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CALM3 affects the prognosis of leukemia and hemorrhoids

Jie He, MD[a], Zhijie Ni, MD[a], Zhongbo Li, MD[a,*]

Abstract

Leukemia is an abnormal proliferation of white blood cells in the bone marrow, resulting in a large accumulation of abnormal leukemia cells in the blood and bone marrow. Hemorrhoids are dilated and swollen veins in the rectum or anal area. However, the relationship between CALM3 and leukemia and hemorrhoids remains unclear. The hemorrhoids dataset GSE154650 and leukemia dataset GSE26294 were downloaded from GEO databases generated by GPL20301 and GPL571.The R package limma was used to screen differentially expressed genes (DEDs). Weighted gene co-expression network analysis (WGCNA) was performed. The construction and analysis of protein–protein interaction (PPI) network, functional enrichment analysis, Gene Set Enrichment Analysis (GSEA) and comparative toxicogenomics database (CTD) analysis were performed. TargetScan was used to screen miRNAs regulating central DEGs. It was verified by western blot basic cell assay. A total of 125 DEGs were co-identified. According to the GO analysis, they are mainly enriched in small molecule catabolic processes, skin development, and chemokine receptor binding. The KEGG analysis results show that the target cells are mainly enriched in the interaction of cytokines and cytokine receptors, as well as butyric acid metabolism. The GSEA analysis results indicate enrichment in small molecule catabolic processes, skin development, and chemokine receptor binding. Six core genes (CALM3, ACE2, PPARGC1A, XCR1, CFTR, PRKCA) were identified. We found that the core gene CALM3 is highly expressed in hemorrhoid samples, low in leukemia samples, and has low expression in normal samples, which may play a regulatory role in hemorrhoids and leukemia. Immunoinfiltration results showed a higher proportion of T_cells_CD4_memory_resting and a correlation with T_cells_CD8. WB experiment verified the result. CALM3 expression is low in leukemia, and the lower the expression is, the worse the prognosis is. CALM3 is highly expressed in hemorrhoids, and the higher the expression, the worse the prognosis.

Abbreviations: CTD = Comparative Toxicogenomics Database, DEGs = differential epigenetic genes, FC = fold change, FDR = false discovery rate, GEO = gene expression omnibus, GO = gene ontology, GSEA = gene set enrichment analysis, KEGG = Kyoto Encyclopedia of Gene and Genome, PPI = protein-protein interaction, STRING = Search Tool for the Retrieval of Interacting Genes, TOM = topological overlap matrix, WGCNA = weighted gene co-expression network analysis.

Keywords: CALM3, gene, hemorrhoids, Leukemia, molecular mechanism

1. Introduction

Leukemia is a malignant blood disease primarily characterized by abnormal proliferation of hematopoietic stem cells in the bone marrow, leading to the accumulation of abnormal leukemia cells in the blood and bone marrow. The exact causes of leukemia are often unclear, but some potential risk factors include radiation exposure, dyes, certain chemicals, Helicobacter pylori infection, and certain genetic factors.[1,2] Leukemia encompasses various subtypes, including acute lymphoblastic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, and chronic myeloid leukemia, affecting both the blood and bone marrow.[3,4] Patients with leukemia may experience symptoms such as fatigue, anemia, infections, lymph node enlargement, bleeding, and bone pain. Pathologically, leukemia is characterized by the excessive presence of abnormal leukemia cells in the bone marrow, which fail to perform normal hematopoietic functions. These abnormal cells can also enter the bloodstream and other tissues.[5] Leukemia is a severe form of cancer that, if left untreated, can lead to serious health problems and even life-threatening conditions.

Hemorrhoids, also known as piles, refer to the dilation and swelling of veins in the rectal or anal region. They can occur internally or externally.[6] Hemorrhoids are often associated with factors such as prolonged sitting, constipation, increased intra-abdominal pressure, and in some cases, genetic factors.[7] Hemorrhoids can be classified into internal hemorrhoids, located within the rectum, and external hemorrhoids, which

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

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occur near the external skin of the rectum. Patients with hemorrhoids may experience symptoms such as anal pain, itching, bleeding, constipation, or diarrhea. Internal hemorrhoids may lead to bleeding, while external hemorrhoids are typically accompanied by pain and swelling. Pathologically, hemorrhoids involve the dilation and congestion of blood vessels in the rectal or anal area, resulting in swelling.[8] Hemorrhoids are usually not life-threatening, but they can cause discomfort and pain. Severe cases of hemorrhoids may require treatment, including medication, surgery, or other interventions.[9]

Research indicates that both of these diseases may be associated with genetic factors, chromosomal abnormalities, gene fusion, and other factors. Therefore, in-depth research into their molecular mechanisms remains essential for understanding their pathogenesis and finding treatment approaches.

In recent years, bioinformatics techniques have been widely applied in various fields, including basic research, medical applications, drug development, and disease treatment. The continuous development and innovation of bioinformatics technology have driven advancements in the life sciences and provided critical support for addressing health and environmental challenges. CALM3, also known as Calmodulin 3, is a human gene that encodes a protein called Calmodulin 3. Calmodulin is a calcium-binding protein that plays a crucial role in various cellular processes, including signal transduction, muscle contraction, and enzyme regulation.[10] Several studies have started to utilize bioinformatics to explore potential therapeutic targets for diseases. For example, research by Lei[11] demonstrated the expression of CAMK1 in pancreatic cancer and its association with clinical pathological features. However, the relationship between CALM3 and leukemia or hemorrhoids is currently unclear.

Therefore, this study aims to employ bioinformatics techniques to uncover core genes that distinguish leukemia and hemorrhoids from normal tissues and conduct enrichment analysis and pathway analysis. Public datasets will be used to validate the significant roles of CALM3 in leukemia and hemorrhoids. Additionally, basic cellular experiments will be conducted to validate these findings.

2. Methods

2.1. Leukemia and Hemorrhoid datasets

In this study, the Hemorrhoid dataset GSE154650 and Leukemia dataset GSE26294 profiles were downloaded from the GEO database (http://www.ncbi.nlm.nih.gov/geo/), generated from GPL20301 and GPL571, respectively. GSE154650 comprised 20 hemorrhoid and 18 control samples, while GSE26294 included 8 leukemia and 8 normal samples. These datasets were utilized to identify the Differential Expressed Genes (DEGs) associated with leukemia and hemorrhoids.

2.2. Selection of differential expressed genes

The R package "limma" was employed for probe summarization and background correction of the matrices from GSE154650 and GSE26294. Benjamini-Hochberg correction was utilized to adjust the raw p-values. Fold Change (FC) was calculated with False Discovery Rate (FDR). The cutoff criteria for DEGs were set as P value < .05 and FC > 1.2. A volcano plot was created for visualization.

2.3. Functional enrichment analysis

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses are computational methods to assess gene functions and biological pathways. In this study, the selected DEG lists were input into the KEGG REST API (https://www.kegg.jp/kegg/rest/keggapi.html) to obtain the latest KEGG Pathway gene annotations, which were used as a background. Genes were mapped to the background set, and enrichment analysis was performed using the R package clusterProfiler (version 3.14.3) to obtain results on gene set enrichment. Additionally, GO annotations of genes from the org.Hs.e.g..db (version 3.1.0) package were used as background, with a minimum gene set size of 5 and a maximum gene set size of 5000. A P value of < .05 and an FDR of < 0.25 were considered statistically significant criteria. Furthermore, the Metascape database was used for comprehensive gene list annotation, functional enrichment analysis, and visualization export (http://metascape.org/gp/index.html).

2.4. Gene set enrichment analysis

For Gene Set Enrichment Analysis (GSEA), the GSEA software (version 3.0) was obtained from the GSEA website (DOI: 10.1073/pnas.0506580102, http://software.broadinstitute.org/gsea/index.jsp). Samples were divided into 2 groups based on disease and normal status, and the c2.cp.kegg.v7.4.symbols.gmt gene subset was downloaded from the Molecular Signatures Database (DOI: 10.1093/bioinformatics/btr260, http://www.gsea-msigdb.org/gsea/downloads.jsp) for pathway and molecular mechanism evaluation. Gene expression profiles and phenotype grouping were used to analyze gene expression patterns. The minimum gene set size was set to 5, the maximum gene set size to 5000, and 1000 permutations were performed. A P value of < .05 and an FDR of < 0.25 were considered statistically significant. GO and KEGG analyses were also conducted for the entire genome using GSEA.

2.5. Weighted gene co-expression network analysis

Initially, the Median Absolute Deviation of each gene was calculated from the gene expression profiles, and the lower 50% of genes with the smallest Median Absolute Deviation were removed. The R package Weighted Gene Co-expression Network Analysis (WGCNA)'s goodSamplesGenes method was used to eliminate outlier genes and samples. WGCNA was then employed to construct a scale-free co-expression network. Specifically, Pearson correlation matrices were computed for all gene pairs, and an adjacency matrix was constructed using a power function, a _ mn = | c _ mn | ^ β (C _ mn = Pearson correlation between gene m and gene n, a _ mn = adjacency between gene m and gene n). β is a soft thresholding parameter that can emphasize strong correlations between genes while attenuating weaker ones. After choosing a power of 7, the adjacency was transformed into a Topological overlap matrix (TOM), which measures the interconnectedness of genes in the network as defined by 1 – TOM. Gene modules were identified using average linkage hierarchical clustering based on TOM-based dissimilarity measure, with a minimum module size of 30. Modules were further merged if their dissimilarity was less than 0.25, and the gray module, representing genes that couldn't be assigned to any other module, was recognized.

