Identification of Intracranial Aneurysm Inflammatory Hub Genes by Machine Learning

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Research Article

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Abstract

Rupture of intracranial aneurysm (IA) has a very high mortality and disability rate, so it is very important to explore the mechanism of IA formation and to find potential drug therapy targets. Inflammatory responses have now been shown to play an important role in the formation and development of IA. The diagnosis, therapeutic target and prognostic marker research of various diseases through machine learning has superior performance, but it has not been reported in IA. In this study, we searched for the core inflammatory genes in the formation of IA through the GEO database, and identified the interaction relationship of these genes and the core regulated gene (TLR4) through the string database. The results of enrichment analysis indicated that our obtained inflammatory genes were associated with inflammatory response, immune response and vascular disease. Then, three key genes (CLEC7A, RTP4, SOX11) were found by two machine learning algorithms. The ROC curve results showed that key genes had high clinical value. In addition, by analyzing the correlation between key genes and immune cell infiltration, we found that inflammatory cells positively correlated with the expression of key genes play an important role in the formation and development of IA. This study is the first time to find the key genes of IA formation by machine learning method. The hub genes obtained in this study can provide new ideas for the formation and molecular mechanism of IA in the future, and provide a new direction for the non-surgical treatment of IA.

1| Introduction

Intracranial aneurysms (IA) arise from cerebral arteries and are characterized by localized dilation of cerebral arteries. Saccular aneurysms are the most common. Studies have shown that rupture of IA leads to subarachnoid hemorrhage (SAH) mortality in excess of 25%.(1) To prevent aneurysm rupture, surgical clipping or endovascular wrapping is offered to patients with unruptured aneurysms.(2) Significant technological advancements and improvements have been made in these invasive treatments. However, the rate of adverse outcomes due to clipping and coiling of unruptured aneurysms cannot be ignored.(3) Therefore, exploring the mechanism of intracranial aneurysm and finding potential therapeutic targets may be a promising alternative for patients with unruptured aneurysms.

IA is pathologically characterized by loss of arterial wall integrity caused by endothelial dysfunction, intimal hyperplasia, disruption of the extracellular matrix (ECM), and inflammatory responses.(4) Inflammation has a strong role in promoting, and many studies have shown that inflammatory response plays an important role in the destruction of arterial wall integrity.(5) Inflammatory chemokines released by the local inflammatory response lead to the infiltration of macrophages in the aneurysm wall. Macrophages express and release MMPs, which destroy the ECM of the arterial wall, which in turn leads to the recruitment of other inflammatory cells, which exacerbate the degeneration and weakening of the arterial wall, and ultimately lead to the formation and growth of aneurysms.(6, 7) Therefore, some studies have been conducted to inhibit the development of IA and reduce the risk of IA rupture by inhibiting the local inflammatory response of aneurysms.(8) Wen et al found that taking aspirin can delay the growth of intracranial aneurysms.(9) The study by Terceño et al. showed that taking acetylsalicylic acid can

reduce the risk of IA rupture.(10) However, the inhibition of aneurysm growth by inhibiting arterial inflammation has not achieved satisfactory results. On the one hand, this is due to the shortage of drugs currently used to suppress inflammation. Another aspect is due to the lack of therapeutic targets.

Medicine is one of the early applications of artificial intelligence (AI), which is gradually changing the way many diseases are diagnosed and treated.(11) Machine learning is an important part of artificial intelligence that uses algorithms to identify patterns of expression in datasets. At present, machine learning has been used in the diagnosis of various diseases, the research of therapeutic targets and prognostic markers. However, there is no research report using machine learning to find the key genes and potential therapeutic targets of intracranial aneurysm. Therefore, this study combined bioinformatics and machine learning to discover potential inflammatory genes associated with intracranial aneurysm formation. In this study, we analyzed the inflammatory DEGs of intracranial aneurysm samples and superficial temporal artery samples, and obtained 4 core genes by machine learning method. Further research identified a relationship between these genes and inflammatory cells. This may help to explain the mechanism of aneurysm formation and the search for therapeutic targets.

2 | Materials And Methods

2.1 | Gene Expression Dataset

mRNA expression profiling datasets GSE54083 and GSE75436 were downloaded from the Gene Expression Comprehensive Database (GEO, https://www.ncbi.nlm.nih.gov/geo/). There are 13 intracranial aneurysm samples and 10 superficial temporal artery samples in the GSE54083 dataset. There are 15 intracranial aneurysm samples and 15 superficial temporal artery samples in the GSE75436 dataset. After merging the 2 datasets, batch effects were eliminated using the limma and sva packages in R software.

