The Identification of Hub Genes in Diagnosing Blunt Trauma with Venous Thromboembolism Using Integrated Bioinformatic Analysis

Qian Sun
Tangshan Second Hospital

Fei Tan
Tangshan Second Hospital

Hui Zheng
Tangshan Second Hospital

Shilin Wei
Tangshan Second Hospital

Lirong Zhang
Tangshan Second Hospital

Hongwei Zhang
Tangshan Second Hospital

Wei Qi (mailto:Tshanqiwei2003@163.com)
Tangshan Second Hospital

Keywords: blunt trauma, venous thromboembolism (VTE), hub genes, bioinformatic analysis

Posted Date: May 24th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2867449/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Background

Whether blunt trauma patient will get venous thromboembolism (VTE) is still a huge challenge to forecast. The objective of our study is to investigate and identify the potential biomarker genes for blunt trauma patients with VTE based on the method of bioinformatics analysis.

Methods

To investigate differentially expressed genes (DEGs) of blunt trauma and VTE, firstly we used the data obtained from the gene expression omnibus (GEO) database, and DEGs were respectively obtained via LIMMA. Next Weighted gene co-expression network analysis (WGCNA) was used to investigate the co-expression network of blunt trauma DEGs and two modules were found. We chose the most significant module as the key one in correlation with blunt trauma. We got intersection of DEGs in blunt trauma and VTE, which were analyzed using Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Then we used STRING to create a protein-protein interaction (PPI) network, and hub genes were screened by Cytoscape software and analyzed of functional enrichment. We chose another GEO database to evaluate the value of hub genes, and receiver operating characteristic (ROC) curves were done at last.

Results

There are a total of 546 DEGs of VTE and 4266 DEGs of blunt trauma were screened out from GSE19151 and GSE36809 respectively. Then 1568 DEGs of blunt trauma were identified in the most significant module. And 101 intersections of DEGs were found to be enriched for various functions and pathways. Among these DEGs, 6 overlapped hub genes with high degrees of stress method were selected. These hub genes include MRPL3, RPLP0, MRPL15, TP53, MYC, CD3D. And the six hub genes were tested to have high diagnostic values.

1. Introduction

In terms of global mortality, trauma contributes 10.1% to the burden of disease. Trauma-related injuries result in the deaths of nearly 4.8 million people every year(1). Blunt trauma provide an account of the majority of chest traumas, which may cause a great number of complications(2). Most abdominal traumas are closed injuries, which account for 80–90% of cases(3). There is a high rate of blunt trauma to the kidneys, which is usually caused by motor vehicle accidents (63%)(4). In blunt abdominal trauma, the spleen is the most commonly injured organ, which is approximately 46%(5).

VTE, as a thromboembolic disease, mainly includes pulmonary embolism and deep vein thrombosis. Most hospitalized patients suffer from this complication, which causes a high mortality rate and morbidity(6). In the ICU, 5.2% of patients had VTE events, while the rate of COVID-19 patients admitted to the intensive care unit increased to 8.3%(7). It is now widely acknowledged that VTE is a prototypical immunothrombotic reaction, and inflammation and elevated plasma CRP levels are associated with a number of these conditions(8).

It is widely recognized that trauma patients without prophylaxis are at risk for DVT at a rate ranging from 5–80%, and traditional risk factors for VTE include lower limb injury, head injury, and pelvic fracture(9). In spite of the fact that all trauma patients are at an increased risk for developing VTE, and what is interesting, in contrast to white patients, Asians experienced significantly fewer VTEs(10). Trauma patients who experience VTE do not only suffer clinical symptoms, but they are also economically burdened as well. Because of this, efforts have never been stopped to prevent VTE after trauma(11).

As far as we know, few research has been published on the identification of hub genes for blunt trauma with VTE. Here, we firstly used the data obtained from the gene expression omnibus (GEO) database of trauma and VTE, and DEGs were respectively obtained via LIMMA. WGCNA was used to investigate the co-expression network of blunt trauma DEGs, intersection of DEGs in blunt trauma and VTE were analyzed using GO and KEGG pathway. Then we used STRING to create a protein-protein interaction (PPI) network, and six hub genes were screened and evaluated.