2.6. Protein-protein interaction (PPI) network construction and analysis

The STRING database (http://string-db.org/) collects, scores, and integrates protein-protein interaction data from various sources and complements it with predicted interactions. In this study, the list of DEGs was input into the STRING database to construct a PPI network for predicting core genes

(confidence > 0.4). Cytoscape software was used for visualization and prediction of core genes in the PPI network formed by STRING. Initially, the PPI network was imported into Cytoscape, and the MCODE algorithm was used to identify the most relevant modules. Additionally, 3 algorithms (BottleNeck, Closeness, and Stress) were used to calculate the top 10 genes with the highest relevance, and their intersection was taken. Finally, core gene lists were exported after visualization.

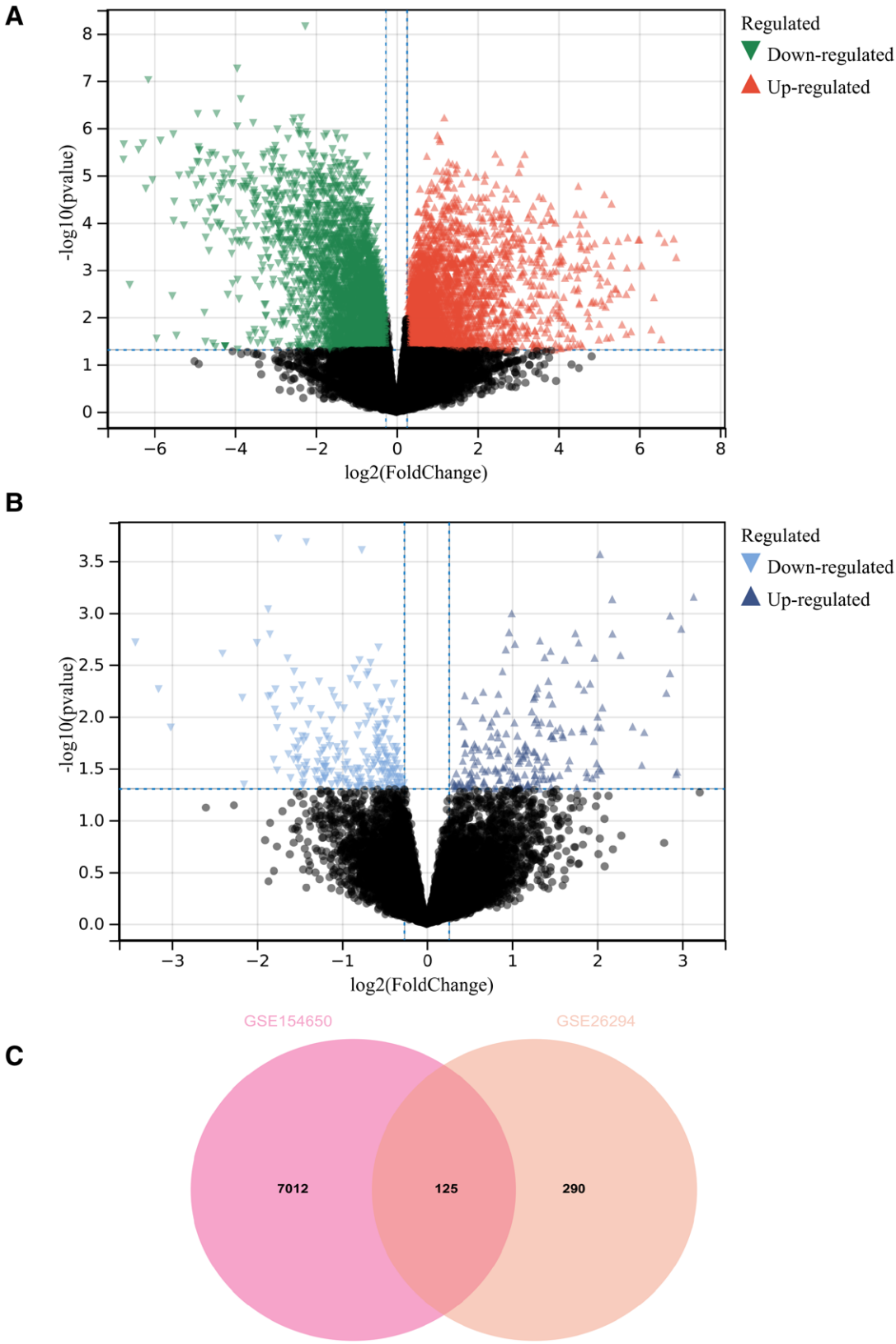

Figure 1. Differential gene analysis. (A) Differential genes of GSE154650. (B) Differential genes of GSE26294. (C)A total of 125 DEGs. DEGs = differential epigenetic genes.

2.7. Gene expression heatmaps

Heatmaps of core genes identified using the 3 algorithms in the PPI network were generated using the R package heatmap. The expression levels of core genes were visualized for leukemia vs. normal samples and hemorrhoids vs. normal samples.

2.8. Immune infiltration analysis

CIBERSORT (http://CIBERSORT.stanford.edu/) is a widely used method for calculating immune cell infiltration. The LM22 gene file defines 22 immune cell subtypes. Integrated bioinformatics methods were applied to analyze matrices from GSE154650 and GSE26294 using the CIBERSORT package. Linear Support Vector Regression was used to deconvolute the expression matrix of immune cell subtypes, and samples with sufficient confidence ($P < .05$) were selected as cutoff criteria.

2.9. CTD Analysis

The Comparative Toxicogenomics Database (CTD) integrates a vast amount of data on interactions between chemicals, genes, functional phenotypes, and diseases. Core genes were input into the CTD website to identify the most relevant diseases, and radar plots were generated for each gene to illustrate expression differences.

2.10. miRNA target prediction

TargetScan (www.targetscan.org) is an online database for predicting miRNA-target interactions. In this study, TargetScan was used to identify miRNAs that regulate the DEGs.

2.11. Western blotting

Western blotting, also known as immunoblotting, is a method to detect the expression of a certain protein in complex samples according to the specific binding of antigens and antibodies, and can qualitatively and semi-quantitatively analyze proteins. Total protein was extracted and the protein content was determined. After SDS-PAGE electrophoresis and membrane transfer, the protein samples were blocked with 5% skim milk for 1 h at room temperature, shaken with Tris Buffered Saline Tween at high speed on a shaker, washed for 5 minutes, and repeated 3 times. The primary antibody was added and incubated overnight at 4 °C, followed by TBST shaking 3 times (5 minutes each time) and TBST shaking 3 times (5 min each time). The results were analyzed after chemiluminescence development.

3. Results

3.1. Analysis of differential expressed genes

In this study, using predefined cutoff criteria, differential expressed genes (DEGs) were identified from the matrices of

