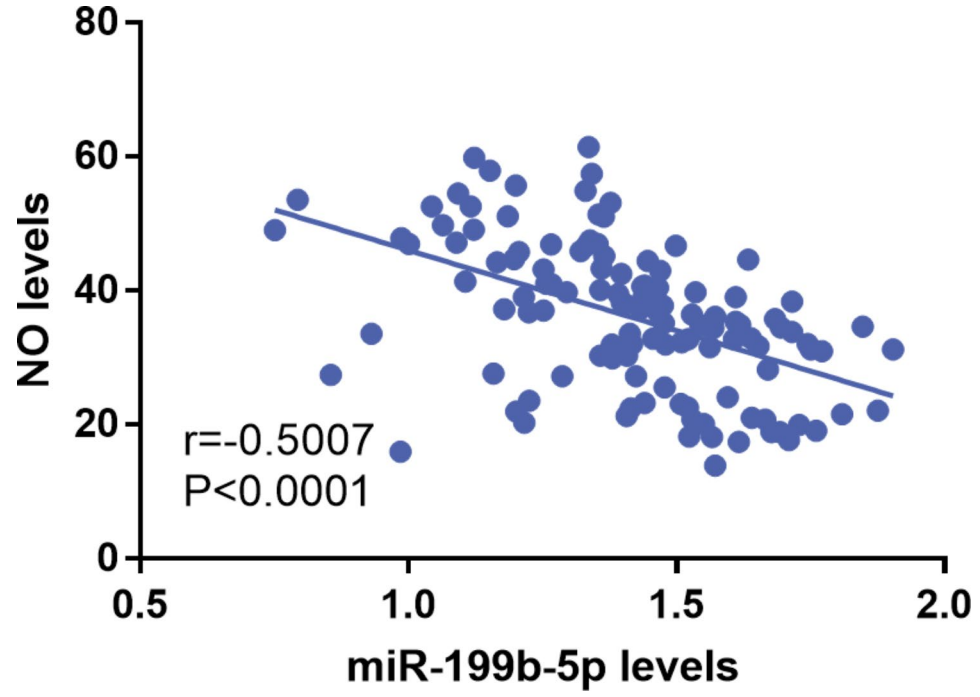

Fig. 2 Pearson correlation coefficient analysis showed that NO level was negatively correlated with the expression level of miR-199b-5p (r = -0.5007, $P < 0.0001$)

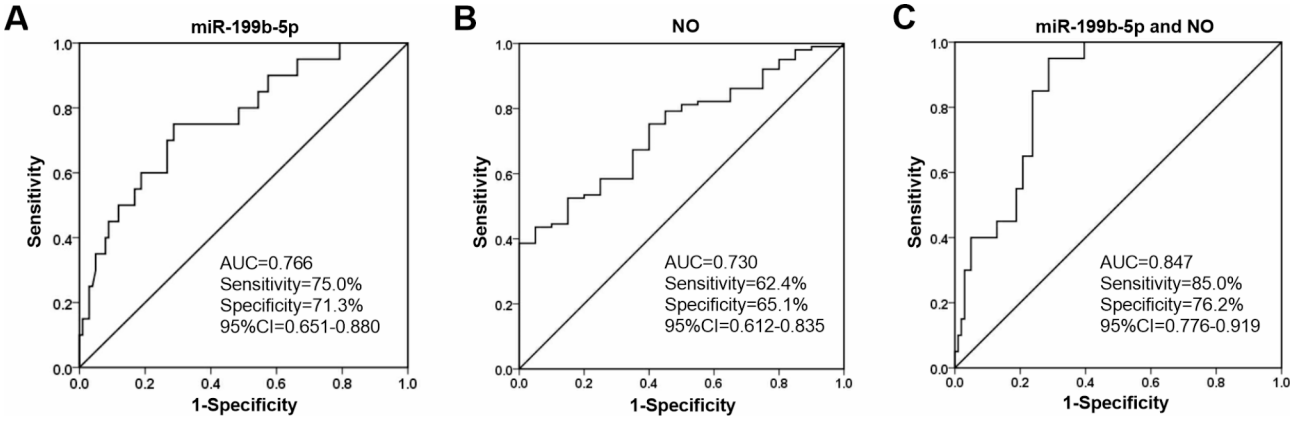

Fig. 3 ROC curves analysis. (**A**) ROC curve of miR-199b-5p. (**B**) ROC curve of NO. (**C**) ROC curve of miR-199b-5p combined with NO

