Abstract. Krüppel-like family (KLF) members are important regulators of proinflammatory activation in the vasculature. A transcriptome study involving RNA sequencing (RNA‑seq) and quantitative PCR (qPCR) was performed to investigate Klf15 and Klf15‑regulated gene levels in C57BL/6 mice with inferior vena cava thrombi and in control (Blank) mice. A total of 2,206 differentially expressed genes (DEGs), including 1,330 upregulated and 876 downregulated genes, were identified between the deep venous thrombosis (DVT) group and the Blank group. Additionally, 1,041 DEGs (235 upregulated and 806 downregulated) were identified between the Klf15‑small interfering RNA (siRNA) and Klf15‑negative control (NC) groups. The DEGs were subjected to Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analyses, and qPCR was conducted to validate the results. A total of seven significant DEGs were selected from the RNA‑seq results. Matrix metalloproteinases (Mmp)12, Mmp13, Mmp19, Arg1, Ccl2, heme oxygenase‑1 and Fmo3 levels were significantly higher, while Klf15 levels were lower, in the DVT group than in the Blank group. Fmo3 and Mmp19 have not been previously identified as DVT‑associated DEGs. Klf15, Mmp12 and Mmp13 levels were compared between the Klf15‑siRNA and Klf15‑NC groups. Mmp12 and Mmp13 expression was significantly higher, while that of Klf15 was lower, in the Klf15‑siRNA group than in the Klf15‑NC group. Critical roles of Klf15, Mmp12 and Mmp13 have not been previously been shown to help regulate DVT initiation and progression. Moreover, Klf15‑mediated regulation of DVT may be modulated by downregulation of various genes, such as Mmp12 and Mmp13, potentially providing a theoretical foundation and diagnostic criteria for DVT treatment.

Introduction
Deep venous thrombosis (DVT) is one of the most common vascular diseases and is associated with high mortality and complex therapeutic processes (1). Thrombolytics and interventional therapies are still the mainstream treatments for DVT, but they are limited by a low cure rate and a high postoperative recurrence rate (2). The current therapeutic methods are restricted, especially regarding the prolonged time for diagnosis and treatment (3). Considering the complex mechanisms and various regulatory factors of DVT, studies on DVT have focused on the underlying regulatory genes, providing a valuable foundation for the diagnosis and treatment of DVT (4‑6).

Krüppel‑like factor 15 (Klf15), a transcriptional regulatory factor, is involved in various pathophysiological processes, such as cell differentiation, apoptosis and tumor formation, which are closely related to cardiovascular diseases such as hypertension, atherosclerosis and coronary heart disease (4). Klf15 is widely expressed in tissues and organs, especially in the heart, liver, kidneys and skeletal muscles (7). Lu et al (8) reported that similarly to other members of the KLF family, Klf15 inhibits NF‑κB activation in vascular smooth muscle by interacting with p300 (Klf15‑p300), thereby inhibiting downstream target genes and inflammatory responses. Moreover, the expression level of Klf15 significantly decreased in mouse aortic smooth muscle cells treated with the oxidized component POVPC and human atherosclerotic tissues, which revealed that Klf15 plays a key role in the formation of atherosclerosis (7,8). Studies revealing the relationship and the genetic interaction between DVT and Klf15 are urgently needed. Therefore, transcriptome analysis of Klf15 in a mouse inferior vena cava (IVC) thrombosis model was performed to identify the functions of Klf15 and its relationship with the regulatory process and formation of DVT.

High‑throughput sequencing, or next‑generation sequencing, is a novel genomic research technique characterized by high data output and involves RNA sequencing (RNA‑seq); high‑throughput sequencing can be utilized in the analysis of various transcriptional and functional regions (9). Strikingly, extensive data resources can be provided via...
high-throughput sequencing to enable the identification and screening of target genes or differentially expressed genes (DEGs) in the whole genome, which is important for analyzing the regulatory relationships between genes and disease pathogenesis (10).

Previous studies have investigated the role of Klf15 in atherosclerosis (8) and vascular smooth muscle cells (VSMCs) (11). Klf15 is a regulator of VSMC proinflammatory activation and overexpression of Klf15 can protect vascular endothelial cells against dysfunction (12). Although the pathogeneses of atherosclerosis and DVT are different, endothelial cells are important for both atherosclerosis and DVT. Disruption of the endothelium and the release of plaque constituents into the lumen of the blood vessel can trigger arterial thrombosis (13,14). Abnormal blood flow, altered properties of the blood itself and changes in the endothelium can trigger venous thrombosis. In contrast to what is observed in atherosclerosis, venous endothelial cells remain intact, but their dysfunction can trigger DVT (15). According to our knowledge, no reports have studied Klf15 in DVT. The research on Klf15 in atherosclerosis prompted the present study to hypothesize that Klf15 can protect against DVT by affecting venous endothelial cells. Preliminary experiments were performed in C57/BL/6 mice and the results showed that inhibition of Klf15 induced DVT. In this study, the regulatory relationship and genetic interactions between DVT and Klf15 were investigated, revealing a new regulatory mechanism in a mouse model that could contribute to the diagnosis and treatment of DVT.

Materials and methods

Mouse and animal studies. The current study was performed with 40 C57BL/6 female mice (age, 8-10 weeks; weight, 20±3 g) that were purchased from the SPF animal laboratory of Kunming Medical University (Kunming, China). The mice were divided into four groups (n=10), according to a random grouping design. Then, the mice were fed at the experimental center of the SPF animal laboratory at Kunming Medical University with free access to food and water, a constant temperature of 21-25°C, a humidity level at 50-65%, under a 12-h light/dark cycle with proper ventilation. Next, a 2-3 week feeding period was conducted until the mice reached ≥25 g per mouse. The mice were observed twice daily to monitor their health and behavior. All animal experiments were performed following approval from the Animal Experiment and Ethics Committee of Kunming Medical University.