2. Methods and Materials

2.1 sources of data

In Fig. 1, you can see the workflow of the study. GSE19151 of VTE and GSE 36809 of blunt trauma were retrieved from a free public database named Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/). In GSE19151 it includes blood from 70 VTE patients and 63 healthy controls. And in GSE36809 it includes blood from 205 blunt trauma samples (whose blood samples collected since injury less than 24h were chosen) and 37 healthy control samples.

2.2 DEGs identification

Firstly, data quality checks and log transformations were performed in order to cancel batches. Based on generalized linear models, LIMMA(12) is a method for screening differential expression data from microarrays, here we use LIMMA (version 3.40.6) to identify DEGs. For statistical significance, an adjusted p-value of 0.05 was used. For visualization of the top 50 DEGs, the Heatmap package was used to plot the volcano map.
Based on the scale-free topology criteria, WGCNA(13) was employed to build the co-expression networks in DEGs of blunt trauma. Firstly, with the soft thresholding power determined, all DEGs were analyzed. Secondly, a weighted co-expression network was created, and two DEG clusters were labeled according to their different colors. A correlation was then explored between the modules and blunt trauma or controls. The most significant module is chosen, which was considered a key component to do further more enrichment analysis.

2.4 intersection of DEGs functional enrichment analysis

Draw Venn Diagram(https://bioinformatics.psb.ugent.be/webtools/Venn/) was employed to identify the intersection of DEGs in VTE and blunt trauma. Both of GO(14) and KEGG(15) pathway analysis were dealt with the intersection of DEGs in order to visualize the terms for the functions and pathways.

2.5 PPI network and potential hub genes identification

The STRING(16) online database was used to create the PPI network of intersection of DEGs, which was processed by Cytoscape software(17) then. Then we used CytoHubba(18) to get the hub genes, and we used Draw Venn Diagram to get six overlapped hub genes.

2.6 hub genes functional enrichment analysis

In order to visualize the functions and pathways of these six overlapped hub genes, GO and KEGG pathway analysis were employed to predicate and identify the function of chosen hub genes.

2.7 the value of hub genes evaluation

We chose GSE48000, a public free database of VTE from GEO, to evaluate the value of these six hub genes. Subsequently the ROC(20) was established, and to quantify its value, the area under the curve (AUC) and 95% confidence interval (CI) were calculated.

2.8 statistical analysis

In order to construct the ROC curve and calculate AUC as well as 95% CI, Sangerbox software was used. P < 0.05 was set as the statistical significance.

3. Result

3.1 identifications of DEGs

There were totally 546 DEGs of VTE identified between VTE patients and controls, with an adjusted p-value of < 0.05, in which 398 DEGs were upregulated and 148 were downregulated. Figure 2A and Fig. 2B show the volcano map of DEGs and the heatmap of the top 50 DEGs. There were totally 4266 DEGs of blunt trauma identified between blunt trauma group and controls, with an adjusted p-value of < 0.05, in which 1799 DEGs were upregulated and 2487 were downregulated. Figure 3A and Fig. 3B show the volcano map of DEGs and the heatmap of the top 50 DEGs.

3.2 WGCNA Analysis

Further processing was performed on the 4266 DEGs of blunt trauma identified with the WGCNA package in R software on SangerBOX. As part of the subsequent analysis, a soft thresholding power of 1 was chosen. And the scale independence and average connectivity showed in Fig. 4A and Fig. 4B. Gene co-expression modules represented by two different colors under the gene tree was showed in Fig. 4C. We found these DEGs were totally clustered into turquoise modules(Fig. 4D). And Fig. 4E showed the correlation between blunt trauma and each module. The results indicated that turquoise (0.86, p < 0.0001) model was the most positive module, which was chose including a total of 1568 DEGs as the key module related to blunt trauma.