NO [24, 25]. This study focused on detecting blood levels of NO. NO is a small molecule vasoactive substance and an important endogenous vasodilator. When endothelial function is impaired, the production and secretion of NO decrease [26]. The results showed that the concentration of NO in patients with DVT was significantly lower than that in the non-DVT group, indicating that the endothelial dysfunction in patients with DVT was more severe than that in the non-DVT group.

The dysregulation of miR-199b-5p was initially found to be significant in the development of human cancers, such as acute myeloid leukemia and hepatocellular carcinoma [27, 28]. A recent study showed that miR-199b-5p was elevated in the serum of patients with osteoarthritis [17]. In this study, after comparing the levels of miR-199b-5p before and after TKA in patients, it was observed that the expression of miR-199b-5p at 24 h after TKA was significantly higher than before TKA. This indicates that miR-199b-5p may be potentially related to the formation of DVT after TKA. Subsequently, the miR-199b-5p index was chosen to predict DVT at 24 h postoperatively. At the same time, preoperative NO index was selected to predict DVT. The results revealed that both indexes were accurate in diagnosing DVT. It was noting that the ROC curve of miR-199b-5p combined with NO had the highest diagnostic value for DVT.

Considering the differential expression of miR-199b-5p in the DVT and non-DVT groups, we explored