2.2 | Identification of Differentially Expressed Inflammatory Genes

1251 inflammatory genes were downloaded from the Gene Set Enrichment Analysis (GSEA) database(http://www.gsea-msigdb.org/gsea/index.jsp)(Supplementary Material 1). Gene difference analysis was performed using the "limma" package on the R language. False discovery rate (FDR) < 0.05 and log2 fold change (log2FC)|≥1 were set as cutoff values for screening DEGs. The intersection of DEGs and inflammatory genes was considered as a group of significantly differentially expressed inflammatory genes.

2.3 | Enrichment Analysis

Using clusterProfiler, org.Hs.eg.db, DOSE, enrichplot and ggplot2 software packages in R language, Gene Ontology(GO), Kyoto Encyclopedia of Genes and Genomes(KEGG) and Disease ontology (DO) enrichment analysesenrichment analysis was performed on 74 inflammatory DEGs.(12, 13) Take p value and q value

< 0.05 as statistical significance test. The Hallmark and C7 gene set v6.2 collections were downloaded from the Molecular Signatures database, and used as the target set for GSEA using the gsea-4.1.2 software downloaded from the Broad Institute.(14)

2.4 | Protein–Protein Interaction (PPI) Network Analysis

A PPI network with a confidence level of 0.4 was established from STRING database (http://string-db.org/). The results were then imported into Cytoscape 3.8.1 to build the network model. Using Cytoscape's cytoHubba plug-in, the top 30 DEGs with high connectivity in the gene expression network were selected as hub genes according to the degree algorithm

2.5 | Identification of Hub Inflammatory DEGs in IA

Two machine learning algorithms were used to screen hub genes for the formation of IA. The Least Absolute Shrinkage and Selection Operator (LASSO) is a regression analysis algorithm that uses regularization to improve prediction accuracy. The LASSO regression algorithm was performed using the "glmnet" package in R to identify hub genes for the formation e of IA. Support Vector Machine (SVM) is a supervised machine learning technique widely used for classification and regression. To avoid overfitting, the RFE algorithm was used to select the best genes from the metadata cohort.(15) Therefore, to identify the gene set with the highest discriminative power, Support Vector Machine Recursive Feature Elimination (SVM-RFE) is applied to select suitable features. The hub genes screened by the two machine learning algorithms at the same time use the venn package of R to obtain the same hub genes. Then principal component analysis (PCA) based on hub genes was performed using the "ggplot2" package, and three-dimensional PCA plots were drawn. In addition, receiver operating characteristic (ROC) curves were constructed and AUC values were calculated to estimate the accuracy of hub genes.

2.6 | The Analysis of Immune Cell Infiltration

To quantify the proportion of immune cells in the samples, we assessed the infiltrating immune cells of each sample using the CIBERSORT algorithm.(16) The proportion of infiltrating immune cells was visualized in R software using the "ggplot2" package and the "pheatmap" package. Correlation heatmaps were created by the 'corrplot' package to visualize the correlation of infiltrating immune cells. Differences in immune cell infiltration between samples are shown in violin plots using the "vioplot" package.

2.7 | Correlation Analysis Between Hub Genes and Infiltrating Immune Cells

The association of the identified hub genes with the level of infiltrating immune cells was explored using Spearman rank correlation analysis in R software. The resulting associations were visualized using the "ggplot2" package.

3 | Result

3.1 | Identification of differentially expressed Inflammatory genes

Differential analysis of gene mRNA levels in IA (n = 28) and superficial temporal arteries (n = 25) in the GEO cohort was analyzed. The obtained differential genes were crossed with inflammatory genes, and finally 74 differentially expressed inflammatory genes were obtained [FDR < 0.05, |log2-fold change (FC)|>1] (Fig. 1A)(Supplementary Material 2).

3.2 | Construction of PPI Network Diagram and Hub Gene Screening

To figure out how overlapping genes interact, we uploaded their information into the STRING database to construct a PPI network. This network contains 57 nodes with 348 edges (Fig. 1B). The results of the PPI network showed that there was a complex relationship between these genes. Bar graphs for the top 30 genes in the PPI network are presented in Fig. 1C. We then used the most connected gene, TLR4, as a hub gene for subsequent analyses.