Generation of IVC thrombus in C57BL/6 mice. Once the weight of the mice exceeded 25 g, modeling of IVC thrombi in C57BL/6 mice was performed in each mouse except the Blank group. Mice were separated into four groups: The Blank group, the DVT group, the Klf15-NC group and the Klf15-small interfering (si)RNA group. The Blank and DVT groups were first generated, and mice in the DVT group underwent an operation to generate an IVC thrombus by utilizing a string to induce artificial stenosis of the IVC for thrombus formation (16). IVC thrombosis in mice was first modeled. After 24 h, the thrombi were acquired. During the perioperative period, the mice were monitored twice daily and they did not appear to be in distress or to exhibit obvious behavioral abnormalities. After the IVC thrombi were collected for further investigation, no other procedures were performed on the mice. Isoflurane was used as the inhaled agent to produce general anesthesia in mice. During the perioperative period of the experiment, the inhalant anesthetic isoflurane was utilized to induce and maintain general anesthesia to minimize animal pain and suffering and limit the discomfort that can accompany scientific research. Isoflurane was first used at 2% for induction and then at 1-1.5% for maintenance. Mice that have undergone IVC removal are likely to experience great pain and distress; thus, euthanasia was considered as the humane option. Euthanasia was conducted 24 h after the IVC thrombus operation. The mice were first anesthetized with 5% isoflurane until they stopped moving or appeared to be unconscious. Next, cervical dislocation was conducted, separating the cervical vertebrae from the skulls of the mice. An array of criteria was used to confirm the success of euthanasia, including arrest of pulse and breathing, lack of corneal reflex and inaudibility of respiratory sounds and heartbeat sounds upon examination with a stethoscope. The same process was performed for the IVC of the Klf15-NC and Klf15-siRNA groups, and there was an additional caudal vein injection with 0.9% normal saline (NS) in the Klf15-NC group and with Klf15 siRNA: 5'-CCT GTG AAG GAGGAACATT-3' (Guangzhou RiboBio Co., Ltd.; 10 nmol per mouse) in the Klf15-siRNA group, which was performed 24 h before the operation. A total of 40 C57BL/6 mice were used in the present experiment, 36 of which were euthanized by cervical dislocation under anesthesia; four died due to hemorrhagic shock. The success of DVT modeling was judged by direct observations of the weights of the thrombi and vessels collected from the mice. In the present experiments, when 7-8 mm thrombi or vessels from mice were examined, most of the thrombi weighed >10 mg and most of the vessels weighed <10 mg.

RNA isolation and RNA-seq. On the basis of morphological experiments, thrombi in the IVC of mice and the vessels themselves were collected for examination. According to the manufacturer's protocol, RNA was extracted with TRIzol Reagent at 4°C. RNA purity was determined using a NanoPhotometer spectrophotometer (IMPLEN) and the concentration was measured using a Qubit® 2.0 Fluorometer (Thermo Fisher Scientific, Inc.). RNA integrity was assessed using the RNA Nano 6000 Assay kit of the Bioanalyzer 2100 system (Agilent Technologies, Inc.). Then, RNA degradation and contamination were monitored on 1% agarose gels. Furthermore, RNA purity was assessed using the RNA Nano 6000 Assay kit of the Bioanalyzer 2100 system (Agilent Technologies, Inc.). Thus, qualified RNA was used as a material for later analyses and provided samples for RNA-seq.

RNA-seq data and bioinformatics analysis. High-throughput sequencing was used to obtain and identify raw reads in samples. Further evaluation of the quality of the clean reads was performed to discard low-quality reads, which had either >50% of bases with a Q value ≤20 or >5% unrecognized sequences (‘N’). After obtaining the high-quality clean reads, the reads were mapped to the human reference genome to enable downstream gene analysis.
For analysis of the expression levels of transcripts and the correlation of replicates, the fragments per kilobases per million mapped reads (FPKM) method was utilized in Pearson's correlation analysis to identify DEGs among each group of transcript sequences, which was determined by genetic length and the reads mapped to the human reference genome.

To detect the DEGs among the groups, DESeq2 was used, which provides statistical routines for determining differential expression in digital gene expression data using a model based on a negative binomial distribution. The resulting P-values were adjusted using Benjamini and Hochberg's approach for controlling the false discovery rate. Genes with an adjusted P<0.05 according to DESeq2 were considered differentially expressed.

GO enrichment analysis of DEGs was implemented by the clusterProfiler R package 3.14.3 (17), in which the gene length bias was corrected. GO terms with corrected P<0.05 were considered differentially enriched with DEGs. KEGG is a database resource used to elucidate the high-level functions and utilities of biological systems, such as the cell, organism and ecosystem, from molecular-level information, especially large-scale molecular datasets generated by genome sequencing and other high-throughput experimental technologies (http://www.genome.jp/kegg/). The clusterProfiler R package was used to test the statistical enrichment of DEGs in KEGG pathways.

qPCR analysis of DEGs. For validation of the expression of genes and the consistency of these two comparisons, qPCR analysis of DEGs was conducted according to the manufacturer's protocol for Maxima® SYBR-Green/ROX qPCR Master Mix (2X) (MBI Fermentas; Thermo Fisher Scientific, Inc.) on an ABI PRISM® 7300HT system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The thermocycling conditions were as follows: Initial denaturation at 95°C for 10 min (1 cycle), followed by denaturation at 95°C for 15 sec and annealing and extension at 60°C for 60 sec (40 cycles). The primer sequences were obtained using the 2 ‑∆∆Cq method (8)

\[\text{ABI DataAssist}^{TM} v3.0\ software (Thermo Fisher Scientific, Inc.)\] and were as follows: KLF15 (77 bp) forward: 5'‑CTT CCC TGA ATT TCT GTC‑3' and reverse: 5'‑ATT CTT CAA TCT CCT CCA‑3'; Mmp12 (88 bp) forward: 5'‑CAG CAT TCC A A T A A T C C A A ‑ 3 '  a n d  r e v e r s e :  5 ' ‑ G T A T G T C A T C A G C A G AGA‑3'; Mmp13 (79 bp) forward: 5'‑GTG ATG ATG ATG ATG ATG ATG A C ‑ 3 '  a n d  r e v e r s e :  5 ' ‑ G C A G G A T G G T A G T A T G A T G T A G ATGAC‑3' and reverse: 5'-GAGAATGGTGGTATGTAGTATT‑3'; Arg1 (71 bp) forward: 5'-AACACGCCAGTGGCTTTA‑3' and reverse: 5'-TCAGTCTTGGGATGCTC‑3'; Ccl2 (120 bp) forward: 5'-TGGGTCCAGACATACT‑3' and reverse: 5'-ACGGGTCAACTTACATC‑3'; Mmp12 (88 bp) forward: 5'-GAGTCTGGGACAGTGCTC‑3' and reverse: 5'-GAG ATGGCCTGGTGTAAG‑3'; heme oxygenase-1 (Hmox1) (88 bp) forward: 5'-TCACAGATGGCCGCAGTT‑3', and reverse: 5'-AGCAGGTGCTTGGGATG‑3'; Mmp19 (113 bp) forward: 5'-GATGCTGCGGTGGTG‑3' and reverse: 5'-GATGGCTGCGGTGGTGTAAG‑3'; β-actin (87 bp) forward: 5'-TGCAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA
thrombi (Figs. 1 and 6) are presented as the mean ± SEM. The experiments were repeated three times. An unpaired two-tailed Student's t-test between two groups was used for statistical significance of differences analyzed. P<0.05 was considered to indicate a statistically significant difference.

Results

Effect of Klf15 on thrombosis formation and the wet weight of the mouse thrombus. Klf15 has been shown to be critical for the initiation and progression of vascular inflammation (6). In this study, to identify the effects of Klf15 on DVT, morphological experiments were conducted on mice with IVC thrombi, which were divided into four groups: The Blank group, the DVT group, the Klf15-NC group with 0.9% NS caudal vein injection and the Klf15-siRNA group with Klf15-siRNA caudal vein injection (Table I). The vessels were removed from the mice of Blank group. If there was a blood clot blocking the vein based on direct observations (Fig. 1A), the clot was removed from the mouse for further examination (Fig. 1B). The results revealed that the weight of the thrombus in the Klf15-siRNA group was increased compared with that in the Klf15-NC group. As shown in Fig. 1C, compared with the Klf15-NC group, the Klf15-siRNA group with Klf15-siRNA injection (and thus with significantly reduced Klf15 expression) exhibited a significantly increased thrombus wet weight (Fig. 1D). Thus, Klf15 is significantly associated with thrombus formation and weight.