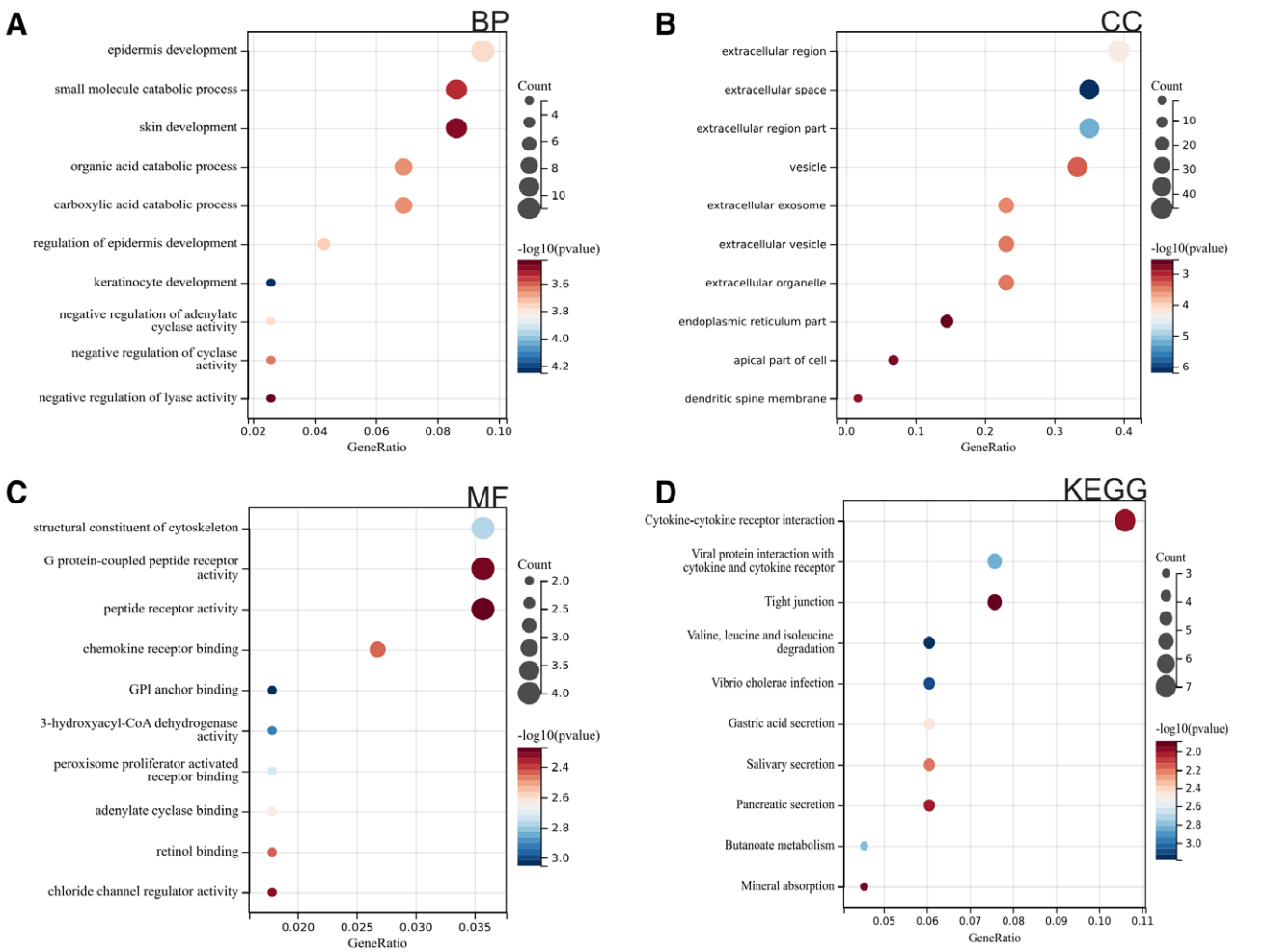

Figure 2. Functional enrichment analysis of DEGs. (A) Biological process analysis. (B) Cellular component analysis. (C) Molecular function analysis. (D) KEGG enrichment analysis. DEGs = differential epigenetic genes, KEGG = Kyoto Encyclopedia of Gene and Genome.

GSE154650 (Fig. 1A) and GSE26294 (Fig. 1B). A total of 125 DEGs were identified (Fig. 1C).

3.2. Functional enrichment analysis

3.2.1. DEGs. We performed GO and KEGG analyses on these DEGs. According to the GO analysis, they were mainly enriched in processes related to small molecule catabolic processes, skin development, and chemokine receptor binding (Fig. 2A–C).

KEGG analysis results showed that the target cells were primarily enriched in interactions between cytokines and cytokine receptors, and butyric acid metabolism (Fig. 2D).

3.2.2. GSEA. Additionally, we conducted GSEA enrichment analysis on the entire genome to identify potential enrichments in non-DEGs and validate the results of DEGs. The intersection of enrichment terms with GO and KEGG enrichment terms of DEGs is shown, primarily enriched in processes related to small molecule catabolic processes, skin development, and chemokine receptor binding (GSE154650, Fig. 3A–D; GSE26294, Fig. 3E–H).

3.3. Metascape enrichment analysis

In the Metascape enrichment analysis, GO enrichment terms included small molecule catabolic processes, epidermis development, and extracellular matrix organization (Fig. 4A). Enrichment networks colored by enrichment terms and p-values were also generated (Fig. 4B–E), visualizing the associations and confidence of each enrichment term.

3.4. WGCNA

The selection of the soft thresholding power is a crucial step in WGCNA analysis. The soft thresholding power for WGCNA analysis was set at 7 (Fig. 5A and B). This generated a hierarchical clustering tree of all genes, leading to the identification of important modules. The interactions between these modules

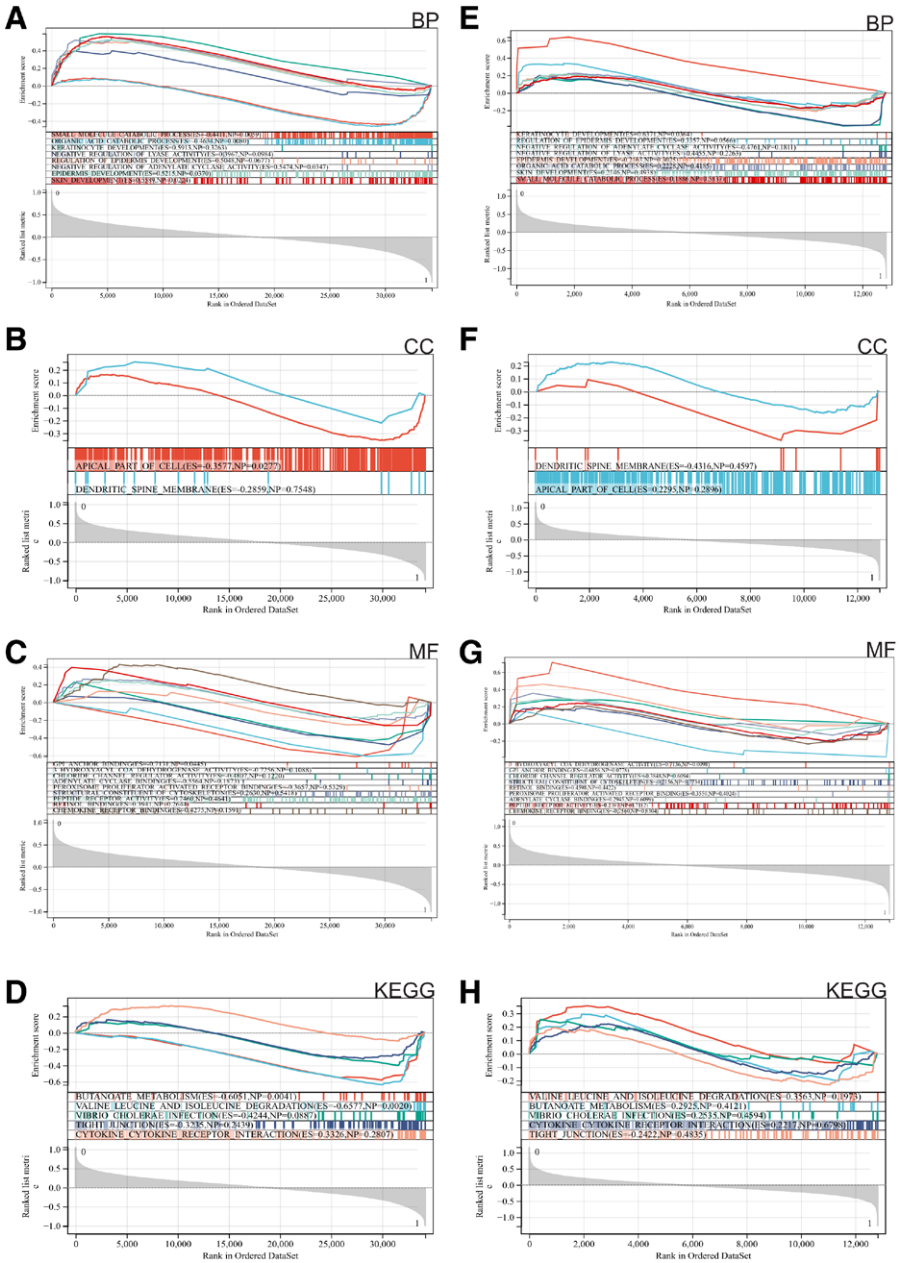

Figure 3. Gene set enrichment analysis. (A–D) GSE154650. (E–H) GSE26294.

