Identification of biomarkers of venous thromboembolism by bioinformatics analyses

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Abstract

Venous thromboembolism (VTE) is a common vascular disease and a major cause of mortality. This study intended to explore the biomarkers associated with VTE by bioinformatics analyses.

Based on Gene Expression Omnibus (GEO) database, the GSE19151 expression profile data were downloaded. The differentially expressed genes (DEGs) between single VTE (sVTE)/recurrent VTE (rVTE) and control were identified. Then, pathway enrichment analysis of DEGs were performed, followed by protein–protein interaction (PPI) network construction.

Total 433 upregulated and 222 downregulated DEGs were obtained between sVTE and control samples. For rVTE versus control, 625 upregulated and 302 downregulated DEGs were identified. The overlap DEGs were mainly enriched in the pathways related to ribosome, cancer, and immune disease. The DEGs specific to rVTE were enriched in several pathways, such as nod-like receptor signaling pathway. In the PPI network, 2 clusters of VTE genes, including ribosomal protein family genes and NADH family-ubiquinol-cytochrome genes, were identified, such as ribosomal protein L9 (RPL9), RPL5, RPS20, RPL23, and tumor protein p53 (TP53).

The nod-like receptor signaling pathway, ribosomal protein family genes, such as RPL9, RPL5, RPS20, and RPL23, and DEG of TP53 may have the potential to be used as targets for diagnosis and treatment of VTE.

Abbreviations: DEG = differentially expressed gene, GEO = Gene Expression Omnibus, IL = interleukin, PPI = protein–protein interaction, RPL9 = ribosomal protein L9, rVTE = recurrent venous thromboembolism, sVTE = single venous thromboembolism, TP53 = tumor protein p53, VTE = venous thromboembolism.

Keywords: differentially expressed gene, pathway enrichment analysis, protein–protein interaction network, venous thromboembolism

1. Introduction

Venous thromboembolism (VTE) is one of the most common vascular disease, as well as a major cause of mortality.\textsuperscript{[1,2]} The incidence of VTE increases dramatically over 45 years old.\textsuperscript{[3]} Its annual incidence is about 1 or 2 cases per 1000 persons in the general population.\textsuperscript{[4]} The most frequent clinical manifestations are deep vein thrombosis of the leg, and pulmonary embolism. VTE is a chronic disease with acute exacerbations, and the major outcomes of VTE are recurrence, postthrombotic syndrome, and death.\textsuperscript{[1]} Approximately, as many as 30% patients develop to recurrent venous thromboembolism (rVTE) within 8 years after stopping a standard anticoagulant therapy.\textsuperscript{[5]} In consideration of the increasing life expectancy, adopting strategies to prevent and treat VTE is becoming more and more important all over the world.\textsuperscript{[6]}

VTE is a multicausal disease involving environmental and genetic risk factors.\textsuperscript{[7]} Family and twin studies have found that genetics play important roles in the occurrence of VTE.\textsuperscript{[8]} An increasing number of genetic variants involved in the fibrolysis and coagulation pathways are consistently linked to VTE.\textsuperscript{[9–11]} Reitsma and Rosendaal\textsuperscript{[12]} reported that some inflammatory mediators, such as interleukin (IL)-6 and tumor necrosis factor α, represented risk determinants for VTE. In addition, a recent study suggested that tissue factor and tissue factor pathway inhibitor played key roles in coagulation and acted as direct risk factors for VTE.\textsuperscript{[13]} Although progresses have been achieved for the pathogenesis of VTE, the genetic mechanisms are far from being understood. Determination of the recurrence risk factors for VTE is still a vital health concern.\textsuperscript{[14]}

In the present study, the microarray data GSE19151\textsuperscript{[2]} including single venous thromboembolism (sVTE), rVTE, and control samples were downloaded from Gene Expression Omnibus (GEO) database for differential expression analysis. In addition, functional enrichment analysis and protein–protein interaction (PPI) network construction were performed for differentially expressed genes (DEGs). The present study aimed to explore the genes and pathways associated with the occurrence and development of sVTE and rVTE, which may be helpful to find out novel biomarkers for the diagnosis and treatment of VTE.
2. Methods

2.1. Affymetrix microarray data
The GSE19151 gene expression profile data were downloaded from GEO, (http://www.ncbi.nlm.nih.gov/geo/), a public functional genomics database. The gene expression profile platform was Affymetrix Human Genome U133A 2.0 Array (Affymetrix Inc., Santa Clara, CA). A total of 63 healthy control, 32 sVTE, and 38 rVTE were available for analysis.