3.3 | Functional, Pathway and GSEA Enrichment Analysis of Inflammatory Genes

To explore the potential functional features of differentially expressed inflammatory genes, we performed GO enrichment analysis, KEGG enrichment analysis, DO enrichment analysis and GSEA enrichment analysis. (Fig. 2A-C). The results of biological process (BP) in GO enrichment analysis indicated that these genes were mainly involved in regulation of inflammatory response, positive regulation of cytokine production, leukocyte migration, activation of immune response, and leukocyte mediated immunity. The results of cellular component (CC) in GO enrichment analysis indicated that these genes were mainly involved in collagen-containing extracellular matrix, blood microparticle, low-density lipoprotein particle, and plasma lipoprotein particle. The results of molecular function (MF) in GO enrichment analysis showed that these genes were mainly involved in receptor ligand activity, signaling receptor activator activity, cytokine activity, and immune receptor activity. The results of GO enrichment analysis showed that these genes mainly caused the formation of aneurysm through inflammatory cells and inflammatory factors, and lipid metabolism was also involved. The results of KEGG enrichment analysis showed that these genes were mainly related to PI3K-Akt signaling pathway, Cytokine-cytokine receptor interaction, Lipid and atherosclerosis, Toll-like receptor signaling pathway. The results of DO enrichment analysis showed that diseases rich in DEGs were mainly associated with vascular diseases or inflammatory diseases such as cerebrovascular disease, atherosclerosis, arteriosclerotic, cardiovascular disease, coronary artery disease, arteriosclerosis, systemic lupus erythematosus. GSEA enrichment analysis showed that regulation of immune system process, immune effector process, leukocyte mediated immunity, etc. were mainly active in intracranial aneurysm samples (Fig. 2D).

3.4 | Identification of hub genes based on machine learning algorithm

To identify hub genes for IA formation, we used two different machine learning algorithms to screen for DEGs of inflammation. In this study, 16 key DEGs were identified using the LASSO algorithm (Fig. 3A). Furthermore, based on the SVM-RFE algorithm, 4 DEGs were identified as biomarkers (Fig. 3B). Then, we obtained three key genes including RTP4, CLEC7A, SOX11 by taking the intersection of the genes obtained by these two algorithms (Fig. 3C). Then we added the gene with the most linked nodes in the PPI network, TLR4, as a hub gene in the following analysis. The results of key gene differential analysis showed that the expression levels of RTP4, CLEC7A, SOX11 and TLR4 in IA were higher than those in the control group (Fig. 3D). The results of PCA analysis showed that the identified hub genes could effectively distinguish IA samples from control samples (Fig. 3E). Receiver operating characteristic (ROC) curves were constructed and AUC values were calculated to assess the predictive value of the model in the study study sample. The results showed that the AUCs of RTP4, CLEC7A, SOX11, and TLR4 were 0.954, 0.990, 0.903, and 0.884, respectively (Fig. 4A-D), indicating that the identified hub genes had good validation performance.

3.5 | Immune Cell Infiltration

We investigated the composition of immune cells in IA tissue versus superficial temporal artery tissue (Fig. 5A). The proportions of T cells regulatory (Tregs) ($P < 0.001$), Mast cells resting ($P = 0.011$) and Neutrophils ($P = 0.017$) in IA tissue were significantly lower than those in superficial temporal artery tissue. However, the proportion of Monocytes ($P < 0.001$) in AMI tissue was significantly higher than that in superficial temporal artery tissue (Fig. 5B). Correlation analysis showed that T cells CD4 naïve had the strongest positive correlation ($r = 0.59$) with Neutrophils ($r = 0.59$), and B cells memory and B cells naive had the strongest negative correlation ($r = 0.57$) (Fig. 5C).

3.6 | Correlation analysis between CLEC7A, RTP4, SOX11, TLR4 and infiltrating immune cells

By analyzing the correlation between hub genes and infiltrating immune cells, we found that CLEC7A was significantly related to Monocytes ($r = 0.49$, $P < 0.001$), Plasma cells ($r = 0.34$, $P = 0.012$), T cells gamma delta ($r = 0.3$, $P = 0.023$), Mast cells resting ($r=-0.27$, $P = 0.043$), and T cells regulatory (Tregs) ($r=-0.57$, $P < 0.001$) were significantly correlated (Fig. 6A). RTP4 and Monocytes ($r = 0.4$, $P = 0.002$), B cells memory ($r=-0.27$, $P = 0.048$), Neutrophils ($r=-0.36$, $P = 0.006$), T cells regulatory (Tregs) ($r=-0.51$, $P < 0.001$) were significantly correlated (Fig. 6B). SOX11 was significantly associated with Monocytes ($r = 0.36$, $P = 0.007$), B cells memory ($r=-0.3$, $P = 0.026$), T cells regulatory (Tregs) ($r=-0.47$, $P < 0.001$) (Fig. 6C). TLR4 and Monocytes ($r = 0.3$, 0.027), T cells gamma delta ($r = 0.29$, $P = 0.031$), Mast cells activated ($r = 0.27$, $P = 0.048$), Mast cells resting ($r=-0.27$, $P = 0.044$), T cells regulatory (Tregs) ($r= -0.52$, $P < 0.001$) were significantly correlated (Fig. 6D).