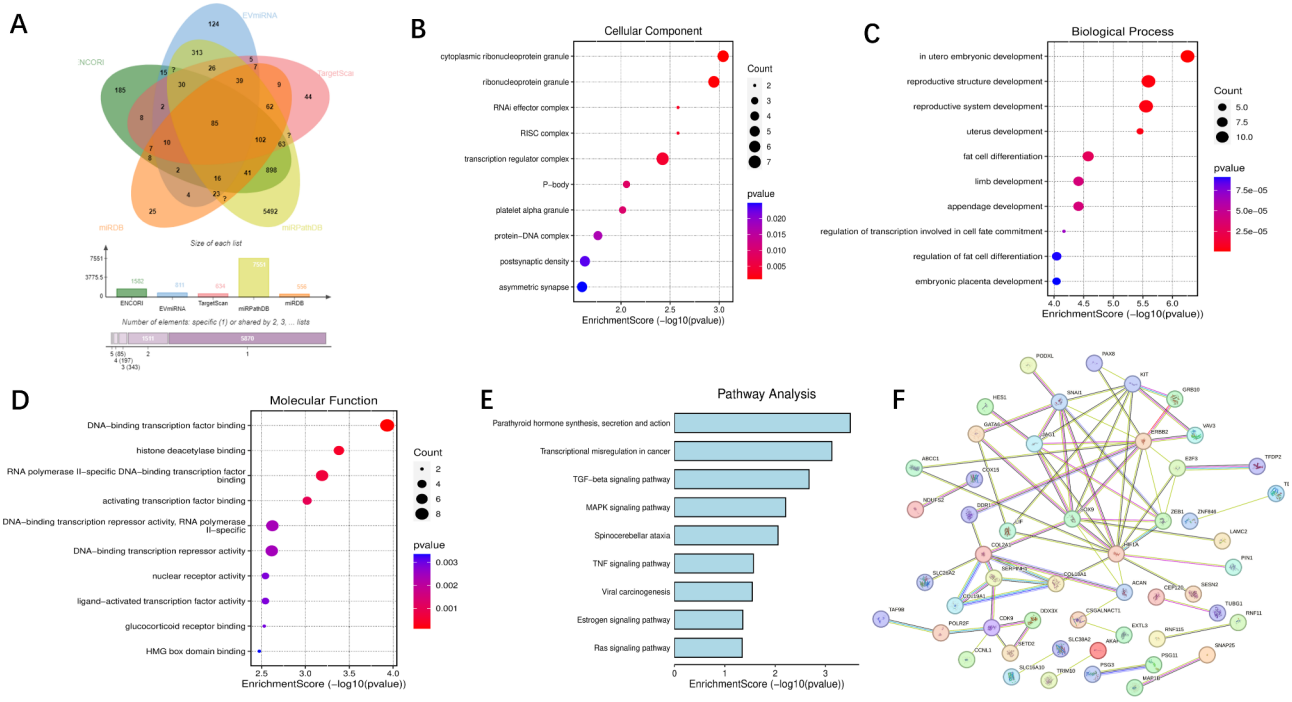

Fig. 4 Bioinformatics analysis of miR-199b-5p. (**A**) Venn diagram of five database-predicted downstream target genes of miR-199b-5p. GO analysis of (**B**) cellular component, (**C**) biological process and (**D**) molecular function. (**E**) KEGG pathway enrichment analysis. (**F**) Protein-protein interaction (PPI) analysis. The miR-199b-5p target regulatory interaction network

Table 3 The names of 85 downstream target genes of miR-199b-5p at the intersection of the Venn diagram

ATP13A2	SIRT1	GIT1	MAP4K3	MYH9	CLCN3	PPP1R9A
ECE1	SHOC2	MINK1	PREPL	CELSR1	MIER3	PDE7A
WDTC1	FZD4	NLK	MCFD2	GSK3B	PDE4D	RUNX1T1
AGO1	CAPRIN1	ITGA3	ALS2	PLXND1	MGAT4B	SNTB1
RNF11	SORL1	AKAP1	USP37	KPNA4	OSTM1	SLC25A37
POGK	ACVR1B	CLTC	NPAS2	ACVR2B	CEP85L	SULF1
LAMC1	SP1	ZNF516	RANBP2	ARIH2	GCNT2	FZD6
TGFB2	RASSF3	ZCCHC2	GPD2	ATXN7	E2F3	SLC24A2
ZBTB18	MAB21L1	SLC35E1	STK4	KIT	SOX4	HSPA5
ITGA8	HIF1A	PNPLA6	DYRK1A	TET2	CDCA7L	RLIM
ANK3	ZBTB42	BCAM	MICAL3	KIAA1109	PODXL	TAF9B
CACNB2	FAM222B	ZNF544	MN1	NAA15	AUTS2	TSPAN6
DDX3X						

the molecular functions of the target genes regulated by miR-199b-5p and the possible pathways involved. We predicted a total of 85 downstream target genes, mainly involved in biological processes such as cell differentiation, transcriptional regulation, and embryonic development. These genes were enriched in multiple signaling pathways, including MAPK, TNF, and Ras signaling pathways. We identified the top 5 hub proteins that interact most with other proteins in the PPI network: SOX9, HIF1A, COL2A1, JAG1, and SNAI1. According to the literature search information, it was found that the expression of these hub proteins was significantly correlated with the occurrence of thrombus. For example, the expression of JAG1 is decreased in the blood of patients with lower limb DVT and is related to the course of DVT [29]. The mechanism of miR-199b-5p's involvement in thrombus regulation remains to be further explored.

This study also has some limitations. Considering that this study is a single-center study with limited samples, the incidence of DVT in the lower limbs after TKA may be unstable, which requires further multi-center and large sample prospective studies to verify the stability of the results of this study. In addition, at present, this study only shows that the increase of miR-199b-5p after TKA is potentially associated with the occurrence of DVT, but in fact, the role and mechanism of miR-199b-5p in DVT

remain unclear. Therefore, in future studies, we will try to verify the mechanism of miR-199b-5p in DVT through in vivo and in vitro experiments. In short, cultured human umbilical vein endothelial cells in vitro were used to investigate the changes of endothelial injury related indexes, inflammation indexes and coagulation indexes. The rat model of DVT was constructed, and miRNA simulators or inhibitors were injected into the tail vein to observe the thrombosis formation and index changes.