RNA-seq results, data quality assessment and mapping. After performing the morphological experiments previously described, genetic analysis was conducted by RNA-seq, a method of high-throughput sequencing, to investigate transcriptional gene abundance and simultaneously study active regions of transcription.

Samples were divided into four groups: The Blank group, the DVT group, the Klf15-NC group (0.9% NS caudal vein injection) and the Klf15-siRNA group (Klf15-siRNA caudal vein injection). A total of ~25.8 and 28.1 million raw reads were collected from the DVT and Blank groups, respectively, while 29.6 and 30.3 million raw reads were obtained from the Klf15-NC and Klf15-siRNA groups, respectively. Then, further analysis was performed to obtain high-quality clean reads and the low-quality reads, which had either >50% of bases with a Q value ≤20 or >5% of unrecognized sequences (‘N’), were discarded. Consequently, ~24.8 and 26.8 million high-quality clean reads were obtained from the DVT and Blank groups, respectively, 29.1 and 29.6 million high-quality clean reads were obtained from the Klf15-NC and Klf15-siRNA groups, respectively. Then, the clean reads were mapped to the human reference genome for downstream gene analyses. As a result, the rates of mapping for the DVT group and the Blank group were 89.40 and 90.22%, respectively, while the rates of mapping for the Klf15-NC group and the Klf15-siRNA group were 89.26 and 90.12%, respectively, which demonstrated the quality of the gene mapping. The results of the RNA-seq reads are listed in Table II and the mapping results are listed in Table III. The high-quality reads for different groups were collected for further analyses.

Identification of DEGs. For analysis of gene expression and the DEGs, data processing was conducted with the essential conditions of an adjusted P<0.05 and log2-fold-change (FC) for the determination of gene regulation, which was log2FC>1 for upregulated genes and log2FC<-1 for downregulated genes. In total, 2,206 DEGs were identified from the comparison of the DVT group and Blank group, including 1,330 upregulated genes and 876 downregulated genes, which are represented as red points and green points in the volcano plot, respectively (Fig. 3A). Gene expression analysis also revealed 1,041 DEGs between the Klf15-siRNA group and the Klf15-NC group, with 235 upregulated genes and 806 downregulated genes (Fig. 3B). The number of DEGs in each comparison is listed in Table IV. As shown in Table V, the expression levels of these genes showed significant differences in the DVT group compared with in the Blank group and the genes mainly clustered into three gene families; there were five genes in the Mmp family, four genes in the IL family, and 13 genes in the chemokine family. The expression levels of Mmp3, Mmp8, Mmp9, Mmp13 and Mmp19 in the DVT group were significantly increased compared with those in the Blank group. The expression of Mmp12 was also increased in the DVT group when compared with the Blank group; however, this finding was not significant. Genes in the interleukin (IL) family, including Il-1r2, Il-1a, Il-1b and Il-6, were expressed more highly in the DVT group than in the Blank group. Moreover, the expression levels of genes in the Cc and Cx families of the DVT group were higher than those of the Blank group, except the expression level of Cc121a, which was lower in the DVT group than in the Blank group.
As shown in Table V, there were four DEGs (Selp, Plaur, Hmox1 and Argl) that were detected from the comparison between the DVT group and the Blank group, which correlated with the previously obtained results.

As shown in Table V, there were four DEGs (Selp, Plaur, Hmox1 and Argl) that were detected from the comparison between the DVT group and the Blank group, which correlated with the previously obtained results.
Notably, two DEGs were identified, Fmo3 and Mmp19, that had not been previously detected in DVT and are listed in Table V.

As shown in Table VI, three genes, Klf15, Mmp12 and Mmp13, showed higher expression in the Klf15-siRNA group than in the Klf15-NC group, except for the level of Klf15 itself due to the caudal vein injection of Klf15 siRNA in mice of the Klf15-siRNA group.

**GO analysis of DEGs.** Previously in this study, DEGs were identified among different groups. Thus, GO enrichment analysis was performed to discover the biological processes of these DEGs, which demonstrated significant functions of gene expression in different groups. Then, the GO terms were divided into three categories: Biological process (BP), cellular component (CC) and molecular function (MF).

As shown in Fig. 4A and B, the top 30 ranked GO terms of the comparison between the DVT group and the Blank group were selected for the bar graph and scatter plot. Consequently, ‘leukocyte migration’ was the most significantly enriched term. Then, ‘positive regulation of locomotion’, ‘positive regulation of cell motility’ and ‘positive regulation of cell migration’ accounted for the most enriched terms in the BP category. In addition, in Fig. 4C and D, the top 30 ranked GO terms from the comparison of the Klf15-siRNA group and the Klf15-NC group showed that ‘axon, postsynapse’ and ‘presynapse’ were the most highly enriched terms. At the same time, ‘the regulation of ion transmembrane transport’, ‘signal release’ and ‘modulation of synaptic transmission’ were abundant in both the CC category and the MF category.

The results of the GO analysis revealed the distribution of genes in different biological functions, from which information regarding DEGs that may be beneficial to further study could be obtained.

**KEGG pathway analysis of DEGs.** To characterize the coordinative relations between genes and the roles of genes in biological functions, DEGs were analyzed by KEGG enrichment analysis, in which biochemical metabolic and signal transduction pathways were detected from the included DEGs.

The results in Table VII revealed that 50 pathways with significant expression (P<0.01) were identified in the comparison between the DVT group and the Blank group. In Fig. 5A and B, the top 20 ranked pathways are listed in the bar graph and bubble diagram; numerous signal transduction pathways were notably enriched, including the ‘HIF-1 signaling pathway’, ‘Th17 cell differentiation’, ‘the intestinal immune network for IgA production’, ‘TNF signaling pathway’, ‘cell adhesion molecules (CAMs)’, ‘the PI3K-Akt signaling pathway’, ‘ECM-receptor interactions’, ‘the Jak-STAT signaling pathway’ and ‘the IL-17 signaling pathway’. Moreover, BP terms, including ‘the cytokine-cytokine receptor interaction’, ‘hematopoietic cell lineage’, ‘Th17 cell differentiation’, ‘CAM’, ‘ECM-receptor interaction’, ‘glycolysis/gluconeogenesis’, ‘osteoclast differentiation’, ‘Staphylococcus aureus infection’, ‘Th1 and Th2 cell differentiation’, and ‘complement and coagulation cascade terms’, were significantly enriched in the analysis.