4 | Discussion

Although the early diagnosis and treatment of IA has been greatly improved, surgical treatment is still the only treatment for IA(17). Surgical risks and postoperative complications are still major problems in the treatment of IA. Therefore, exploring the formation and development mechanism of IA and finding potential drug therapy targets is expected to become a new therapeutic direction for IA(3). Inflammation and immune cell infiltration as important mechanisms for the growth and expansion of IA have been confirmed by many studies(4, 18–20). At present, although there are studies targeting inflammatory genes to treat IA, the results are not satisfactory(10). Therefore, this study will use bioinformatics combined with machine learning to find the core genes of inflammatory aneurysm formation, explore the potential mechanism of IA formation and discover IA inflammatory therapeutic targets, and provide a new direction for non-surgical treatment of IA.

We downloaded 2 datasets from the GEO database, and obtained a total of 74 differentially expressed inflammatory genes, including 58 up-regulated genes and 16 down-regulated genes, through differential analysis and intersection with inflammatory genes. We constructed a PPI network of these genes from the STRING database. Through the analysis of the PPI network, we found that there are complex regulatory relationships among these differentially expressed genes, and TLR4, as the core gene of the PPI network, has regulatory relationships with most of the genes in the network. At present, many studies have found that TLR4 plays an important role in the formation, expansion and rupture of IA. Kazuha Mitsui et al found that the TLR4 pathway promotes the development of intracranial aneurysm rupture by accelerating aneurysm wall inflammation, and TLR4 inhibition significantly reduced the rupture rate and proinflammatory cytokine levels of aneurysm rupture(21). Liang Liu et al. also found that TLR4 is associated with susceptibility to IA(22). Subsequently, we performed functional enrichment analysis on DEGs of inflammation. Results of BP and MF in GO enrichment analysis These genes were associated with inflammatory response, expression of inflammatory factors and regulation of inflammatory cells. The results of the MF analysis were mainly related to lipid metabolism. The study of Zhao et al. showed that inflammation-dependent hyperlipidemia is one of the mechanisms of aneurysm formation(23). The results of KEGG enrichment analysis were also related to inflammatory pathways and lipid metabolism pathways. Li et al. show that SRPK1 gene silencing increases vascular smooth muscle cell proliferation and vascular remodeling in IA through the PI3 K/Akt signaling pathway, which helps inhibit progression in arteries(24). By analyzing ruptured IA, Korostynski et al. found that Cytokine-cytokine receptor interaction is an important mechanism of IA rupture(25). The results of DO enrichment analysis indicated that the inflammatory DEGs obtained by our analysis were associated with vascular disease. Subsequently, we performed GSEA enrichment analysis, which showed that these genes were associated with inflammatory responses, immune regulation, and regulation of immune cells. The above analysis confirmed the accuracy of our results. It also demonstrated the important role of inflammatory response and immune cell infiltration in the formation, development and rupture of IA. Therefore, it is expected to be a safe and effective treatment for IA by regulating the expression of inflammatory genes and local immune cell infiltration. Identifying the core genes in the formation of IA through bioinformatics and machine learning,

and clarifying the correlation between the expression of core genes and immune cells, is helpful for the non-surgical treatment of IA.

Based on two machine learning algorithms, 3 core genes were identified. Due to the important regulatory role of TLR4 in the PPI network, we also included it as a core gene in subsequent analyses. RTP4 encodes a protein associated with cell surface opioid receptor expression. No research on RTP4 in IA has been found so far. Of note is the important role of RTP4 in the immune response. For example, RTP4 can induce the body's immune response to malaria, reduce the virus titer in the brain of infected mice, and alleviate the neurological symptoms of the mice(26). Furthermore, RTP4 is required for antigen-dependent immunoediting of cancer cells using CRISPR screens(27). Therefore, we judged that RTP4 may play an important role in the formation of IA. However, further in vivo and in vitro experiments are still needed to prove it. CLEC7A, also known as dectin-1, is involved in various pathophysiological processes including infection, allergy, regulation of inflammation, cancer and other diseases(28). Studies have shown that Dectin-1 plays an important role in the immune regulation of the central system and can promote tolerance, anti-inflammatory and neuroprotective responses(29). Dectin-1 Regulating Macrophage Polarization and Neutrophil Infiltration in Myocardial Ischemia-Reperfusion Injury(30). Interestingly, the two types of immune cells mentioned above confirm important inflammatory cells in the formation and progression of intracranial aneurysms(31, 32).SOX11, a member of the SRY box (SOX) family, emerges as an important transcriptional regulator that controls cell fate and differentiation as a whole(33). It is also involved in regulating the inflammatory response in the body. The study by Feng et al found that the decrease of SOX11 expression can alleviate the inflammatory response in spinal cord injury(34).