2.2. Data preprocessing and DEGs identification
The original data were performed background correction, quantile normalization, and log transformation using the robust multiarray average algorithm. The probe was removed when it corresponded to multiple gene symbols. Finally, the gene expression matrix including 12,754 genes was obtained.

The DEGs of sVTE versus control and rVTE versus control were screened out based on the limma package in R/Bioconductor. The P-values regarding the significance of differences were calculated using paired-samples t test. The obtained P-values were adjusted with Benjamini and Hochberg (BH) method, obtaining the false discovery rate (FDR). Additionally, the log$_2$-fold change (log$_2$FC) was calculated. log$_2$FC > 1.5 and FDR < 0.05 were considered as the cutoff values for DEGs screening.

2.3. Functional enrichment analysis
ClueGO is a plugin of cytoscape used to facilitate the biological interpretation and to visualize functionally grouped terms in the form of networks. In the present study, we performed Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis based on the ClueGO plugin with P-value <.05 and kappa score=0.4. The DEGs enriched in the pathways were displayed using CluePedia plugin in cytoscape.

2.4. PPI network construction
We downloaded the comprehensive interaction information of human proteins from the Search Tool for the Retrieval of Interacting Genes database (http://string-db.org/). The interaction relationship pairs of DEGs associated with VTE with combined score >0.9 were extracted to construct the PPI network using cytoscape (http://www.cytoscape.org/).

3. Results

3.1. Identification of DEGs
After preprocessing, 655 DEGs including 433 upregulated and 222 downregulated DEGs were obtained between sVTE and control samples. For rVTE versus control group, there were 625 upregulated and 302 downregulated DEGs. Additionally, 357 DEGs were upregulated and 176 ones were downregulated in both sVTE and rVTE samples (Fig. 1).

3.2. Functional enrichment analysis
As shown in Fig. 1, 357 overlapped upregulated and 176 overlapped downregulated DEGs were identified in between sVTE and rVTE samples. Functional enrichment analysis of these DEGs revealed that they were significantly enriched in
Figure 2. Pathways enriched by the overlap DEGs of rVTE versus control, and sVTE versus control. Larger dot represents pathway; small dot represents DEG enriched in the pathway. Pathway node size represents the significant level, and a bigger size represents a more significant level. Pathway dot color represents function group, and the same group has the same color. Gene node color is the same with its function group. DEG = differentially expressed gene, sVTE = single venous thromboembolism, rVTE = recurrent venous thromboembolism.

Table 1

| Pathway ID | Pathway name                                | Count | P           |
|------------|---------------------------------------------|-------|-------------|
| Up         | Ribosome                                   | 42    | 9.56E-36    |
| hsa00190   | Oxidative phosphorylation                   | 24    | 2.37E-14    |
| hsa05012   | Parkinson’s disease                         | 21    | 6.69E-11    |
| hsa05010   | Alzheimer’s disease                         | 20    | 9.11E-09    |
| hsa05016   | Huntington’s disease                        | 21    | 1.51E-08    |
| hsa04092   | Nonalcoholic fatty liver disease (NAFLD)    | 17    | 3.62E-07    |
| hsa04260   | Cardiac muscle contraction                  | 11    | 8.03E-06    |
| hsa04062   | Chemokine signaling pathway                 | 10    | 2.94E-02    |
| Down       | Hepatitis B                                | 11    | 6.38E-05    |
| hsa05161   | Acute myeloid leukemia                      | 6     | 1.52E-03    |
| hsa04919   | Thyroid hormone signaling pathway           | 8     | 1.66E-03    |
| hsa05200   | Pathways in cancer                          | 15    | 2.34E-03    |
| hsa05162   | Colorectal cancer                           | 6     | 2.40E-03    |
| hsa05205   | Proteoglycans in cancer                     | 10    | 3.25E-03    |
| hsa04510   | Focal adhesion                              | 10    | 3.95E-03    |
| hsa04810   | Regulation of actin cytoskeleton            | 10    | 4.63E-03    |
| hsa05166   | HTLV-I infection                            | 11    | 5.33E-03    |
| hsa05213   | Endometrial cancer                          | 5     | 7.79E-03    |