In the comparison between the Klf15-siRNA group and the Klf15-NC group, 23 pathways with significant expression (P<0.01) were identified and are listed in Table VIII. The top 20 ranked pathways are shown in Fig. 5C and D. The results revealed that several signal transduction pathways...
Figure 4. Bubble diagram and bar diagram of the DEG GO terms. Bubble diagram of the top 20 ranked GO terms of the DEGs. In the bubble diagram, the vertical axis indicates GO terms and the horizontal axis represents the enrichment factor. The sizes of dots indicate the number of genes in the GO term. In the bar diagram, GO terms were divided into three categories: The red bar represents BP, the green bar represents CC and the blue bar indicates MF. (A) Bubble diagram of the top 30 ranked DEGs from the comparison between the Blank group and the DVT group. (B) Bar diagram of GO terms from the comparison between the Blank group and the DVT group.
Figure 4. Continued. Bubble diagram and bar diagram of the DEG GO terms. (A) Bubble diagram of the top 20 ranked GO terms of the DEGs. In the bubble diagram, the vertical axis indicates GO terms and the horizontal axis represents the enrichment factor. The sizes of dots indicate the number of genes in the GO term. In the bar diagram, GO terms were divided into three categories: The red bar represents BP, the green bar represents CC and the blue bar indicates MF. (C) Bubble diagram of the top 30 ranked DEGs from the comparison of the Klf15-NC group and the Klf15-siRNA group. (D) Bar diagram of GO terms from the comparison of the Klf15-NC group and the Klf15-siRNA group. si, small interfering; Klf15, Krüppel-like family 15; DVT, deep venous thrombosis; GO, gene ontology; BP, Biological process; MF, molecular function; CC, cellular component; DEGs, differentially expressed genes.
Figure 5. Bar diagram and bubble diagram of KEGG pathway enrichment analysis of DEGs. In the bar diagram, KEGG pathways are listed in the order of the enrichment ratio. A bubble diagram of the top 20 ranked KEGG pathways of DEGs. In the bubble diagram, the vertical axis indicates the KEGG pathways and the horizontal axis represents the enrichment ratio. The sizes of the dots indicate the number of genes in the Gene Ontology term. (A) Bar diagram of KEGG pathways from the comparison between the Blank group and the DVT group. (B) Bubble diagram of the top 20 ranked KEGG pathways from the comparison between the Blank group and the DVT group.
Figure 5. Continued. Bar diagram and bubble diagram of KEGG pathway enrichment analysis of DEGs. In the bar diagram, KEGG pathways are listed in the order of the enrichment ratio. A bubble diagram of the top 20 ranked KEGG pathways of DEGs. In the bubble diagram, the vertical axis indicates the KEGG pathways and the horizontal axis represents the enrichment ratio. The sizes of the dots indicate the number of genes in the Gene Ontology term. (C) Bar diagram of KEGG pathways from the comparison between the Klf15-NC group and the Klf15-siRNA group. (D) Bubble diagram of the top 20 ranked KEGG pathways from the comparison between the Klf15-NC group and the Klf15-siRNA group. si, small interfering; Klf15, Krüppel-like family 15; DVT, deep venous thrombosis; KEGG, Kyoto Encyclopedia of Genes and Genomes.
were significantly enriched, including ‘the PI3K-Akt signaling pathway’, ‘the Hippo signaling pathway’, ‘the cAMP signaling pathway’ and ‘the relaxin signaling pathway’. In addition, BP terms were identified; these terms included ‘the cholinergic synapse’, ‘neuroactive ligand-receptor interaction’, ‘ECM-receptor interaction’, ‘dopaminergic synapse’,
'nicotine addiction', 'rheumatoid arthritis', 'synaptic vesicle cycle', 'taste transduction', 'vascular smooth muscle contraction', 'hypertrophic cardiomyopathy (HCM)', 'serotonergic synapse', 'mucin type O-glycan biosynthesis', 'dilated cardiomyopathy (DCM)' and 'protein digestion and absorption' terms. Among these terms, the 'vascular smooth muscle contraction', 'HCM' and 'DCM' terms provide crucial information regarding the roles of Klf15 in the formation and pathophysiological processes of vascular disease, especially DVT.

Table I. Number of successful injection of mice and successful modeling of DVT mice.

| Group          | Blank | DVT  | Klf15-NC | Klf15-siRNA |
|----------------|-------|------|----------|-------------|
| Sample size    | 10    | 10   | 10       | 10          |
| Number of successful injection of mice | -     | -    | 9        | 8           |
| Number of successful modeling of DVT mice | -     | 7    | 5        | 5           |

si, small interfering; Klf15, Krüppel-like family 15; DVT, deep venous thrombosis.

Table II. Quality assessment of the raw RNA-sequences reads results of the sequences.

| Sample      | Raw_reads | Clean_reads | Clean_bases | Error_rate | Q20 | Q30 | GC_pct |
|-------------|-----------|-------------|-------------|------------|-----|-----|--------|
| DVT1        | 26177314  | 25683459    | 7.71G       | 0.03       | 97.39 | 92.99 | 50.06  |
| DVT2        | 23970627  | 22164628    | 6.65G       | 0.03       | 97.01 | 92.28 | 50.38  |
| DVT3        | 27492800  | 26662217    | 8.0G        | 0.03       | 97.55 | 93.50 | 50.96  |
| Blank1      | 30056705  | 27671970    | 8.3G        | 0.03       | 97.73 | 93.81 | 50.95  |
| Blank2      | 26587894  | 26131760    | 7.84G       | 0.03       | 97.30 | 92.87 | 50.33  |
| Blank3      | 27518969  | 26623013    | 7.99G       | 0.03       | 96.92 | 92.03 | 50.23  |
| Klf15-NC1   | 23873304  | 23344387    | 7.0G        | 0.02       | 98.19 | 94.79 | 50.64  |
| Klf15-NC3   | 31553809  | 31061688    | 9.32G       | 0.02       | 98.28 | 95.07 | 51.05  |
| Klf15-NC4   | 33418796  | 32815907    | 9.84G       | 0.02       | 98.19 | 94.84 | 51.31  |
| Klf15-siRNA_2 | 28443883 | 27658994    | 8.3G        | 0.02       | 98.18 | 94.81 | 50.36  |
| Klf15-siRNA_3 | 31256846 | 30573498    | 9.17G       | 0.02       | 98.26 | 94.90 | 51.13  |
| Klf15-siRNA_5 | 31209875 | 30496859    | 9.15G       | 0.02       | 98.05 | 94.40 | 50.41  |

si, small interfering; Klf15, Krüppel-like family 15; DVT, deep venous thrombosis; NC, negative control.