Types of immune cell infiltration in IA and superficial temporal artery samples were assessed using CIBERSOTR. It was found that various immune cell subtypes were closely related to the important biological processes of IA. T cells regulatory (Tregs), Mast cells resting, decreased infiltration of Neutrophils and increased infiltration of Monocytes were found to be related to the formation and development of IA. In addition, through correlation analysis of CLEC7A, RTP4, SOX11, TLR4 and immune cells, it was found that CLEC7A, RTP4, SOX11 and TLR4 were all associated with T cells regulatory (Tregs) and Monocytes. Our findings were also confirmed in other studies. T cells regulatory (Tregs) can significantly reduce the incidence and severity of IA, but it is often present at low levels or insufficient activity in IA tissues T cells regulatory (Tregs) can significantly reduce the incidence and severity of IA, but it is often present at low levels or insufficient activity in IA tissues(35, 36). Sun et al. found that increasing the number of T cells regulatory (Tregs) is beneficial to improve the remodeling of IA cases(37). During homeostasis and inflammation, circulating monocytes can infiltrate the vasculature, where they develop into macrophages and regulate immune responses(20). Subsequently, the polarization of macrophages regulates the formation and progression of intracranial aneurysms by releasing cytokines and modulating the inflammatory response of other immune cells, as well as releasing different cytokines to regulate the process of extracellular matrix remodeling. Increased phagocytic infiltration is responsible for the formation and progression of IA(38). Zhang et al. found that monocytes promote a higher inflammatory state in IA by causing a loss of balance of CD4(+) T cell subsets(39).

However, the limitations of this study should also be acknowledged. First of all, our data are obtained from public datasets, and the reproducibility needs to be further verified. Secondly, the sample size of this study may still be insufficient, and the key genes and immune cell infiltration of IA cannot be well identified. In addition, some of the inflammatory hub genes we identified have not been studied in IA and need further experimental verification..

Conclusion

Based on machine learning methods, we found that RTP4, CLEC7A, SOX11, and TLR4 may be the inflammatory hub genes of IA formation. Immune cell infiltration in IA patients was measured in detail. In addition, the correlation between RTP4, CLEC7A, SOX11, TLR4 and immune cells may play an important role in IA. These hub genes and immune cells may be potential targets for non-surgical treatment of IA patients.

Declarations

Conflict 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.

Author contribution

Jianping Chen designed experiments, analyzed and interprets data, and writes manuscripts. Penggao Dai, Qi Lin analyzed and interprets data. All authors participated in the drafting of this article, and reviewed and approved the final manuscript.

Data Availability Statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

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References

1. Macdonald RL, Schweizer TA (2017) Spontaneous subarachnoid haemorrhage. Lancet (London England) 389(10069):655–666
2. Tawk RG, Hasan TF, D'Souza CE, Peel JB, Freeman WD (2021) Diagnosis and Treatment of Unruptured Intracranial Aneurysms and Aneurysmal Subarachnoid Hemorrhage. Mayo Clinic