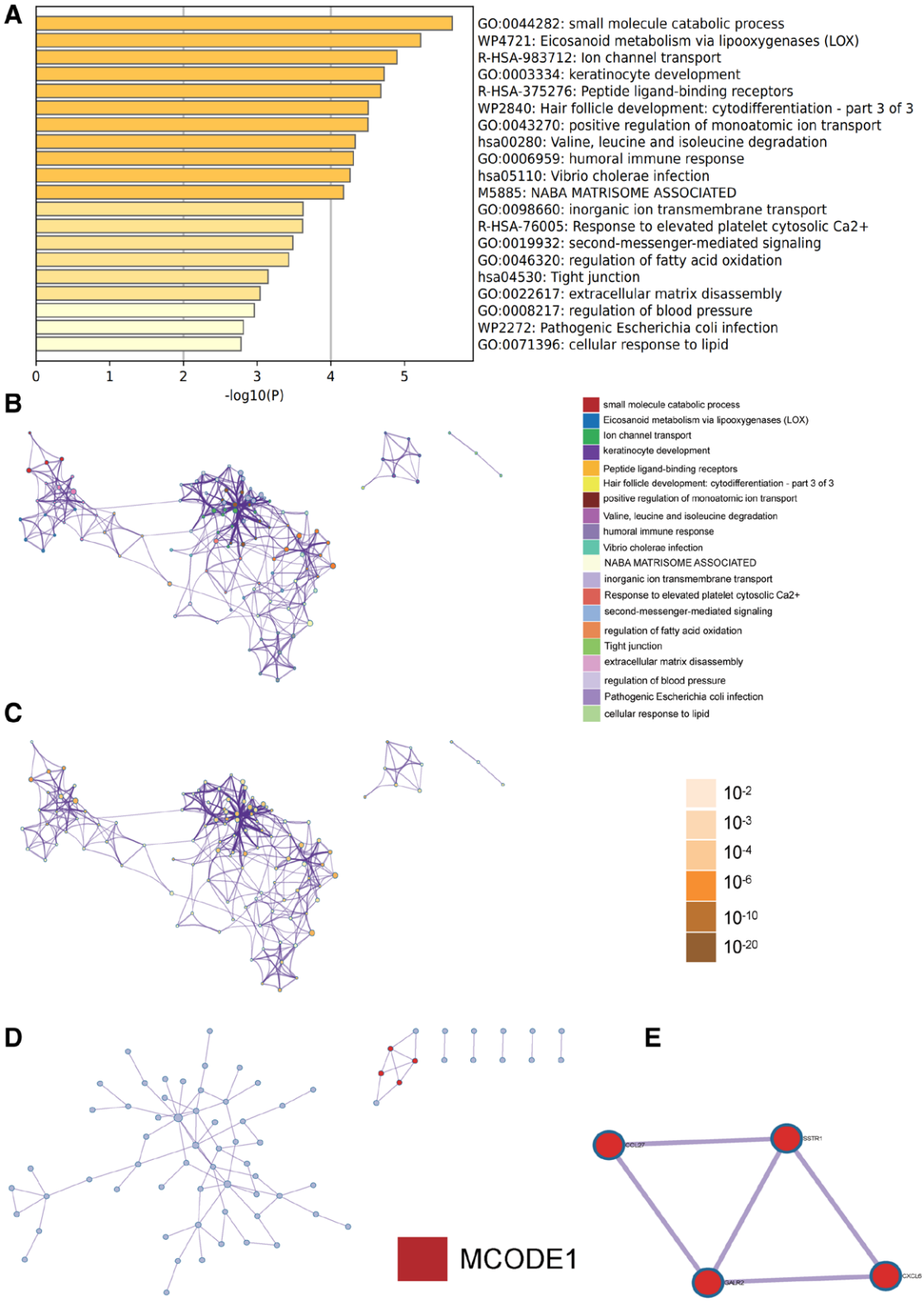

Figure 4. Metascape enrichment analysis. (A) Bar graph of enriched terms across input gene lists, colored by p-values. (B) Network of enriched terms: colored by cluster ID, where nodes that share the same cluster ID are typically close to each other. (C) colored by *P* value, where terms containing more genes tend to have a more significant *P* value. (D) Protein-protein interaction network. (E) MCODE components identified in the gene lists.

were analyzed (Fig. 5C and D), and a heatmap of module-trait correlations (Fig. 6A) and scatter plots showing the correlation between gene significance (GS) and module membership (MM) for relevant hub genes (Fig. 6B–E) were generated.

We calculated the module eigengene and gene expression correlation to obtain MM, and based on the cutoff criterion ($|MM| > 0.9$), 516 highly connected genes in clinically significant modules were identified as hub genes.

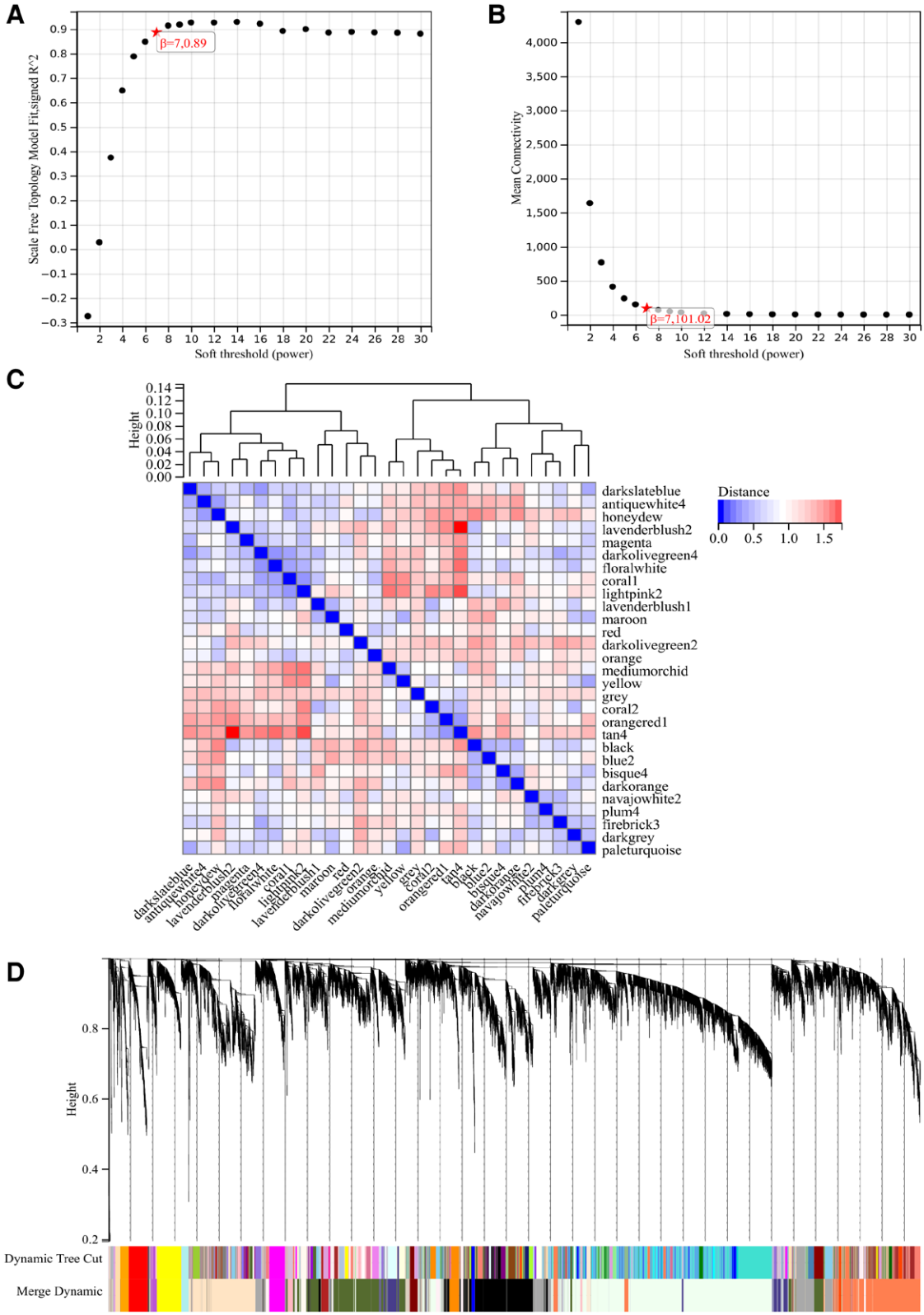

Figure 5. WGCNA. (A) β = 7,0.89. (B) β = 7101.02. (C, D) The hierarchical clustering tree of all genes was constructed, and important modules were generated. WGCNA = weighted gene co-expression network analysis.

3.5. Protein-protein interaction (PPI) network construction and analysis

The DEGs' PPI network was constructed using the STRING online database and analyzed with Cytoscape software (Fig. 7A). Central genes were identified using 3 different algorithms (Fig. 7B–D), and the Venn diagram illustrated the intersection (Fig. 7E), revealing 6 core genes (CALM3, ACE2, PPARGC1A, XCR1, CFTR, PRKCA).