Table III. Read mapping results of the sequences

| Sample      | Total_reads | Total_map (%) | Unique_map (%) | Multi_map (%) | Splice_map (%) |
|-------------|-------------|---------------|----------------|---------------|----------------|
| DVT1        | 51366918    | 49046085 (95.48) | 46528972 (90.58) | 2517113 (4.9)  | 17368593 (33.81) |
| DVT2        | 44329256    | 41980259 (94.7)  | 39085822 (88.17) | 2894437 (6.53)  | 15925579 (35.93)  |
| DVT3        | 53324434    | 50898142 (95.45) | 47701078 (89.45) | 3197064 (6.0)   | 19203249 (36.01)  |
| Blank1      | 55343940    | 53021663 (95.8)  | 49481609 (89.41) | 3540054 (6.4)   | 20032572 (36.2)   |
| Blank2      | 52263520    | 49811834 (95.31) | 47560000 (91.0)  | 2251834 (4.31)  | 18114051 (34.66)  |
| Blank3      | 53246026    | 50413825 (94.68) | 48052390 (90.25) | 2361435 (4.43)  | 18879029 (35.46)  |
| Klf15-NC1   | 46688774    | 45469186 (97.39) | 42152166 (90.28) | 3317020 (7.1)   | 16922773 (36.25)  |
| Klf15-NC3   | 62123376    | 60297028 (97.06) | 55302462 (89.02) | 4994566 (8.04)  | 21761027 (35.03)  |
| Klf15-NC4   | 65631814    | 63630957 (96.95) | 58085965 (88.5)  | 5544992 (8.45)  | 2369020 (36.11)   |
| Klf15-siRNA_2 | 55317988 | 53743158 (97.15) | 50929624 (92.07) | 2813534 (5.09)  | 19622245 (35.47)  |
| Klf15-siRNA_3 | 61146996 | 59638348 (97.61) | 53836311 (88.04) | 5847537 (9.56)  | 22426460 (36.68)  |
| Klf15-siRNA_5 | 60993718 | 59329105 (97.27) | 55020590 (90.21) | 4308515 (7.06)  | 21394177 (35.08)  |

si, small interfering; Klf15, Krüppel-like family 15; NC, negative control; DEG, differentially expressed genes; DVT, deep venous thrombosis.
Next, to confirm the results of the DEG analyses, eight significant DEGs were selected from the RNA-seq results from the comparison of the DVT and Blank groups for further qPCR validation. As shown in Fig. 6, the expression levels of Mmp12 and Mmp13 in the DVT group were significantly increased compared with those in the Blank group. The expression level of Klf15 in the DVT group decreased significantly compared with that in the Blank group. Moreover,

| DEG set          | DEGs | Upregulated | Downregulated |
|------------------|------|-------------|---------------|
| DVT_vs_Blank     | 2,206| 1,330       | 876           |
| Klf15-siRNA_vs_NC| 1,041| 235         | 806           |

si, small interfering; Klf15, Krüppel-like family 15; DVT, deep venous thrombosis; NC, negative control; DEG, differentially expressed genes.

| Gene_id | DVT | Blank | log2 Fold change | Padj   | Gene_name |
|---------|-----|-------|------------------|--------|-----------|
| ENSMUSG00000049723 | 78.077668 | 47.9396 | 0.707139 | 0.566752 | Mmp12     |
| ENSMUSG00000050578 | 204.5046 | 4.875547 | 5.391707 | 3.03x10⁻⁹ | Mmp13     |
| ENSMUSG00000043613 | 394.1668 | 290.8774 | 3.759948 | 3.10x10⁻⁹ | Mmp3      |
| ENSMUSG00000058000 | 3114.6048 | 63.94969 | 5.606005 | 1.10x10⁻¹⁰| Mmp8      |
| ENSMUSG00000017737 | 7694.2132 | 732.7235 | 3.392543 | 1.06x10⁻²⁹| Mmp9      |
| ENSMUSG00000025355 | 1703.9523 | 203.5787 | 3.064868 | 1.52x10⁻¹³| Mmp19     |
| ENSMUSG00000026073 | 3607.8063 | 48.75941 | 6.20899 | 3.12x10⁻⁶³| Il1r2     |
| ENSMUSG00000027399 | 553.44397 | 39.4537 | 3.811049 | 9.93x10⁻¹⁹| Il1a      |
| ENSMUSG00000027398 | 5679.7852 | 139.7907 | 5.344781 | 9.60x10⁻⁷⁶| Il1b      |
| ENSMUSG00000025746 | 1369.395 | 5.758426 | 7.893124 | 2.59x10⁻⁹ | Il6       |
| ENSMUSG00000035352 | 906.99266 | 100.0886 | 3.17938 | 6.97x10⁻²¹ | Ccl12     |
| ENSMUSG00000035385 | 3020.6374 | 95.41017 | 4.984662 | 1.14x10⁻⁴⁸ | Ccl2      |
| ENSMUSG00000094686 | 7.4708094 | 119.9455 | -4.02534 | 2.10x10⁻⁶⁸ | Ccl21a    |
| ENSMUSG00000009082 | 3120.6479 | 31.87038 | 6.61243 | 3.97x10⁻⁷⁶ | Ccl3      |
| ENSMUSG00000018930 | 1205.0351 | 32.31576 | 5.220409 | 1.03x10⁻³² | Ccl4      |
| ENSMUSG00000035042 | 712.57684 | 2817.25 | -1.98337 | 1.21x10⁻¹¹ | Ccl5      |
| ENSMUSG00000018927 | 8371.4662 | 1210.417 | 2.789986 | 1.64x10⁻⁴⁴ | Ccl6      |
| ENSMUSG00000035373 | 2485.7371 | 79.3094 | 5.497069 | 3.32x10⁻⁰⁷ | Ccl7      |
| ENSMUSG00000019122 | 5063.0309 | 95.41017 | 3.095273 | 2.35x10⁻⁵⁰ | Ccl9      |
| ENSMUSG00000029380 | 2017.182 | 19.95151 | 6.659775 | 1.9x10⁻⁰⁴ | Cxcl1     |
| ENSMUSG00000061353 | 6200.4582 | 10594.49 | -0.77288 | 4.82x10⁻⁰⁵ | Cxcl12    |
| ENSMUSG00000021508 | 3590.1969 | 118.9011 | 4.916188 | 6.83x10⁻⁰⁷ | Cxcl14    |
| ENSMUSG00000018920 | 523.29727 | 975.7794 | -0.89848 | 0.00284 | Cxcl16    |
| ENSMUSG00000058427 | 16451.23 | 10.2436 | 10.65536 | 8.05x10⁻¹⁴⁸ | Cxcl12    |
| ENSMUSG00000029379 | 9750.5765 | 0.886821 | 13.4245 | 9.92x10⁻³⁵ | Cxcl3     |
| ENSMUSG00000029371 | 329.7922 | 4.430165 | 9.552655 | 7.59x10⁻⁷⁹ | Cxcl5     |
| ENSMUSG00000029417 | 721.6049 | 682.6357 | 0.079769 | 0.889672 | Cxcl9     |
| ENSMUSG00000026180 | 3454.5181 | 333.2202 | 3.3741 | 5.89x10⁻⁴⁶ | Cxcr2     |
| ENSMUSG00000045382 | 3109.524 | 812.4151 | 1.93664 | 2.84x10⁻¹⁵ | Cxcr4     |
| ENSMUSG00000048521 | 152.50759 | 568.3105 | -1.89793 | 1.83x10⁻⁰⁷ | Cxcr6     |
| ENSMUSG00000026691 | 114.11276 | 14.20097 | 3.004407 | 0.005941 | Fmo3      |
| ENSMUSG00000026580 | 1733.686 | 129.0424 | 3.747548 | 5.07x10⁻²⁸ | Selp      |
| ENSMUSG00000046223 | 3734.2897 | 435.4963 | 3.100282 | 1.12x10⁻⁴⁷ | Plaur     |
| ENSMUSG00000005413 | 12311.935 | 735.4273 | 4.065414 | 7.15x10⁻⁴² | Hmox1     |
| ENSMUSG00000019987 | 9944.886 | 38.99255 | 7.994212 | 1.56x10⁻¹⁶ | Arg1      |

si, small interfering; Klf15, Krüppel-like family 15; DVT, deep venous thrombosis.