In summary, this study found that the expression of miR-199b-5p increased after TKA compared to before TKA. Multivariate regression analysis showed that age, family history of DVT, decrease of NO and increase of mIR-199b-5p were risk factors for DVT after TKA. miR-199b-5p combined with NO has good diagnostic value for the occurrence of DVT after TKA.

Acknowledgements
Not applicable.

Author contributions
Conceptualization, S.Z., W.M., Y.L., L.L. and S.L.; Data curation, Y.L. and L.L.; Formal analysis, Y.L.; Funding acquisition, L.L.; Investigation, S.Z., W.M. and Y.L.; Methodology, Y.L. and L.L.; Project administration, Y.L.; Resources, Y.L.; Software, Y.L.; Supervision, L.L. and S.L.; Validation, Y.L.; Visualization, L.L. and S.L.; Roles/Writing - original draft, Y.L.; Writing - review & editing, S.Z., W.M., L.L. and S.L.

Funding
No funding was received to assist with the preparation of this work.

Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Affiliated Hospital of Panzhihua University before the study began. The participants' right to be informed about the study was ensured and agreed to participate in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 21 June 2024 / Accepted: 16 August 2024
Published online: 24 August 2024

References

1. Gargano G, Oliva F, Oliviero A, Maffulli N. Small interfering RNAs in the management of human rheumatoid arthritis. Br Med Bull. 2022;142(1):34–43.
2. Gargano G, Asparago G, Spiezia F, Oliva F, Maffulli N. Small interfering RNAs in the management of human osteoporosis. Br Med Bull. 2023;148(1):58–69.
3. Zhao Z, Wang C, Xu Y, Wang X, Jia B, Yu T et al. Effects of the local bone renin-angiotensin system on Titanium-Particle-Induced Periprosthetic Osteolysis. Front Pharmacol. 2021; 12684375.
4. Yan J, Zhou J, Zhen J, Li L, Care Clinical Research Group ZPC. Knowledge and awareness of venous thromboembolism in Intensive Care Units in Zhejiang Province, China: a cross-sectional survey. Med Sci Monit. 2020; 26e923378.
5. Xiong X, Xu S, Li T, Cheng B. Correlation of the severity of anemia in patients undergoing total joint arthroplasty with preoperative deep vein thrombosis: a retrospective cohort study. J Orthop Surg Res. 2022;17(1):554.
6. Li Q, Dai B, Yao Y, Song K, Chen D, Jiang Q. Chronic kidney dysfunction can increase the risk of deep vein thrombosis after total hip and knee arthroplasty. Biomed Res Int. 2017; 20178260487.
7. Hamad S, Derichsweiler D, Gaspar JA, Brockmeier K, Hescheler J, Sachinidis A, et al. High-efficient serum-free differentiation of endothelial cells from human iPS cells. Stem Cell Res Ther. 2022;13(1):251.
8. El Hasbani G, Taher AT, Jawad A, Uthman I. COVID-19, Antiphospholipid Antibodies, and Catastrophic Antiphospholipid Syndrome: a possible association? Clin Med Insights Arthritis Musculoskelet Disord. 2020; 131179544120978667.
9. Myatt L, Review. Reactive oxygen and nitrogen species and functional adaptation of the placenta. Placenta. 2010;31(SupplSuppl):S66–9.
10. Meng P, Yang R, Jiang F, Guo J, Lu X, Yang T et al. Molecular mechanism of Astragaloside IV in improving endothelial dysfunction of Cardiovascular diseases mediated by oxidative stress. Oxid Med Cell Longev. 2021; 20211481236.