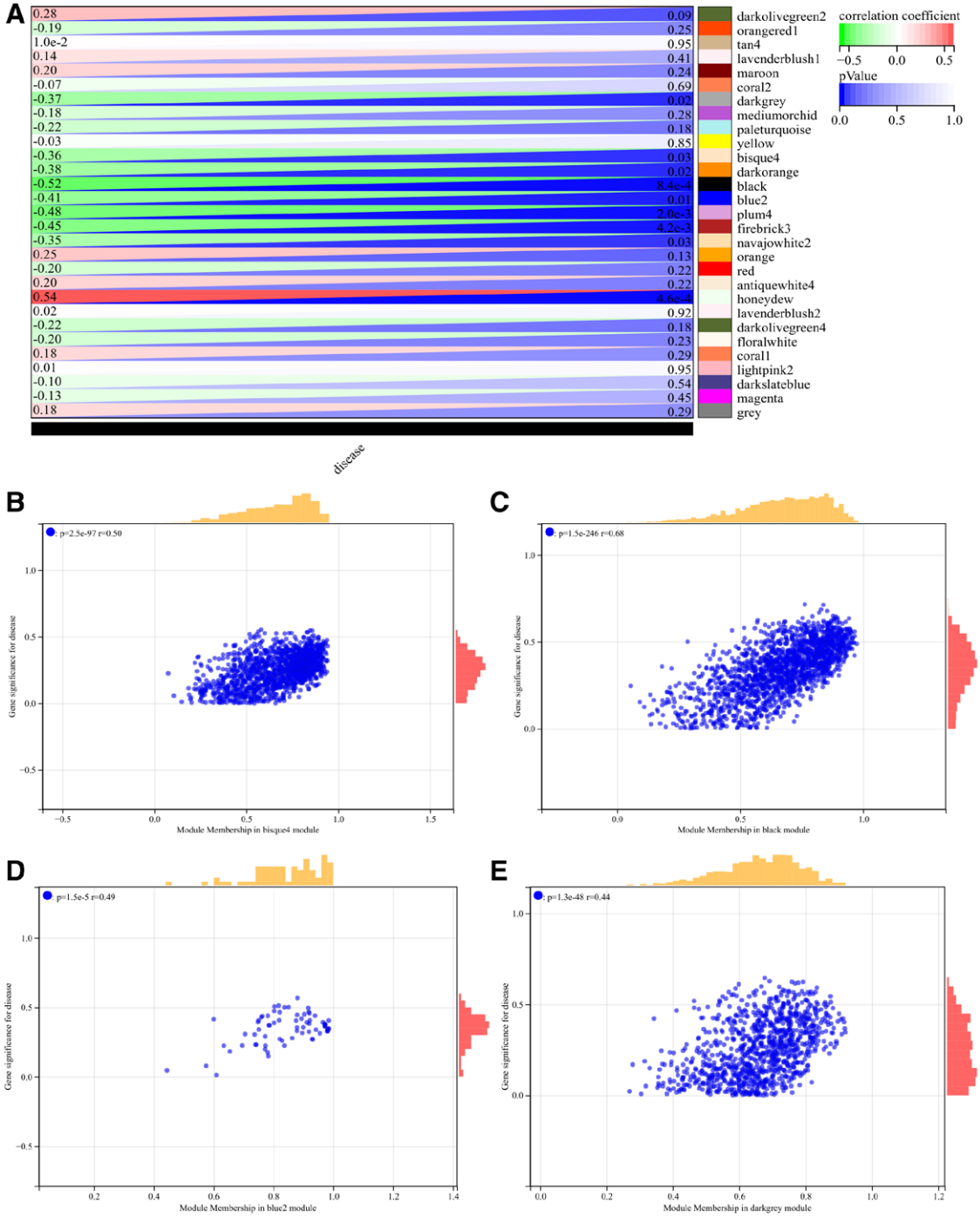

Figure 6. (A) The heat map of correlation between modules and phenotypes. (B–E) The scatter map of correlation between GS and MM of related hub genes.

Additionally, we used the Metascape website to output a protein-protein interaction network and identified core modules, validating the PPI network results from STRING. Genes CCL27, SSTR1, CXCL6, and GALR2 were identified as core genes.

3.6. Gene expression heatmaps

We visualized the expression of core genes in samples using heatmaps for GSE154650 (Fig. 8A) and GSE26294 (Fig. 8B). We observed that the core gene CALM3 was highly expressed in hemorrhoid samples and lowly expressed in control samples. In leukemia samples, CALM3 showed low expression, while in normal samples, it exhibited high expression, indicating a potential regulatory role in hemorrhoids and leukemia.

3.7. CTD analysis

In this study, the core gene list was input into the CTD website to search for diseases related to the core genes, enhancing the understanding of gene-disease associations. Genes (CALM3) were found to be associated with pain, fever, constipation, anemia, leukemia, early myeloid cells, acute lymph node metastasis, inflammation, and proliferation (Fig. 8C).

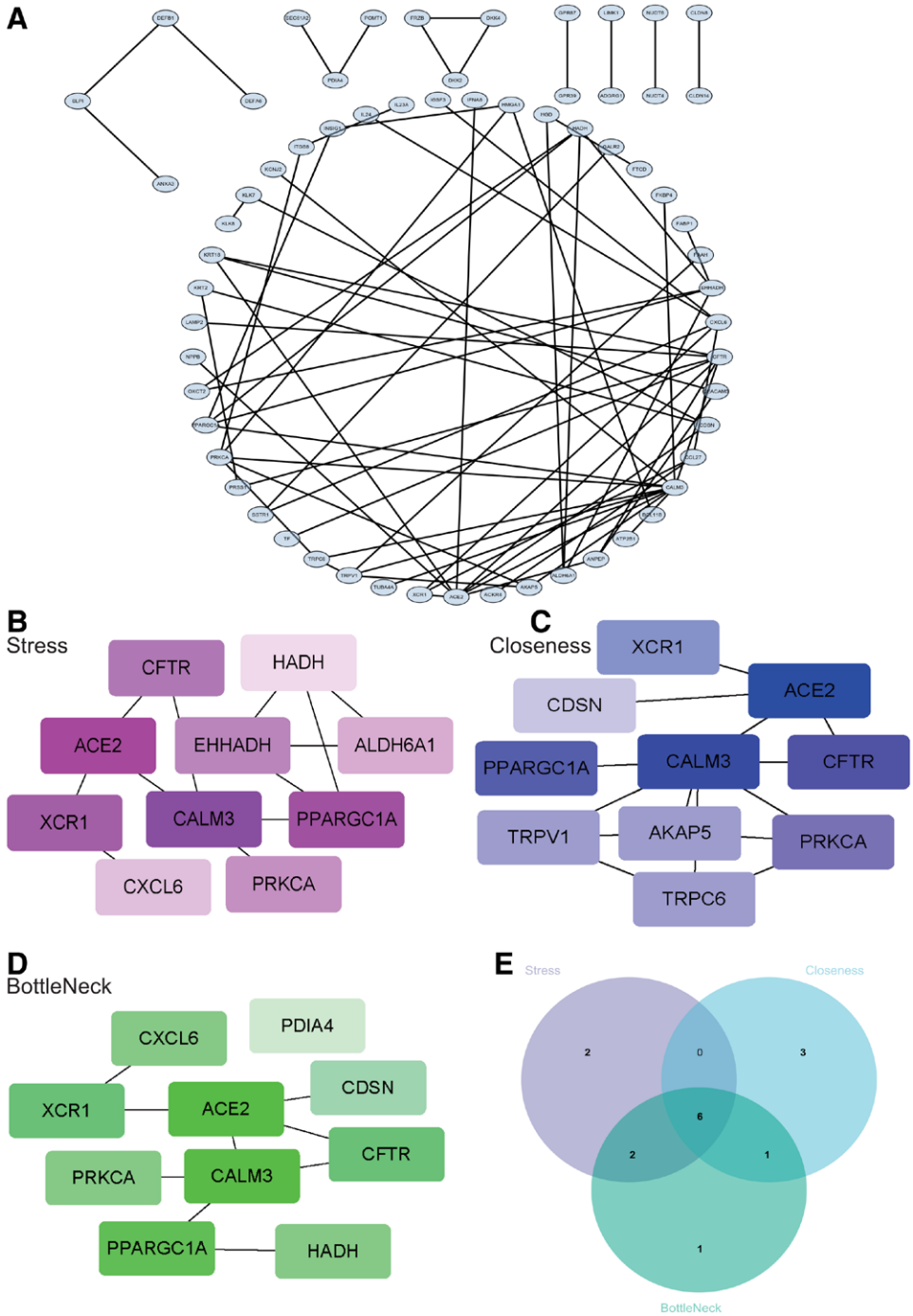

Figure 7. Construction and analysis of protein-protein interaction (PPI) networks. (A) PPI network of DEGs. (B) BottleNeck was used to identify the central gene. (C) Closeness was used to identify the central gene. (D) Stress was used to identify the central gene. (E) Merge using Venn diagrams. DEGs = differential epigenetic genes.

3.8. Immune infiltration analysis

We used the CIBERSORT package to analyze matrices from GSE154650 and GSE26294. For GSE154650, at a 95% confidence level, the proportions of immune cells in the full gene expression matrix were obtained (Fig. 9A), along with a heatmap of immune cell expression in the dataset (Fig. 9B). Co-expression correlation analysis of infiltrating immune cells generated a pattern of co-expression between immune cell components (Fig. 9C), indicating a high proportion of T_cells_CD4_memory_resting and a high correlation with T_cells_CD8.

For GSE26294, the proportion of immune cells in the whole gene expression matrix (Fig. 10A) and the heat map of immune cell expression in the dataset (Fig. 10B) were obtained with 95% confidence, and the co-expression correlation analysis of infiltrating immune cells was also performed. The co-expression pattern of immune cell components was obtained (Fig. 10C). The results showed that the proportion of Macrophages_M0 was high, and it was highly correlated with Macrophages_M2.