3.3 intersection of DEGs functional enrichment analysis

101 DEGs intersected in VTE and blunt trauma were screened and showed in Fig. 5A. KEGG analysis revealed DEGs was enriched in 184 pathways showed in Fig. 5B. All DEGs were found enriched in 349 molecular functions (MF), 228 cellular components (CC), and 1000 biological processes (BP). Figure 5C–E showed the top 9 BPs, CCs, and MFs. The GO category showed that 'IMMUNE_EFFECTOR_PROCESS', 'CELL_ACTIVATION', and 'CELL_ACTIVATION_INVOLVED_IN_IMMUNE_RESPONSE' were enriched markedly.

3.4 PPI network construction and overlapped hub genes analysis

We used STRING database in order to explore the interaction between these intersected genes. And the STRING PPI network was constructed by setting 0.4 as the minimum interaction score. Figure 6A showed that the PPI network included totally 101 nodes and 142 edges. Figure 6B showed these DEGs analyzed using cytoscape software. The top ten hub genes (Table 1) were screened out by twelve different calculation algorithms, such as MNC, MCC, EPC, DMNC, Degree, and so on. Using the cytoHubba plug-in in Cytoscape. And six overlapped hub genes (Fig. 6C), including MRPL3, RPLP0, MRPL15, TP53, MYC, CD3D were identified, using calculate and draw custom venn diagrams, which were among the four algorithms (MNC, Degree, EPC, Stress).

3.5 diagnostic value assessment of hub genes

GSE48000 was retrieved from GEO to evaluate the diagnostic value of hub genes. For each hub genes, ROC curves were developed to evaluate those diagnostic specificity and sensitivity, showed in Fig. 7A-G. Each item's AUC and 95% CI were calculated. Here are the results: CD3D (AUC 0.66, CI 0.77–0.54), MRPL15 (AUC 0.67, CI 0.78–0.55), MRPL3(AUC 0.71, CI 0.83–0.50), MYC (AUC 0.76, CI 0.86–0.65), RPLP0(AUC 0.61, CI 0.74–0.48), and TP53(AUC 0.64, CI 0.77–0.51). MRPL3 and MYC pose a high diagnostic value.
4. Discussion

For hospitalized trauma patients, VTE remains a major cause of morbidity and mortality(10). Among trauma patients, VTE is the most frequent cause of death in the hospital and is also the most preventable(9). Biomarkers, also known as biological indicators, are used to determine a disease’s development or risk. It has been reported that there are few biomarkers that correlate blunt trauma with VTE disease in previous studies. Then it is crucial to elucidate the effective diagnostic markers of these two diseases. Integrated bioinformatics analyses were used here, and the diagnostic value for VTE in blunt trauma patients was evaluated. A notable discovery was the identification of six potential hub genes, including MRPL3, RPLP0, MRPL15, TP53, MYC, CD3D. And we found these overlapped hub genes were related with ‘ribosomal subunit’, ‘ribosome’ and ‘large ribosomal subunit’ markedly. Among them MRPL3 and MYC pose a high diagnostic value.

Using animal models of trauma, most studies have concluded that trauma activates autophagy in osteoblasts, cardiomyocytes, or lung tissue(1). The role of autophagy in immunity is broad, and includes cell-autonomous defense as well as multicellular immune coordination(21). VTE prognosis and onset are predicted by the neutrophil/lymphocyte ratio, in clinical practice this should be extensively applied. There is also some diagnostic and prognostic value to platelet/lymphocyte ratio, however, further studies are necessary to determine its reliability and stability(22). Previous studies showed that in early thrombus the heme is the main source of vascular endothelial cell oxidation, and it can lead to thrombosis further(23). Due to its high sensitivity, only D-dimer is used as a screening test for VTE. However further research is needed to evaluate readily available biomarkers that have high sensitivity and specificity for trauma cases due to the limitation of D-dimer(9).