**Table IV.** The number of DEGs identified from four groups.

**Table V.** DEGs identified in a comparison of the DVT group and the Blank group by RNA-sequencing.

qPCR validation of DEGs. Next, to confirm the results of the DEG analyses, eight significant DEGs were selected from the RNA-seq results from the comparison of the DVT and Blank groups for further qPCR validation. As shown in Fig. 6, the expression levels of Mmp12 and Mmp13 in the DVT group were significantly increased compared with those in the Blank group. The expression level of Klf15 in the DVT group decreased significantly compared with that in the Blank group. Moreover,
The levels of Mmp 19, Arg1, Ccl2, Fmo3 and Hmox1 in the DVT group were all significantly increased compared with those in the Blank group, which demonstrated the correlation of the results.

**Discussion**

DVT is one of the most common vascular diseases and has a high mortality rate (1). Nevertheless, the current diagnostic and therapeutic methods are limited (8). In current DVT studies, the mechanism and regulatory factors involved in the formation and pathological process of DVT should be investigated to provide a foundation for the diagnosis and treatment of DVT (19).

Klf15 was shown to be closely related to cardiovascular diseases such as hypertension, atherosclerosis and coronary heart disease (20). Klf15 is a transcriptional regulatory factor involved in various functions, including cell differentiation, apoptosis and tumor formation, and is expressed in various tissues and organs, including the heart, liver, and kidneys (21). Moreover, Klf15 plays a key role in the development of atherosclerosis (12). According to the authors' preliminary experiments, it was found that Klf15 might also affect thrombosis. To promote knowledge about the relationship and genetic interaction between DVT and Klf15, this study was performed.

Numerous obstacles prevent the complete understanding of the pathology, diagnosis and treatment of DVT. The present study aimed to examine factors regulating the initiation and progression of DVT and factors related to effective and utilisable measures.

To the best of our knowledge, this is the first study to perform high-throughput sequencing in a mouse DVT model and to investigate the effect of Klf15 on DVT formation. The data and analyses in the current study suggest that pathways including TNF, PI3K-Akt, IL-17, Jak-STAT, NF-κB, and MAPK should be considered, as such pathways were correlated with the formation of thrombi according to the KEGG enrichment analysis of the DEGs between the DVT and Blank groups. Previous studies (22,23) have reported that MAPK pathways are related to vascular endothelial venous thrombosis and our colleagues have suggested that resveratrol may exert an in vitro antithrombotic activity by inactivating MAPK signaling pathways (24). Moreover, KEGG analysis of the comparison of the Klf15-siRNA group and the Klf15-NC group indicated that PI3K-Akt pathway play a central role in the regulatory pathway involved in DVT formation.

The DEGs revealed by these comparisons indicated the crucial role of certain genes in the regulation of DVT. In the comparison of the DVT and Blank groups, the identified genes ranged from members of the Mmp family, the IL family, and the chemokine family to Selp, Plaur, Hmox1 and Arg1. Fonseca et al. (25) indicated that Mmp plays a critical role in numerous cellular processes. Li et al. (26) discovered that Mmp3 polymorphisms and upregulated protein expression in the Chinese Han population may provide new markers associated with the evaluation of DVT diagnosis and risk.

Lenglet et al. (27) performed a study on mice by subjecting their brains to ischemic stroke and revealed differentially expressed levels of Mmp family genes, including significantly upregulated expression of Mmp9, 10, and 13 and Timp1. Xiao et al. (28) indicated that Mmp8 enhanced vascular smooth muscle cell (VSMC) proliferation and played an important role in neointima formation via ADAM10-, N-cadherin-, and β-catenin-mediated pathways. Mmp8 enhances VSMC proliferation, according to a study of WT and Mmp9-/− mice that underwent stasis venous thrombosis (VT) by ligation of the IVC. The tissues were harvested at different time points and the results showed that the midterm vein wall collagen content was regulated by Mmp9 (28). Thus, Mmp9 plays a role in both vein wall responses and late thrombus resolution. Quillard et al. (29) found that Mmp13 prevailed over Mmp8 in collagen degradation in atheromata, thus identifying a selective target for plaque structure formation. Based on the current analysis and previous reports, the present study believes that the role of the MMP family in DVT deserves further study.

Genes in the IL family were identified, including Il-lr2, Il-la, Il-lb and Il-6, that showed higher expression in the DVT group than in the Blank group. Gupta et al. (30) demonstrated the increased expression of NLRP3, caspase-1 and IL-1β in individuals with clinically established VT. van Minkelen et al. (31) found that IL1RN-H5H5 carriership increases the risk of VT. Analyses of the DEGs in the chemokine families revealed that these DEGs had generally higher expression in the DVT group than in the Blank group, with the exception of some members mentioned in qPCR Validation of DEGs. Among those genes in the Cc and Cx families, a study of Cxcr2 was previously performed. Henke et al. (32) found that normal DVT resolution involved Cxcr2-mediated neovascularization, collagen turnover and fibronolysis and that this process is probably primarily monocyte-dependent. Henke and Wakefield (33) indicated that early thrombus resolution primarily involves Cxcr2-associated plasmin activation and Mmp-9, while later resolution involves both Cxcr2- and Ccr2-mediated uPA cell influx and thrombus angiogenesis. According to the above reports and our data, inflammation plays an important role in the formation of DVT. The present study speculated that Ccl2, a downstream gene of Klf15, may be the key factor in the effects of Klf15 on DVT formation.