3.9. Western blotting experimental results

In comparison to the control group, CALM3, GPCR, Gq, PLCβ, IP3R, ASPH, RYR, TRDN, CAMK, TFEB, NFAT genes are upregulated in leukemia, in addition to IL-18 and C-Myc. When

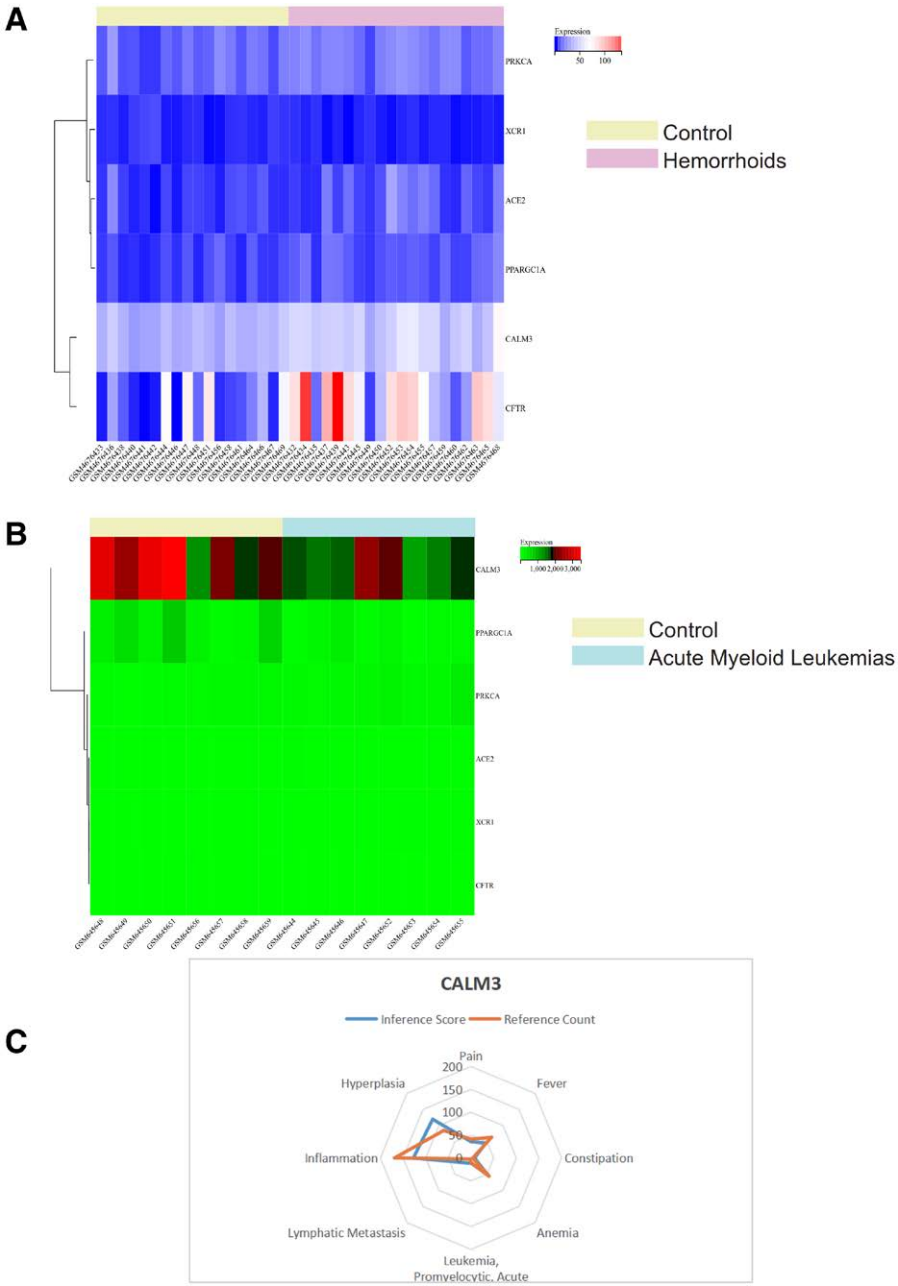

Figure 8. Heat map of gene expression. (A) GSE154650 core gene expression heat map. (B) GSE26294 core gene expression heat map. (C) CTD analysis. The core genes (CALM3). CTD = Comparative Toxicogenomics Database.

compared to the leukemia group, all genes are upregulated in the Leukemia_OE group and downregulated in the Leukemia_KO group (Fig. 11).

Similarly, when compared to the control group, CALM3, GPCR, Gq, PLCβ, IP3R, ASPH, RYR, TRDN, CAMK, TFEB, NFAT, IL-18, and C-Myc are upregulated in hemorrhoids. In contrast, when compared to the hemorrhoids group, all genes are upregulated in the Hemorrhoids_OE group and downregulated in the Hemorrhoids_KO group (Fig. 12).

4. Discussion

Leukemia is a malignant tumor of the blood system, and its primary danger lies in its interference with the production and function of normal blood cells, leading to excessive proliferation of leukemia cells in the bone marrow.[12,13] Patients with leukemia may experience symptoms such as anemia, bleeding tendencies, and susceptibility to infections, all of which are related to abnormalities in blood cells. Leukemia can rapidly spread to other tissues and organs, posing a serious threat to the body, and in severe cases, it can be life-threatening. Hemorrhoids, on the other hand, are a common problem in the rectal or anal area, mainly causing pain, discomfort, and bleeding in the anus or rectum. Hemorrhoids can lead to swelling and inflammation of the tissues around the anus and may affect bowel movements and quality of life when severe. While hemorrhoids are typically not life-threatening, they can significantly impact a patient's comfort and daily life.[14,15] The results of this study indicate that the expression levels of CALM3 are related to the prognosis in both leukemia and hemorrhoids. CALM3 is underexpressed in leukemia, with lower levels associated with worse prognosis, while it is overexpressed in hemorrhoids, with higher levels

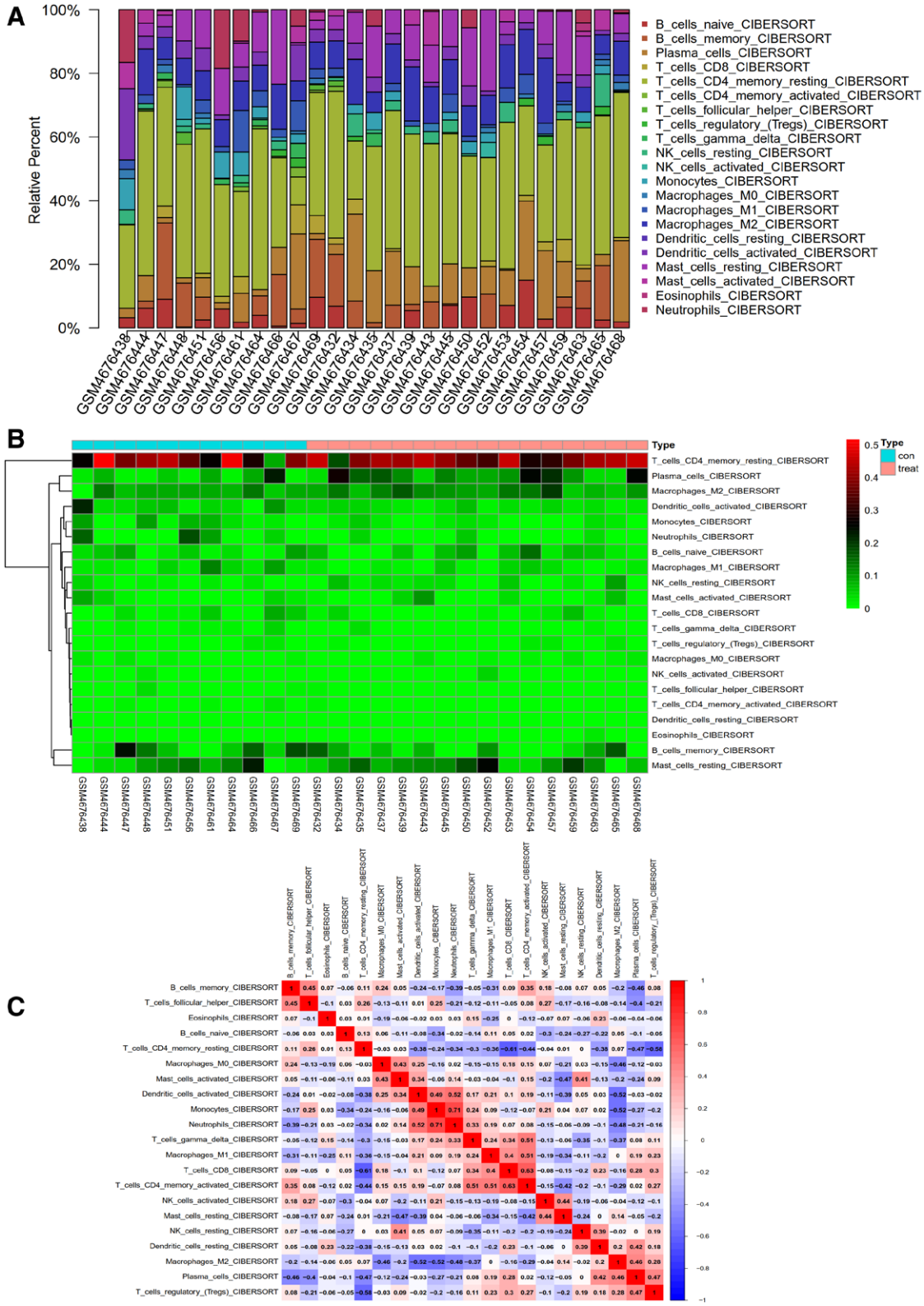

Figure 9. Immune infiltration. GSE154650. (A) The full gene expression matrix results in the proportion of immune cells. (B) Heat map of immune cell expression. (C) Co-expression patterns among immune cell components.

associated with poorer outcomes. This provides new directions for future disease management and treatment[16,17]