rVTE = recurrent venous thromboembolism, sVTE = single venous thromboembolism.
some pathways which could be divided into several function groups including ribosome, oxidative phosphorylation, cancer, and immune disease. Specially, some DEGs were involved in multiple function groups, such as tumor protein p53 (TP53), transforming growth factor beta 1, and v-myc avian myelocytomatosis viral oncogene homolog (Fig. 2). In addition, we analyzed the pathways enriched by up- and downregulated DEGs, respectively. As shown in Table 1, the upregulated DEGs were significantly enriched in ribosome (hsa03010), oxidative phosphorylation (hsa00190), and Parkinson disease (hsa05012), and the downregulated DEGs were involved in hepatitis B (hsa05161), acute myeloid leukemia (hsa05221), thyroid hormone signaling pathway (hsa04919), and pathways in cancer (hsa05200).

3.3. rVTE-specific pathway

After functional annotation for the 393 DEGs specific to rVTE, we found that they were enriched in several pathways such as notch signaling pathway, nod-like receptor signaling pathway, and adherens junction (Fig. 3). Furthermore, the upregulated genes were enriched in pathways of ribosome, proteasome, and
Huntington disease. Downregulated genes involved in pathways in cancer, hematopoietic cell lineage, chemokine signaling pathway, and adherens junction (Table 2).

### 3.4. PPI network construction

The PPI network was constructed with 466 nodes and 1356 edges. Among these nodes, 166 were specific to rVTE, 37 were specific to sVTE, and 263 were overlap genes. For the 1356 edges, 567 ones were specific to cancer, 567 ones were specific to sVTE, and 679 ones belong to overlap genes. The PPI network included 2 clusters of VTE genes, including ribosomal protein family genes such as ribosomal protein L9 (RPL9), RPL5, RPS20, and RPL23, and NADH family-ubiquinol-cytochrome genes. In addition, the top 5 hub genes of each class (overlap/rVTE/sVTE) were shown in Table 3, such as RPL9, TP53, and eukaryotic initiation factor 4e.

### 4. Discussion

In the present study, 655 DEGs were identified between sVTE and control samples, and 927 DEGs were obtained in rVTE versus control group. The overlap DEGs of the 2 comparison groups were significantly enriched in several pathway function groups: ribosome, cancer, and immune disease. TP53 was involved in multiple function groups. The rVTE specific DEGs were mainly enriched in nod-like receptor signaling pathway and notch signaling pathway. RPL9, TP53, RPL5, RPS20, and RPL23 were top 5 hub genes in the PPI network. Among the top 5 hub genes in the PPI network, 4 ones including RPL9, RPL5, RPS20, and RPL23 belonged to the ribosomal protein family, besides they were upregulated in both rVTE and sVTE samples. Early studies have identified ribosomes in platelet preparations. The ribosomal protein RPS6, for instance, has been reported to present in circulating platelets, the unique cellular effectors of hemostasis and thrombosis. Platelets support the accumulation of innate immune cells and promote the formation of neutrophil extracellular trap, which contribute to the propagation of deep vein thrombosis. Currently, there are few reports about the relationships between these ribosomal protein family genes and thrombosis, so we speculate that DEGs of RPL9, RPL5, RPS20, and RPL23 and VTE may be closely linked in light of their relationships with platelets.

In addition, the present study showed that TP53 involved in multiple function groups, besides it was a downregulated hub gene in the PPI network. TP53 encodes a tumor suppressor protein p53 which regulates cell cycle and negatively regulates cell division, and acts as a tumor suppressor in many tumor types. Mutations of TP53 are the most common genetic alterations in human tumors, which have been suggested as a molecular marker for the prognosis of tumor. Interestingly, VTE occurs commonly in patients with cancer. For instance, the disseminated intravascular coagulation, a kind of clinical manifestation of VTE, is most commonly observed in patients with widespread metastatic cancer and hematological malignant disorders. In addition, as Cermak et al. reported that tissue factor has been found to be a target of the inactivation of p53. Therefore, we speculated that TP53 might be a key biomarker of VTE.