**Table VI. Differentially expressed genes identified in a comparison of the Klf15-siRNA group and the Klf15-NC group.**

| Gene_id | siRNA       | NC        | log2 Fold change | Padj      | Gene_name |
|---------|-------------|-----------|------------------|-----------|-----------|
| ENSMUSG00000030087 | 100.70496   | 301.1934  | -1.58155         | 0.000497  | Klf15     |
| ENSMUSG00000049723 | 647.4817    | 101.9324  | 2.665213         | 6.61x10^-6 | Mmp12     |
| ENSMUSG00000050578 | 401.39705   | 115.9458  | 1.796271         | 0.000549  | Mmp13     |

si, small interfering; Klf15, Krüppel-like family 15; NC, negative control.
Table VII. KEGG pathway enrichment analysis of the DVT group vs. the Blank group.

| KEGGID   | Description                                      | Gene ratio  | BgRatio  | P-value          | Count | Up | Down |
|----------|--------------------------------------------------|-------------|----------|------------------|-------|----|------|
| mmu04060 | Cytokine-cytokine receptor interaction            | 100/907     | 234/6352 | 2.48x10^-27     | 100   | 60 | 40   |
| mmu04640 | Hematopoietic cell lineage                        | 50/907      | 89/6352  | 1.65x10^-20     | 50    | 26 | 24   |
| mmu05323 | Rheumatoid arthritis                              | 30/907      | 77/6352  | 7.01x10^-08     | 30    | 21 | 9    |
| mmu05144 | Malaria                                           | 22/907      | 47/6352  | 8.39x10^-08     | 22    | 20 | 2    |
| mmu04066 | HIF-1 signaling pathway                           | 34/907      | 99/6352  | 3.48x10^-07     | 34    | 31 | 3    |
| mmu04659 | Th17 cell differentiation                         | 34/907      | 99/6352  | 3.48x10^-07     | 34    | 13 | 21   |
| mmu04672 | Intestinal immune network for IgA production      | 19/907      | 41/6352  | 7.86x10^-07     | 19    | 9  | 14   |
| mmu04668 | TNF signaling pathway                             | 35/907      | 107/6352 | 8.86x10^-07     | 35    | 32 | 3    |
| mmu04514 | Cell adhesion molecules                           | 42/907      | 146/6352 | 3.46x10^-06     | 42    | 18 | 24   |
| mmu04656 | HIF-1 signaling pathway                           | 34/907      | 99/6352  | 3.48x10^-07     | 34    | 31 | 3    |
| mmu04657 | IL-17 signaling pathway                           | 29/907      | 87/6352  | 5.01x10^-06     | 29    | 27 | 2    |
| mmu04512 | ECM-receptor interaction                          | 28/907      | 82/6352  | 4.28x10^-06     | 28    | 19 | 9    |
| mmu04658 | Th1 and Th2 cell differentiation                  | 27/907      | 73/6352  | 4.20x10^-05     | 27    | 7  | 20   |
| mmu05321 | Inflammatory bowel disease                        | 20/907      | 57/6352  | 6.44x10^-05     | 20    | 10 | 10   |
| mmu04630 | Jak-STAT signaling pathway                        | 38/907      | 143/6352 | 7.05x10^-05     | 38    | 25 | 13   |
| mmu05152 | Tuberculosis                                      | 41/907      | 160/6352 | 9.04x10^-05     | 41    | 29 | 12   |
| mmu04064 | NF-kB signaling pathway                           | 27/907      | 92/6352  | 0.0001319       | 27    | 16 | 11   |
| mmu05340 | Primary immunodeficiency                          | 14/907      | 35/6352  | 0.0001645       | 14    | 0  | 14   |
| mmu05202 | Transcriptional misregulation in cancer           | 41/907      | 169/6352 | 0.0003243       | 41    | 27 | 14   |
| mmu05162 | Measles                                           | 32/907      | 122/6352 | 0.0003335       | 32    | 19 | 13   |
| mmu04010 | MAPK signaling pathway                            | 61/907      | 281/6352 | 0.0003692       | 61    | 49 | 12   |
| mmu05166 | HTLV-I infection                                  | 57/907      | 262/6352 | 0.0005396       | 57    | 31 | 26   |
| mmu05164 | Influenza A                                       | 36/907      | 147/6352 | 0.0006105       | 36    | 27 | 9    |
| mmu00052 | Galactose metabolism                              | 12/907      | 31/6352  | 0.0006857       | 12    | 11 | 1    |
| mmu05418 | Fluid shear stress and atherosclerosis            | 34/907      | 138/6352 | 0.0007639       | 34    | 31 | 3    |
| mmu05320 | Autoimmune thyroid disease                        | 16/907      | 50/6352  | 0.0010955       | 16    | 6  | 10   |
| mmu00220 | Arginine biosynthesis                             | 8/907       | 17/6352  | 0.001229        | 8     | 6  | 2    |
| mmu05145 | Toxoplasmosis                                     | 27/907      | 105/6352 | 0.0013127       | 27    | 15 | 12   |
| mmu04062 | Chemokine signaling pathway                       | 40/907      | 176/6352 | 0.0015037       | 40    | 27 | 13   |
| mmu04216 | Ferroptosis                                       | 13/907      | 39/6352  | 0.0020876       | 13    | 11 | 2    |
| mmu04612 | Antigen processing and presentation              | 20/907      | 74/6352  | 0.0028603       | 20    | 9  | 11   |
| mmu05332 | Graft-versus-host disease                         | 15/907      | 50/6352  | 0.0031691       | 15    | 7  | 8    |
| mmu05133 | Pertussis                                         | 19/907      | 70/6352  | 0.0034227       | 19    | 17 | 2    |
| mmu04145 | Phagosome                                         | 35/907      | 156/6352 | 0.0036189       | 35    | 25 | 10   |
| mmu04621 | NOD-like receptor signaling pathway               | 34/907      | 151/6352 | 0.0038603       | 34    | 30 | 4    |
| mmu05134 | Legionellosis                                     | 16/907      | 56/6352  | 0.0040295       | 16    | 15 | 1    |
| mmu00590 | Arachidonic acid metabolism                       | 17/907      | 61/6352  | 0.0040877       | 17    | 14 | 3    |
| mmu04620 | Toll-like receptor signaling pathway              | 22/907      | 87/6352  | 0.0044241       | 22    | 19 | 3    |
| mmu05230 | Central carbon metabolism in cancer               | 17/907      | 62/6352  | 0.0049021       | 17    | 16 | 1    |
| mmu05330 | Allograft rejection                               | 14/907      | 48/6352  | 0.0056832       | 14    | 4  | 10   |
| mmu04940 | Type 1 diabetes mellitus                          | 15/907      | 53/6352  | 0.0058193       | 15    | 6  | 9    |
| mmu04931 | Insulin resistance                                | 25/907      | 105/6352 | 0.0058616       | 25    | 18 | 7    |
| mmu05310 | Asthma                                           | 8/907       | 21/6352  | 0.0060726       | 8     | 7  | 1    |
| mmu05142 | Chagas disease (American trypanosomiasis)        | 24/907      | 100/6352 | 0.0061752       | 24    | 18 | 6    |
| mmu01230 | Biosynthesis of amino acids                       | 18/907      | 70/6352  | 0.0079558       | 18    | 15 | 3    |

KEGG, Kyoto Encyclopedia of Genes and Genomes; DVT, deep venous thrombosis.
Table VIII. KEGG Pathways enrichment analysis of KLF15-siRNA group vs. KLF15-NC group.