CALM3 is a gene that encodes the calmodulin protein, which plays a crucial regulatory role in intracellular calcium signaling processes[18] Calmodulin is a highly conserved protein found in all eukaryotes, including mammals. It regulates many different biological processes inside cells and is essential for maintaining the balance of calcium ions within cells.[19] Calmodulin has a unique structure that includes 4 EF-hand structural domains, which are capable of binding to calcium ions.[20] Each EF-hand domain can

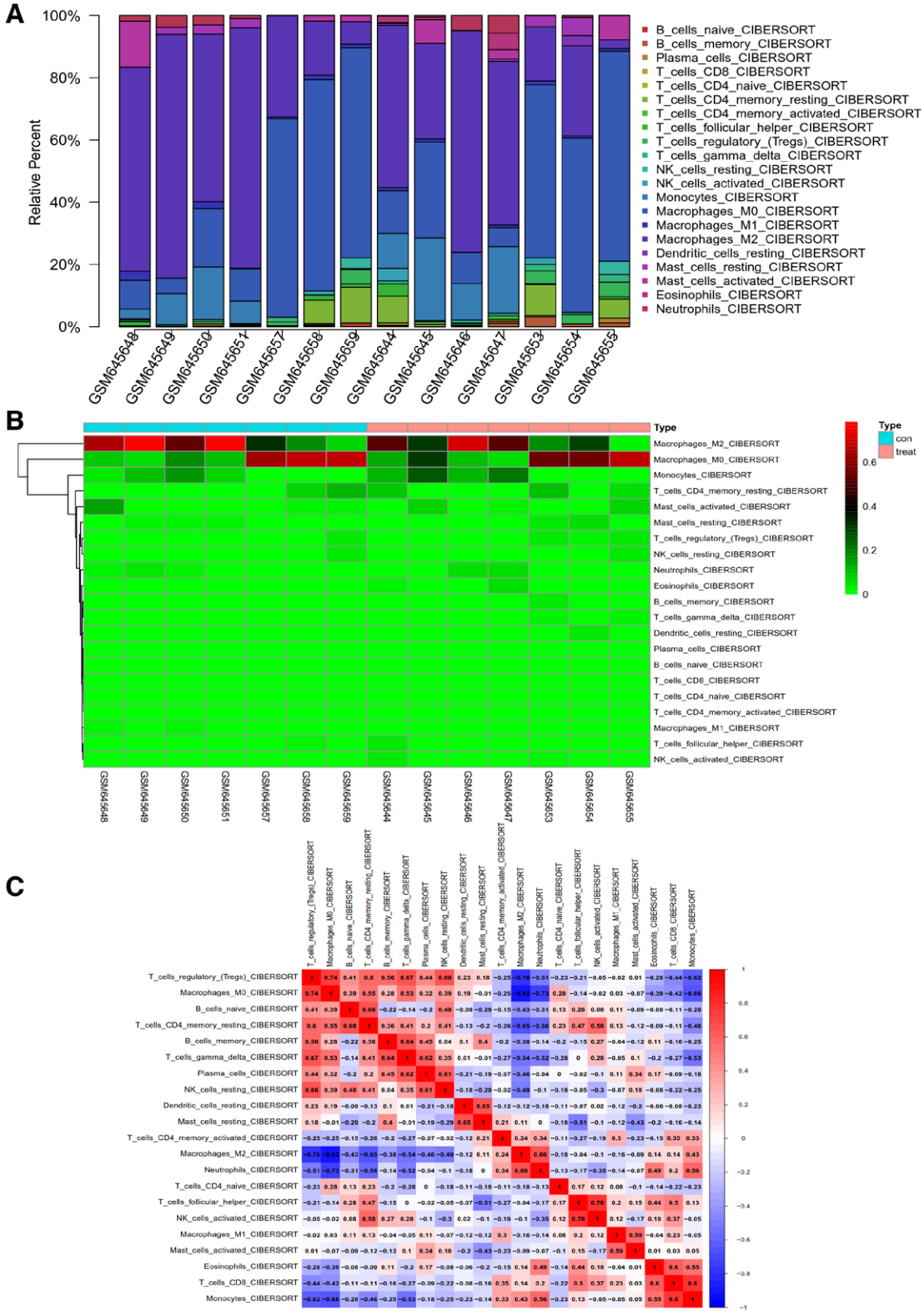

Figure 10. Immune infiltration. GSE26294. (A) The full gene expression matrix results in the proportion of immune cells. (B) Heat map of immune cell expression. (C) Co-expression patterns among immune cell components.

bind to one calcium ion, leading to conformational changes in calmodulin. After calcium binding, calmodulin can interact with a variety of proteins, including kinases, phosphatases, ion channels, and other regulatory molecules. These interactions can modulate the activities of these proteins, thereby affecting various biological processes within cells. CALM3's primary role in cells is its involvement in calcium signal transduction. When intracellular calcium ion levels increase, calmodulin binds to calcium ions and, through interactions with other proteins, transmits signals to regulate a wide range of biological processes.[21,22]

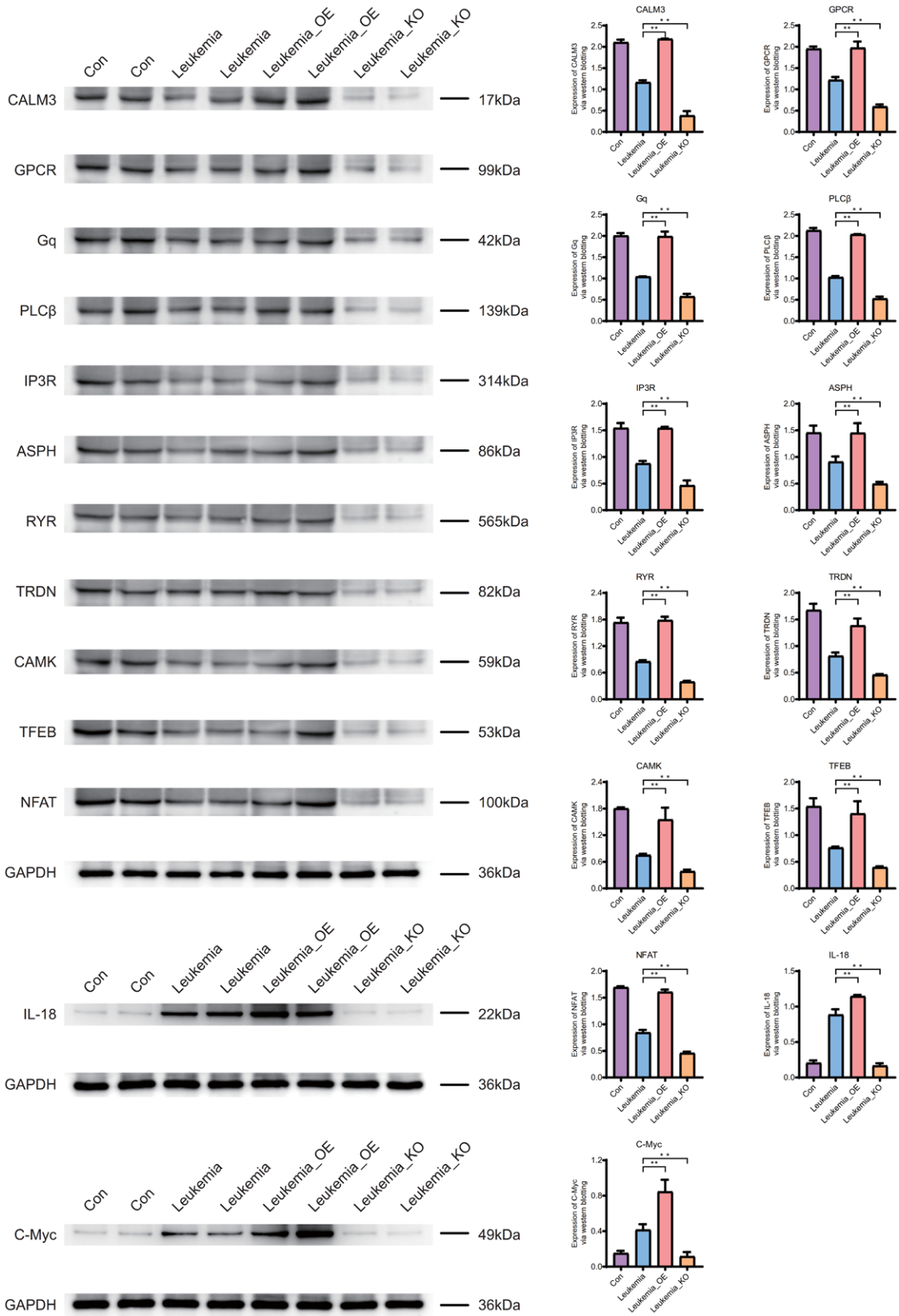

Figure 11. Results of Western blotting test for leukemia.