Nod-like receptor signaling pathway was a significant pathway specific to rVTE. The nod-like receptors are a family of intracellular receptors that represent critical components of the innate immune responses and inflammation in mammals. Chen et al. have reported that nod-like receptors regulate nuclear factor-kappa B signaling, and IL-1β production,

### Table 2

| Pathway ID | Pathway name | Count | P   |
|------------|--------------|-------|-----|
| Up         | hsa03010     | Ribosome | 7  | 2.65E-02 |
|            | hsa03050     | Proteasome | 4  | 3.71E-02 |
|            | hsa06016     | Huntington’s disease | 8  | 4.22E-02 |
| Down       | hsa05200     | Pathways in cancer | 14 | 2.68E-04 |
|            | hsa04640     | Hematopoietic cell lineage | 7  | 2.96E-04 |
|            | hsa04062     | Chemokine signaling pathway | 8  | 3.86E-03 |
|            | hsa04662     | B cell receptor signaling pathway | 5  | 6.50E-03 |
|            | hsa04144     | Endocytosis | 9  | 6.56E-03 |
|            | hsa04520     | Adherens junction | 5  | 7.19E-03 |
|            | hsa04810     | Regulation of actin cytoskeleton | 8  | 7.66E-03 |
|            | hsa04666     | Fc gamma R-mediated phagocytosis | 5  | 1.28E-02 |
|            | hsa04919     | Thyroid hormone signaling pathway | 5  | 3.40E-02 |

### Table 3

| Node       | Type   | Up/down | Degree |
|------------|--------|---------|--------|
| RPL9       | Overlap| Up      | 40     |
| TP53       | Overlap| Down    | 39     |
| RPL5       | Overlap| Up      | 36     |
| RPS20      | Overlap| Up      | 34     |
| RPL23      | Overlap| Up      | 34     |
| EIF4E      | rVTE   | Up      | 27     |
| SRP14      | rVTE   | Up      | 24     |
| RPS25      | rVTE   | Up      | 20     |
| STAT3      | rVTE   | Down    | 19     |
| KAT2B      | rVTE   | Up      | 18     |
| RPL37A     | sVTE   | Up      | 25     |
| NCOA3      | sVTE   | Up      | 9      |
| MED13L     | sVTE   | Up      | 8      |
| SF3B1      | sVTE   | Up      | 7      |
| IDCP2      | sVTE   | Up      | 6      |

**Table 2**

The pathways enriched by the up- and downregulated differentially expressed genes in recurrent venous thromboembolism (rVTE) group.

**Table 3**

Degree top 5 genes in each class.

?VTE represents DEGs in recurrent venous thromboembolism, ?sVTE represents DEGs in single venous thromboembolism, and overlap represents the overlap DEGs of ?VTE and ?sVTE. Up/down represents up/downregulation. DEG = differentially expressed gene, sVTE = single venous thromboembolism, rVTE = recurrent venous thromboembolism.
indicating their crucial role in the pathogenesis of inflammatory diseases. Specially, Fox and Kahn[32] reported that patients with VTE manifested 4 common signs of inflammation, namely redness, heat, pain, and swelling, which might indicate that inflammation play an important role in VTE. Furthermore, in our study, IL8, encoding a proinflammatory mediator, was found to be enriched in the nod-like receptor signaling pathway. Specially, IL8 has been reported to be capable of activating coagulation.[33]

Taken together, nod-like receptor signaling pathway and IL8 may play important roles in the progression of VTE.

In summary, we have used a comprehensive bioinformatics method to analyze the DEGs and pathways related to VTE. The nod-like receptor signaling pathway and its enriched DEGs including RPL9, RPL5, RPS20, TP53, and RPL23 may have the potential to be used as targets for VTE diagnosis and treatment. However, further genetic and experimental studies with larger sample size are still needed to confirm our results.

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