11. Oliviero A, Della Porta G, Peretti GM, Maffulli N. MicroRNA in osteoarthritis: physiopathology, diagnosis and therapeutic challenge. Br Med Bull. 2019;130(1):137–47.
12. Giordano L, Porta GD, Peretti GM, Maffulli N. Therapeutic potential of microRNA in tendon injuries. Br Med Bull. 2020;133(1):79–94.
13. Gargano G, Oliviero A, Oliva F, Maffulli N. Small interfering RNAs in tendon homeostasis. Br Med Bull. 2021;138(1):58–67.
14. Xie X, Liu C, Lin W, Zhan B, Dong C, Song Z, et al. Deep vein thrombosis is accurately predicted by comprehensive analysis of the levels of microRNA-96 and plasma D-dimer. Exp Ther Med. 2016;12(3):1896–900.
15. Lin X, Qiu W, Xiao Y, Ma J, Xu F, Zhang K et al. MiR-199b-5p suppresses Tumor Angiogenesis mediated by vascular endothelial cells in breast Cancer by targeting ALK1. Front Genet. 2019; 101397.
16. Chen S, Wu H, Zhu L, Jiang M, Wei S, Luo J et al. MiR-199b-5p promotes gastric Cancer Progression by regulating HHIP expression. Front Oncol. 2021; 11728393.
17. Feng T, Zhang Q, Li SH, Ping YL, Tian MQ, Zhou SH et al. Inhibition of miR-199b-5p reduces pathological alterations in osteoarthritis by potentially targeting Fzd6 and Gcnt2. Elife. 2024; 12.
18. Wang Q, Chang Y, Yang X, Han Z. Deep sequencing of circulating miRNAs and target mRNAs level in deep venous thrombosis patients. IET Syst Biol. 2023;17(4):212–27.
19. Lee KA, Ramaswamy RS. Interventional approaches to deep venous thrombosis. Curr Opin Cardiol. 2017;32(6):679–86.
20. Ageno W, Beyer-Westendorf J, Garcia DA, Lazo-Langner A, McBane RD, Paciaroni M. Guidance for the management of venous thrombosis in unusual sites. J Thromb Thrombolysis. 2016;41(1):129–43.
21. Moed BR, Miller JR, Tabaie SA. Sequential duplex ultrasound screening for proximal deep venous thrombosis in asymptomatic patients with acetabular and pelvic fractures treated operatively. J Trauma Acute Care Surg. 2012;72(2):443–7.
22. Noboa S, Le Gal G, Lacut K, Mercier B, Leroyer C, Nowak E, et al. Family history as a risk factor for venous thromboembolism. Thromb Res. 2008;122(5):624–9.
23. Kiang JG, Olabisi AO. Radiation: a poly-traumatic hit leading to multi-organ injury. Cell Biosci. 2019; 925.
24. Wang J, Niu N, Xu S, Jin ZG. A simple protocol for isolating mouse lung endothelial cells. Sci Rep. 2019;9(1):1458.
25. Yoshimatsu Y, Watabe T. Emerging roles of inflammation-mediated endothelial-mesenchymal transition in health and disease. Inflamm Regen. 2022;42(1):9.
26. Yang X, Xiang Y, Wang F, Cai G, Li Y, Zhong L, et al. Expressions and relationship of Kruppel-like factor 15 and endothelial nitric oxide synthase in experimental deep venous thrombosis. Ann Transl Med. 2020;8(17):1090.
27. Favreau AJ, McGlauflin RE, Duarte CW, Sathyanarayana P. miR-199b, a novel tumor suppressor miRNA in acute myeloid leukemia with prognostic implications. Exp Hematol Oncol. 2015; 54.

28. Zhou SJ, Liu FY, Zhang AH, Liang HF, Wang Y, Ma R, et al. MicroRNA-199b-5p attenuates TGF-beta1-induced epithelial-mesenchymal transition in hepatocellular carcinoma. Br J Cancer. 2017;117(2):233–44.
29. Lu J, Fang Q, Ge X. Role and mechanism of mir-5189-3p in deep vein thrombosis of Lower extremities. Ann Vasc Surg. 2021; 77288–95.

Publisher's note