CALM3 is located in the human genome, usually on chromosome 19, and is a member of the calmodulin family. It plays an important role in maintaining cell growth and development, and maintaining muscle contraction and relaxation.[23] Calmodulin-3, encoded by CALM3, assists in muscle-cell contraction and relaxation by regulating calcium-dependent muscle protein kinases and other proteins. CALM3 may interact with some proteins that regulate apoptosis and affect cell survival

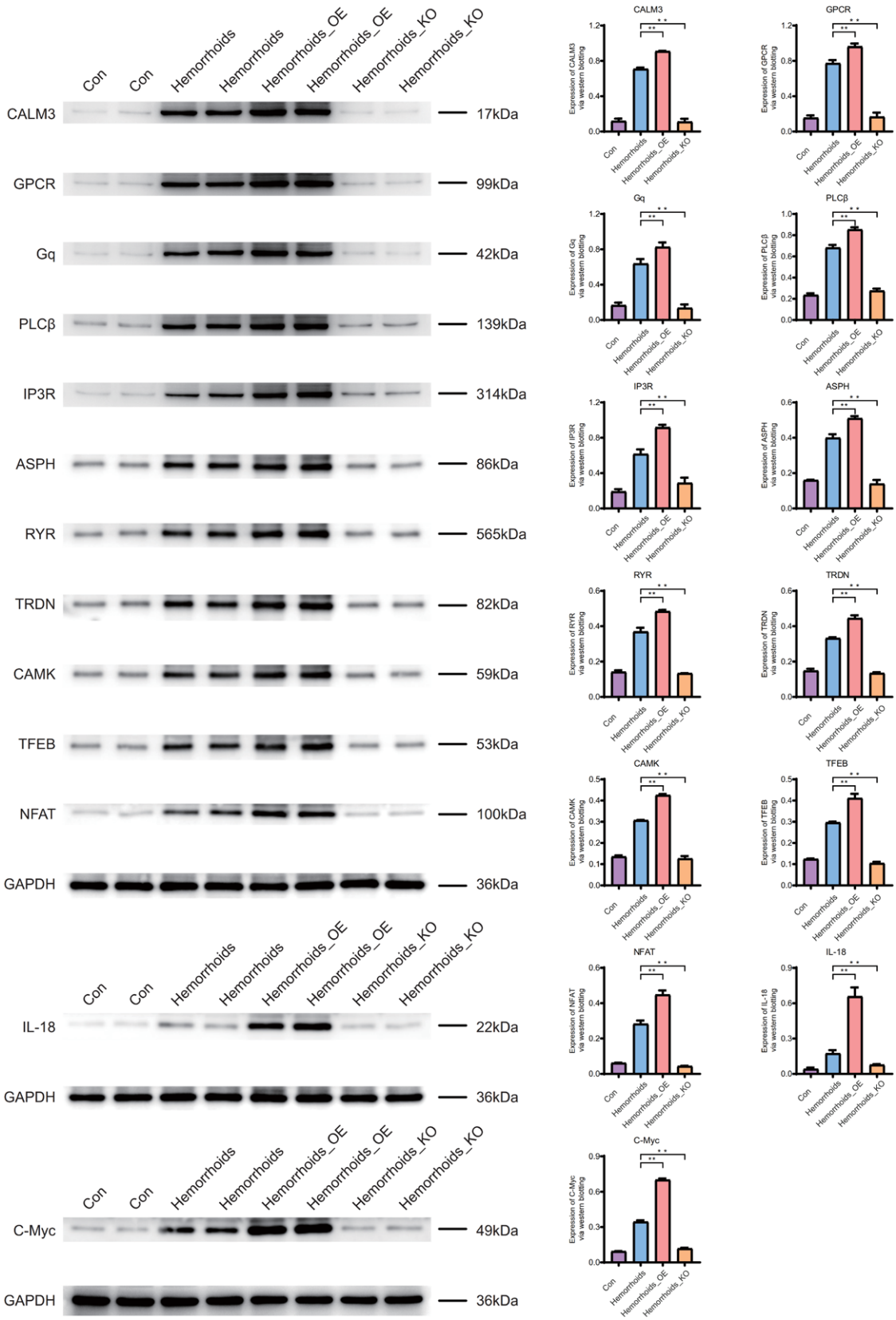

Figure 12. Results of Western blotting test for hemorrhoids.

and death decisions. CALM3 may have an effect on nerve conduction in neurons. By affecting calcium signaling, it plays a role in synaptic transmission and affects the transfer of information between neurons. CALM3 may interact with proteins involved in cell division and growth, thereby having an impact on the regulation of the cell cycle[24–26]

Aberrant expression or mutations of CALM3 may be associated with various diseases, including neurological disorders,

cardiovascular diseases, and cancers.[27,28] Some subtypes of leukemia may be related to calcium signaling, and as CALM3 functions as a calmodulin involved in intracellular calcium signaling, its abnormal expression may impact the normal functioning of these signaling pathways. In some leukemia studies, abnormal expression of CALM3 may be associated with abnormal proliferation and differentiation of leukemia cells, and the function of CALM3 is also related to gene transcription and cell proliferation. Studies such as the one by Sunitha[29] have shown that ARRB1, FLNA, CALM3, and HTT genes are commonly expressed in oral cancer and are involved in carcinogenic pathways. Among them, CALM3 gene binding energy is the lowest, making it easy to bind to target ligands, and it exhibits high affinity binding, suggesting that CALM3 and HTT are promising targets for oral cancer treatment. In leukemia patients, platelet counts may be affected; for example, acute lymphocytic leukemia and acute myeloid leukemia may lead to decreased platelet counts. In some cases, leukemia treatment itself may also have a negative impact on platelets, as chemotherapy drugs can disrupt the production of normal blood cells in the bone marrow. CALM3, to some extent, can regulate platelet levels. Research by Balkenhol et al[30] found that among common proteins, TLN1, CALM3, PRKCB, APP, SOD2, and TIMP1 have higher levels in humans, while RASGRP2, ITGB2, MYL9, EIF4EBP1, ADAM17, ARRB2, CD9, and ZYX have higher levels in mice. The hub kinase SRC exhibits different regulatory effects at the mRNA and protein levels, as well as on the ADP receptor P2RY12. CALM3, as a gene encoding a protein, can bind to various enzymes and co-regulate different physiological processes. Low CALM3 levels are unfavorable for leukemia recovery, pushing it in the opposite direction.

The pathogenesis of hemorrhoids may involve various molecular and cellular processes, including vasodilation and inflammatory responses, some of which may be related to CALM3 or calcium signaling.[31,32] Munk's research[33] suggests that the regulation of CaM gene expression is achieved through various regulatory elements present in 3 genes, including different promoter/insulator elements and 3' and 5' non-coding regions with varying lengths and sequences, as well as epigenetic factors and miRNA regulation. CALM3, expected to produce relatively stable mRNA, may be essential in situations of low or transient transcription, such as during sperm development, and these genes affect the function of the central nervous system and other organs. The nervous system plays a crucial role in perceiving and transmitting the pain caused by hemorrhoids. When hemorrhoid tissues are stimulated or damaged, pain signals are transmitted through neurons to the brain, leading to the sensation of pain. CALM3 binds to calcium ions, and subsequently, it activates or inhibits proteins associated with neuronal excitability, such as ion channels and protein kinases, participating in the regulation of the nervous system. Additionally, the inflammatory response is also a significant part of the gene's regulatory involvement. Research has shown that CALM3 can serve as a regulatory gene for the intestinal microbiome to alleviate ulcerative colitis.[34] CALM3 can affect the nuclear factor κB (NF-κB) signaling pathway, a major pathway involved in inflammation regulation. In an inactive state, NF-κB protein is bound to IκB (NF-κB inhibitory protein) and is in an inactive state. When cells are stimulated by inflammation, calcium ion levels rise. After CALM3 binds to calcium ions, it can associate with IκB, leading to the degradation of IκB, thereby releasing NF-κB and allowing it to enter the cell nucleus, subsequently activating the transcription of inflammation-related genes.[35] Hemorrhoids, in many cases, are aggravated by inflammation[36] and when CALM3 is overexpressed in hemorrhoids, the inflammatory response becomes more intense, leading to poor prognosis.

CALM3 is involved in multiple signaling pathways within cells, including kinase signaling pathways, regulation of ion channels, and regulation of gene expression. Its role is to modulate the activity of these pathways, thereby influencing cellular responses and adaptability. While leukemia and hemorrhoids are 2 different diseases, both are regulated by CALM3 and exhibit opposite states. CALM3 is underexpressed in leukemia, with lower levels associated with worse prognosis, while it is overexpressed in hemorrhoids, with higher levels associated with poorer outcomes. Future research directions mainly focus on the effect of CALM3 expression level on leukemia and hemorrhoids patients to improve medical care, disease treatment, and quality of life. We will continue to explore how to use genomics, transcriptomics, proteomics and other high-throughput technologies to better understand the biological characteristics of individuals, so as to develop more precise and personalized treatment plans. The CALM3 gene has a variety of potential roles in biological processes, which mainly involve its calcium binding and signaling properties. Calmodulin-3 in CALM3 can affect gene expression in some cells. It may help regulate the activity of specific genes by participating in transcriptional regulation together with calmodulin-related proteins.

5. Conclusion

In summary, CALM3 likely plays an important role in the cell proliferation processes of leukemia, regulation of platelet levels, and other aspects. It is characterized by low expression in leukemia, associated with worse prognosis, and high expression in hemorrhoids, associated with poorer outcomes.

Author contributions

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