Using mRNA as a template, and using amino acids as raw materials to synthesize proteins, is the ribosome's primary function(24). Previous studies have showed that the ribosome has a great deal of functions, including affecting protein synthesis and taking a significant role in cell differentiation, proliferation, transformation and apoptosis(25). VTE may be diagnosed and treated by using ribosomal protein family genes(26). MRPL3, as a hub gene identified, was related to acute mountain sickness, future use as a biomarker and therapeutic target may lead to accurate diagnoses and treatments(27). The RPLP0 is present in tissues subject to remote organ dysfunction after extremity trauma(28). Researchers have identified MRPL15 as a methylation factor that can be used to diagnose Alzheimer’s disease(29). As a signaling hub, the Tp53 protein might be capable of evaluating the neuronal microenvironment, assessing the severity and types of injury sustained(30). Liver tumorigenesis is induced by MYC expression in hepatocytes, even without pre-existing chronic diseases(31). An important role for CD3D may be played in the microenvironment of tumor immunity(32). MRPL3, as one of four hub genes, whose expression was increased in tumor tissues and was linked to cancer progression, indicating that it may be as a prognostic and diagnostic marker for breast cancer(33). In mice, MRPL3 causes adult-onset neurodegeneration with memory impairment(34). By targeting MYC directly and indirectly, tumor regression can be achieved, and MYC signaling pathway can help tumor cells to mis-regulate their microenvironment and avoid immune recognition by the host(35).

Compared to the previous studies we screened out six potential hub genes, MRPL3, RPLP0, MRPL15, TP53, MYC, CD3D, which have a high diagnostic value of blunt trauma with VTE. But it is still required that more sample size is needed to validate the efficacy of these overlapped genes as biomarkers in the immediate future.

5. Conclusion

To conclude, we re-analyzed the expression profile GSE19151 and GSE36809. And we screened out six overlapped hub genes in blunt trauma with VTE, including MRPL3, RPLP0, MRPL15, TP53, MYC, CD3D. However, research on the role of these hub genes remains limited. The results show that MRPL3, RPLP0, MRPL15, TP53, MYC, and CD3D can be served as potential target biomarker genes for diagnosis for blunt trauma with VTE.

Declarations

Acknowledgements Not applicable
Ethics approval and consent to participate Not applicable

Patient consent for publication Not applicable

Author Contributions: QS contributed to write the manuscript. QS and FT performed data selection and data analysis. QS and HZ contributed to methodology. QS, FT, and SW contributed to write original draft preparation. QS, WQ and HWZ contributed to visualize. QS, LZ and WQ contributed to the conceptualization, writing review and editing, supervision and design of the research. All authors contributed to the article and approved the submitted version. All authors have read and agreed to the published version of the manuscript.

Funding: The present study was funded by none.

Data Availability Statement: The mRNA expression dataset used in our study was downloaded from the Gene Expression Omnibus (GEO) under accession number GSE19151, GSE36809 and GSE48000(https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE, accessed on 12 April 2023).

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References


29. <MRPL15.pdf>.


Figures
Figure 1

Workflow chart of the study

Figure 2

volcano plot and heatmap for the VTE DEGs. (A) Volcano map of DEGs expression levels. (B) Heatmap of top 50 DEGs.
Figure 3

volcano plot and heatmap for the blunt trauma DEGs. (A) Volcano map of DEGs expression levels. (B) Heatmap of top 50 DEGs.
Figure 4

Identification of module genes via WGCNA in blunt trauma. (A, B) Estimation of the soft thresholding value for a scale-free co-expression network. (C) Cluster dendrogram of all DEGs. (D) Module feature vector. (E) Correlation between each module and blunt trauma.
Figure 5

Enrichment analysis of the intersection of genes in VTE and blunt trauma. (A) Venn diagram. (B) KEGG enrichment analysis. (C) Biological process. (D) Cellular component. (E) Molecular function.
Figure 6

PPI network and enrichment analysis of hub gene. (A, B) PPI network. (C) Venn diagram of the overlapped six hub genes. (D-F) GO functional enrichment analysis of six hub genes. (G) six hub genes analyzed in GeneMANIA.
Figure 7

the diagnostic value evaluation. (A-F) the ROC curve of each hub gene. (G) the ROC curve of all hub genes.