proceedings. ;96(7):1970–2000
3. Gruszka W, Zbroszczyk M, Komenda J, Gruszczyńska K, Baron J (2018) The role of inflammation and potential pharmacological therapy in intracranial aneurysms. Neurol Neurochir Pol 52(6):662–669
4. Suzuki H, Mikami T (2020) Inflammation promotes progression of thrombi in intracranial thrombotic aneurysms. 43:1565–15736
5. Frösen J, Cebral J, Robertson AM, Aoki T (2019) Flow-induced, inflammation-mediated arterial wall remodeling in the formation and progression of intracranial aneurysms. NeuroSurg Focus 47(1):E21
6. Giotta Lucifero A, Baldoncini M, Bruno N, Galzio R (2021) Shedding the Light on the Natural History of Intracranial Aneurysms:An Updated Overview. ; 57(8)
7. Muhammad S, Chaudhry SR, Dobreva G, Lawton MT, Niemelä M, Hänggi D (2021) Vascular Macrophages as Therapeutic Targets to Treat Intracranial Aneurysms. Front Immunol 12:630381
8. Fisher CL, Demel SL (2019) Nonsteroidal Anti-Inflammatory Drugs: A Potential Pharmacological Treatment for Intracranial Aneurysm. Cerebrovasc Dis extra 9(1):31–45
9. Weng JC, Wang J, Li H, Jiao YM, Fu WL, Huo R et al (2020) Aspirin and Growth of Small Unruptured Intracranial Aneurysm: Results of a Prospective Cohort Study. Stroke 51(10):3045–3054
10. Terceño M, Remollo S (2021) Effect of combined acetylsalicylic acid and statins treatment on intracranial aneurysm rupture. 16:e02471532
11. Kassam A, Kassam N (2020) Artificial intelligence in healthcare: A Canadian context. Healthc Manage forum 33(1):5–9
12. Yu G, Wang LG, Han Y, He QY (2012) clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS 16(5):284–287
13. Yu G, Wang LG, Yan GR, He QY (2015) DOSE: an R/Bioconductor package for disease ontology semantic and enrichment analysis. Bioinf (Oxford England) 31(4):608–609
14. Powers RK, Goodspeed A, Pielke-Lombardo H, Tan AC, Costello JC (2018) GSEA-InContext: identifying novel and common patterns in expression experiments. Bioinf (Oxford England) 34(13):i555–i64
15. Sanz H, Valim C, Vegas E, Oller JM, Reverter F (2018) SVM-RFE: selection and visualization of the most relevant features through non-linear kernels. BMC Bioinformatics 19(1):432
16. Newman AM, Liu CL, Green MR (2015) Robust enumeration of cell subsets from tissue expression profiles. 12:453–4575
17. Malhotra A, Wu X, Gandhi D (2021) Management of Unruptured Intracranial Aneurysms. Neuroimaging Clin N Am 31(2):139–146
18. Zhang M, Peng F, Tong X (2021) Associations between haemodynamics and wall enhancement of intracranial aneurysm. 6:467–4753
19. Liu Q, Zhang Y, Yang J, Yang Y, Li M, Chen S et al (2022) The Relationship of Morphological-Hemodynamic Characteristics, Inflammation, and Remodeling of Aneurysm Wall in Unruptured

Intracranial Aneurysms. 13:88–991

20. Wang J, Cao Y (2020) Characteristics of circulating monocytes at baseline and after activation in patients with intracranial aneurysm. Hum Immunol 81(1):41–47
21. Mitsui K, Ikedo T, Kamio Y, Furukawa H, Lawton MT, Hashimoto T, Hypertension (1979) 2020;75(2):468 – 76
22. Liu L, Zhang Q, Xiong XY, Gong QW, Liao MF, Yang QW (2018) TLR4 gene polymorphisms rs11536889 is associated with intracranial aneurysm susceptibility. J Clin neuroscience: official J Neurosurgical Soc Australasia 53:165–170
23. Zhao L, Moos MP, Gräbner R, Pédrono F, Fan J, Kaiser B et al (2004) The 5-lipoxygenase pathway promotes pathogenesis of hyperlipidemia-dependent aortic aneurysm. Nat Med 10(9):966–973
24. Li XG, Wang YB (2019) SRPK1 gene silencing promotes vascular smooth muscle cell proliferation and vascular remodeling via inhibition of the PI3K/Akt signaling pathway in a rat model of intracranial aneurysms. 25:233–2442
25. Korostynski M, Morga R, Piechota M, Hoinkis D, Golda S, Dziedzic T et al (2020) Inflammatory Responses Induced by the Rupture of Intracranial Aneurysms Are Modulated by miRNAs. 57:988–9962
26. He X, Ashbrook AW (2020) RTP4 inhibits IFN-I response and enhances experimental cerebral malaria and neuropathology. 117:19465–1947432
27. Wroblewska A, Dhainaut M, Ben-Zvi B, Rose SA, Park ES, Amir ED et al (2018) Protein Barcodes Enable High-Dimensional Single-Cell CRISPR Screens. Cell 175(4):1141–55e16
28. Tone K, Stappers MHT (2019) C-type lectin receptors of the Dectin-1 cluster: Physiological roles and involvement in disease. 49:2127–213312
29. Deerhake ME, Shinohara ML (2021) Emerging roles of Dectin-1 in noninfectious settings and in the CNS. Trends Immunol 42(10):891–903
30. Fan Q, Tao R, Zhang H, Xie H, Lu L, Wang T et al (2019) Dectin-1 Contributes to Myocardial Ischemia/Reperfusion Injury by Regulating Macrophage Polarization and Neutrophil Infiltration. Circulation 139(5):663–678
31. Korai M, Purcell J, Kamio Y, Mitsui K, Furukawa H, Yokosuka K, Hypertension et al (1979) 2021;77(6):2084-93
32. Ikedo T, Minami M, Kataoka H, Hayashi K, Nagata M, Fujikawa R et al (2017) Dipeptidyl Peptidase-4 Inhibitor Anagliptin Prevents Intracranial Aneurysm Growth by Suppressing Macrophage Infiltration and Activation.Journal of the American Heart Association. ; 6(6)
33. Lefebvre V, Dumitriu B, Penzo-Méndez A, Han Y, Pallavi B (2007) Control of cell fate and differentiation by Sry-related high-mobility-group box (Sox) transcription factors. Int J Biochem Cell Biol 39(12):2195–2214
34. Feng JS, Sun JD, Wang XD, Fu CH, Gan LL, Ma R (2019) MicroRNA-204-5p targets SOX11 to regulate the inflammatory response in spinal cord injury. Eur Rev Med Pharmacol Sci 23(10):4089–4096