| KEGGID   | Description                          | Gene ratio | BgRatio  | P-value | Count | Up  | Down |
|----------|--------------------------------------|------------|----------|---------|-------|-----|------|
| mmu04514 | Cell adhesion molecules              | 25/352     | 144/6351 | 2.50x10^-27 | 25    | 8   | 17   |
| mmu04725 | Cholinergic synapse                   | 20/352     | 107/6351 | 1.23x10^-26 | 20    | 0   | 20   |
| mmu04080 | Neuroactive ligand-receptor interaction | 30/352 | 225/6351 | 5.42x10^-26 | 30    | 3   | 27   |
| mmu04512 | ECM-receptor interaction              | 15/352     | 82/6351  | 3.52x10^-26 | 15    | 0   | 15   |
| mmu04728 | Dopaminergic synapse                  | 19/352     | 126/6351 | 5.60x10^-26 | 19    | 0   | 19   |
| mmu05033 | Nicotine addiction                   | 8/352      | 35/6351  | 0.0005167  | 8     | 0   | 8    |
| mmu05323 | Rheumatoid arthritis                 | 12/352     | 76/6351  | 0.0008645  | 12    | 8   | 4    |
| mmu04151 | PI3K-Akt signaling pathway            | 32/352     | 325/6351 | 0.0009717  | 32    | 5   | 27   |
| mmu04721 | Synaptic vesicle cycle                | 10/352     | 59/6351  | 0.0013226  | 10    | 1   | 9    |
| mmu04742 | Taste transduction                   | 9/352      | 50/6351  | 0.0014766  | 9     | 0   | 9    |
| mmu04270 | Vascular smooth muscle contraction    | 15/352     | 115/6351 | 0.0015912  | 15    | 1   | 14   |
| mmu05410 | Hypertrophic cardiomyopathy          | 12/352     | 82/6351  | 0.0017112  | 12    | 1   | 11   |
| mmu04390 | Hippo signaling pathway              | 18/352     | 152/6351 | 0.0017475  | 18    | 1   | 17   |
| mmu04726 | Serotonergic synapse                 | 14/352     | 106/6351 | 0.0020024  | 14    | 0   | 14   |
| mmu00512 | Mucin type O-glycan biosynthesis     | 6/352      | 26/6351  | 0.0024822  | 6     | 1   | 5    |
| mmu05414 | Dilated cardiomyopathy               | 12/352     | 86/6351  | 0.0025876  | 12    | 0   | 12   |
| mmu04024 | cAMP signaling pathway               | 20/352     | 186/6351 | 0.003181   | 20    | 0   | 20   |
| mmu05321 | Inflammatory bowel disease           | 9/352      | 56/6351  | 0.003331   | 9     | 7   | 2    |
| mmu04974 | Protein digestion and absorption     | 11/352     | 78/6351  | 0.0035469  | 11    | 2   | 9    |
| mmu05144 | Malaria                              | 8/352      | 47/6351  | 0.0038607  | 8     | 2   | 6    |
| mmu04727 | GABAergic synapse                    | 11/352     | 79/6351  | 0.0039219  | 11    | 0   | 11   |
| mmu04911 | Insulin secretion                    | 11/352     | 79/6351  | 0.0039219  | 11    | 1   | 10   |
| mmu04926 | Relaxin signaling pathway            | 15/352     | 126/6351 | 0.0039259  | 15    | 1   | 14   |

KEGG, Kyoto Encyclopedia of Genes and Genomes; DVT, deep venous thrombosis; si, small interfering; NC, negative control.

A study of the relationship between Hmox1 and DVT was conducted by Bean et al (34) who identified a critical cytoprotective enzyme encoded by the inducible Hmox1 gene with anti-inflammatory, antioxidant and anticoagulant activities in the vascular system. A (GT)n microsatellite located in the promoter of the Hmox1 gene influences the level of the response. Peng et al (35) stimulated HO-1 (Hmox1) production and revealed the inhibition of platelet-dependent thrombus formation in HO-1−/− mice compared with that in WT mice, and this inhibition may represent an adaptive response mechanism to reduce platelet activation.

Bojic et al (36) conducted a study on mice regarding the relationships between the peroxisome proliferator-activated receptor (PPAR)δ agonist GW1516 in aortic inflammation and atherosclerosis via intervention by the PPARδ agonist; this study revealed that the progression of preestablished atherosclerosis was inhibited by aortic inflammation and attenuated by the progression of preestablished atherosclerosis. Furthermore, GW1516 intervention decreased the expression of aortic proinflammatory M1 cytokines, increased the expression of the anti-inflammatory M2 cytokine Arg1 and attenuated the iNos/Arg1 ratio. Samsoondar et al (37) performed hepatic gene expression analysis on Ldlr−/− mice fed a high-fat, high-cholesterol diet (42% kcal fat, 0.2% cholesterol) supplemented with bempedoic acid at 0, 3, 10 and 30 mg/kg body weight. These results showed that in vivo thombosis potential.

Shih et al (39) treated WT and FMO3KO mice with control or FMO-3 ASOs. FMO-3-ASO treatment led to the same extent of lipid-lowering effects in the FMO3KO mice as it did in the WT mice, indicating off-target effects. This study revealed that both FMO3KO and WT mice fed a 0.5% choline diet showed a substantial reduction in both TMAO and in vivo thrombosis potential.
In conclusion, a transcriptome study consisting of two parts was performed to investigate the expression levels of Klf15 and other related genes in C57BL/6 mice with IVC thrombi for the first time. The experimental results indicated that 2,206 genes were differentially expressed between the DVT group and the Blank group, and 1,041 DEGs were identified by comparing the Klf15-siRNA group with the Klf15-NC group. The present study confirmed that Arg1, Ccl2 and Hmox1 are related to DVT, as previously identified, and new genes related to the formation of DVT were identified, including Fmo3 and Mmp19. Furthermore, to the best of our knowledge, the present study is the first to reveal that genes such as Mmp12 and Mmp13 are involved in the regulation of DVT; the current results obtained via comparison of the Klf15-siRNA group and the Klf15-NC group are especially revealing. Given the data obtained in the present experiments, it is speculated that Klf15 may play a role in DVT by regulating inflammatory genes, some members of the MMP family or other DEGs; however, this speculation needs to be confirmed in the future. In the next study, cell experiments, clinical experiments and additional animal experiments will be performed to confirm the role of Klf15 in DVT, including pathway regulation and whether DVT formation is regulated by Klf15 via Mmp12 and Mmp13. The present research provides new insights and prospects for studying the mechanism of thrombosis and possible drug targets.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

XZ and RZ conceived the present study. RZ designed the experiments. JZ and SX prepared the materials and conducted the experiments on mice with IVC thrombi. XZ processed the data. RZ contributed substantially to the data analysis and manuscript revision. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All animal experiments were performed following approval from the Animal Experimental Ethical Committee of Kunming Medical University (Kunming, China).

Patient consent for publication

Not applicable.

Competing interest

The authors declare that they have no conflicts of interest.

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