35. Zhang HF, Liang GB, Zhao MG, Zhao GF, Luo YH (2018) Patients with intracranial aneurysms presented defects in regulatory T cells, which were associated with impairment in Tim-3 upregulation. Int Immunopharmacol 64:350–355
36. Zhao MG, Peng C, Li LM, Chen L, Zhang HF (2020) Circulating Treg cells from patients with cerebral aneurysms displayed deficiency in ICOS expression and function. 47:1923–193112
37. Sun X, Zheng X, Zhang X, Zhang Y, Luo G (2020) Exosomal microRNA-23b-3p from bone marrow mesenchymal stem cells maintains T helper/Treg balance by downregulating the PI3k/Akt/NF-κB signaling pathway in intracranial aneurysm. Brain Res Bull 165:305–315
38. Wang Y, Jin J (2019) [Roles of macrophages in formation and progression of intracranial aneurysms]. Zhejiang da xue xue bao Yi xue ban = Journal of Zhejiang University Medical sciences. 48:204–2132
39. Zhang HF, Zhao MG, Liang GB, Yu CY, He W, Li ZQ et al (2016) Dysregulation of CD4(+) T Cell Subsets in Intracranial Aneurysm. DNA Cell Biol 35(2):96–103

Figures

Figure 1

(A) Heatmap of differentially expressed inflammatory genes between IA and STA(superficial temporal arteries). (B) PPI network of inflammatory DEGs. (C) The top 30 inflammatory DEGs in the PPI network and their counts.

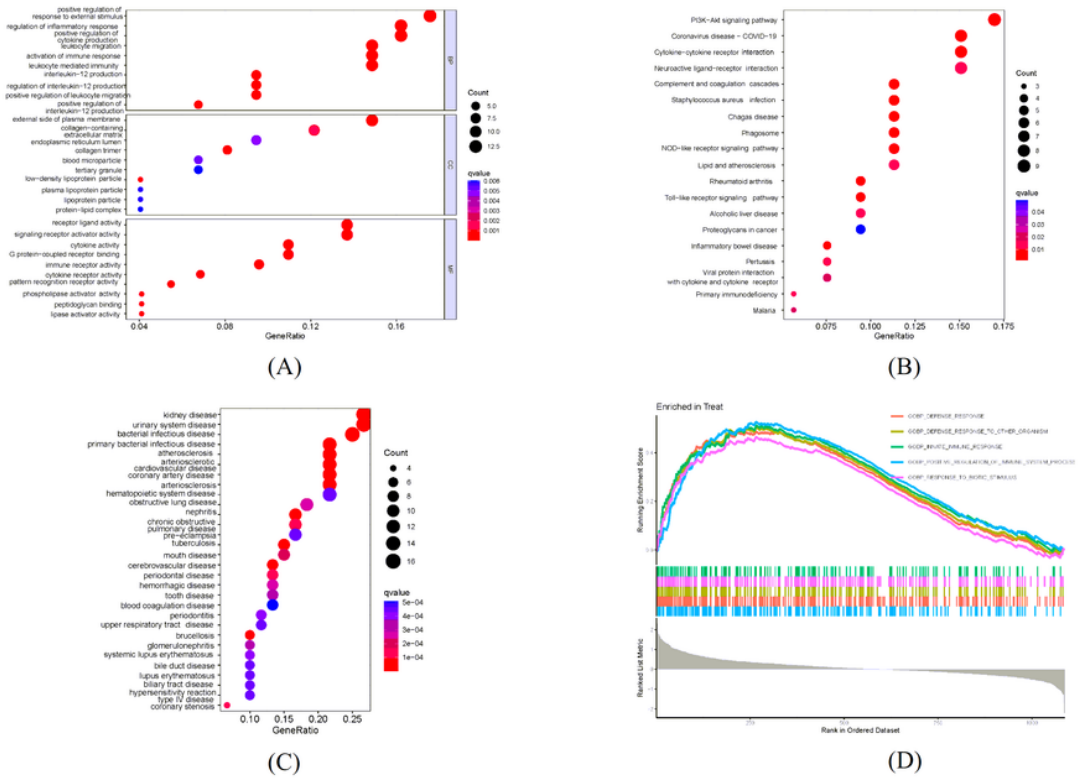

Figure 2

Enrichment analysis of DEGs (A) Gene ontology enrichment analysis. (B) KEGG enrichment analysis. (C) Disease ontology enrichment analysis. (D) Enrichment analyses via gene set enrichment analysis.

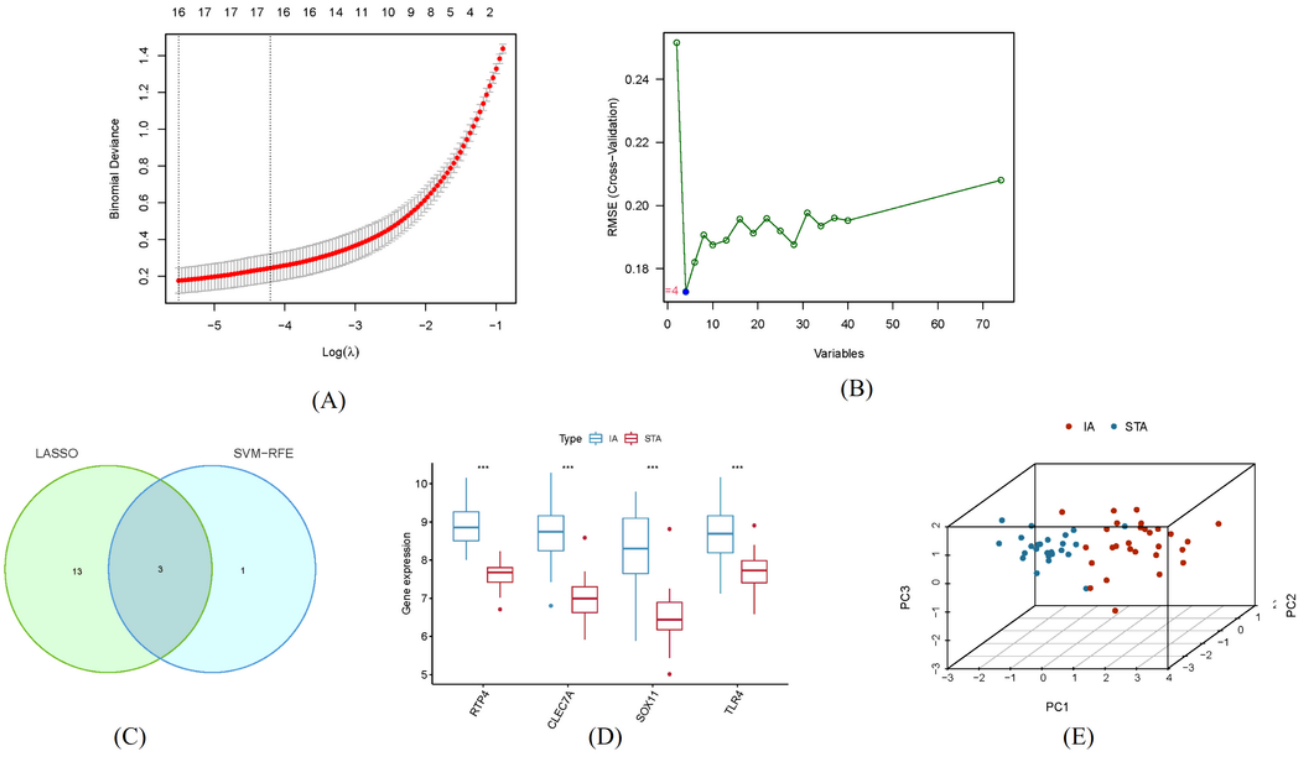

Figure 3

Identification of hub genes for IA based on machine learning algorithms. (A) hub genes by LASSO algorithm. (B) hub genes by SVM-RFE algorithm. (C) Venn plot of the overlapping genes identified by the LASSO algorithm and SVM-RFE algorithm. (D) the expression of hub genes between IA and STA(superficial temporal arteries). (E) PCA plot of AF and IA and STA.

Figure 4

Evaluation of the diagnostic effectiveness of the four hub genes. (A-D) ROC curve of CLEC7A, RTP4, SOX11 and TLR4

Figure 5

Evaluation and visualization of immune cells infiltration in IA and STA(superficial temporal arteries). (A) The proportion of infiltrating immune cells in

IA and STA. (B) The difference of 22 subpopulations of immune cells between IA and STA. (C) Correlation heatmap shows the correlation between 22 immune cell subpopulations.

Figure 6

Correlation between CLEC7A (A), RTP4 (B), SOX11 (C), TLR (D), and infiltrating immune cells in acute myocardial infarction.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterial1.txt
- SupplementaryMaterial2.pdf