

# Diagnostic biomarkers for invasive aspergillosis utilizing weighted gene co-expression network analysis

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## Research

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# Abstract

**Background:** Invasive aspergillosis (IA) has a significant mortality in immunocompromised patients. In recent years, with more aggressive immunosuppressed therapies, the incidence of IA was increasing. However, diagnostic biomarkers with high sensitivity and specificity remain rare. To get new diagnostic biomarkers, microarray dataset GSE78000 was analyzed.

**Methods:** Weighted gene co-expression network analysis (WGCNA) was used to identify hub genes. Roc curves were employed for investigating diagnostic biomarkers for IA.

**Results:** Hub genes were *TLR4*, *TP53I3/PIG3*, *TMTC1*, *ITGAM*, *CYSTM1*, *FAR1*, *GAS7* and *MKNK1*. However, after we compared gene expression of hematological patients suffering from IA with non-IA patients, only *TLR4*, *TP53I3/PIG3* and *TMTC1* were significantly high expression in IA patients. At the optimal cut-off value, *TLR4* can diagnose patients with IA with 78.3% sensitivity and 72.7% specificity. *TP53I3/PIG3* can diagnose patients with IA with 91.3% sensitivity and 54.5% specificity. *TMTC1* can diagnose patients with IA with 78.3% sensitivity and 81.8% specificity. In addition, the data of hematological patients suffering from *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E.coli*) infections were also analyzed. The results showed that *TLR4* and *TP53I3/PIG3* were also significantly high expression in *S. aureus* and *E.coli* infections, while only *TP53I3/PIG3* was obviously higher expression in patients with bacterial infections compared with IA. As for *TMTC1*, we cannot annotate the gene from the microarray data.

**Conclusions:** our results suggested that *TLR4*, *TP53I3/PIG3* and *TMTC1* might be used for the diagnosis of IA, and *TP53I3/PIG3* can also be used to discriminate hematological aspergillosis and bacterial infections.

## Background

Immunocompromised patients with hematologic malignancies (HM), hematopoietic stem cell transplant (HSCT), solid organ transplantation and other immunosuppressed therapies are susceptible to IA[1, 2]. Worldwide, IA is mainly caused by *Aspergillus fumigatus* (*A. fumigatus*) [3] with high mortality and morbidity especially in patients with HM and HSCT [4–6]. Although healthy people inhale conidia of *Aspergillus* daily, we don't show illness. Healthy people can clear *Aspergillus* via their immune system, primarily including alveolar macrophages, neutrophils, monocytes, and natural killer cells [7–9]. While, immunocompromised patients cannot clear *Aspergillus* spores in time because of the deficiency of local and systemic immune defences. As a result, *Aspergillus* can invade the lung and disseminate to other organs and tissues through hematogenous spread, for example the brain [10, 11].

Given the serious harm of IA, the early diagnosis and therapy are crucial for survival rates of IA. However, the early symptoms and signs of IA are lack of specificity [12]. In addition, because the conventional methods for the diagnosis of IA have low sensitivity and specificity, early diagnosis of IA is extremely difficult. Therefore, new diagnostic biomarkers to detect IA are urgent.

To address this challenge, we analyzed the expression profile of 23 samples of hematological patients with IA, 11 samples of hematological patients with non-IA and 9 normal samples via bioinformatics. The gene expression data were uploaded by Andreas Dix et al [13]. The data were downloaded from the GEO database and reanalyzed by bioinformatics. We identified a total of 2303 differentially expressed genes (DEGs) between hematological patients with IA and normal samples. These DEGs were analyzed by gene ontology (GO) analysis, Kyoto encyclopedia of genes and genomes (KEGG) analysis, gene set enrichment analysis (GSEA) and WGCNA. The results of WGCNA showed that 8 co-expression modules were constructed. A black module was founded significantly correlated with IA. Hub genes in black module were *TLR4*, *TP53I3/PIG3*, *TMTC1*, *ITGAM*, *CYSTM1*, *FAR1*, *GAS7* and *MKNK1*. Then, diagnostic biomarkers were identified by comparing the gene expression of hematological patients suffering from IA with non-IA. Then, the data of hematological patients with bacterial infections (the data was uploaded by SH Ahn et al [14]) was also analyzed to validate the gene expression of diagnostic biomarkers. Finally, we identified *TLR4*, *TP53I3/PIG3* and *TMTC1*, which were high sensitivity and specificity, may be used as new diagnostic biomarkers for IA, and *TP53I3/PIG3* may also be used to discriminate hematological aspergillosis and bacterial infections.

## Methods

### GEO dataset

Gene expression data of GSE78000 [13] and GSE33341 [14] were downloaded from the National Center for Biotechnology Information (NCBI) GEO database. In GSE78000 [13], there are 43 samples of expression profile including 23 hematological patients with IA, 11 hematological patients with non-IA and 9 normal samples obtained from whole blood. In GSE33341 [14], there are 94 samples including 43 hematological patients, 32 hematological patients with *S. aureus* infections and 19 hematological patients with *E.coli* infections.

### Identification Of DEGs

The data were downloaded by R package GEOquery [15]. The data were normalized using Limma package and we used eBayes analysis to analyze DEGs [16]. We employed  $|\log_2\text{Fold change}| \geq 0.5$  and  $p \leq 0.05$  as parameters to screen DEGs. Volcano map was generated using ggplot2 package [17], and heatmap was produced using pheatmap package [18].

### GO, KEGG, GSEA And Disease Ontology Semantic Enrichment Analysis

DEGs and module genes were analyzed by ClusterProfiler package [19], which was also used for GO, KEGG and GSEA analysis. Disease Ontology Semantic enrichment (DOSE) analysis was processed

utilizing DOSE package [20]. All results are at a significant level ( $p < 0.05$ ).

## WGCNA Analysis

WGCNA package [21] was used to construct the co-expression net. First, we identified soft-thresholding power  $\beta$  using the function of WGCNA package [21]. Second, WGCNA algorithms were employed to construct co-expression modules and blockwiseModules function of WGCNA was used to determine co-expression modules. Then, the modules were assessed to identify the correlations between modules and clinical traits. The module which was significantly correlated with clinical traits was analyzed using GO, KEGG and DOSE analysis. The string database [22] was further used to identify the genes, which may play an important role in black module. Finally, hub genes were identified using the reported methods [23]. Cytoscape [24] was used to visualize module genes and hub genes. Statistical analysis of hub genes was conducted by ggstatsplot package in R. In addition, receiver operating characteristic (ROC) curves of possible biomarkers were drawn using pROC package [25].

## Results

### The expression profile analysis of microarray data

We identified 2303 DEGs, including 1076 up-regulated genes and 1227 down-regulated genes. The heatmap of DEGs was shown in Fig. 1A. The volcano plot (Fig. 1B) showed the distribution of all genes, the results showed that red plots represented up-regulated genes, green plots represented down-regulated genes and the symbol of DEGs, whose fold change (FC) beyond 8 folds were labeled. The GO analysis was performed to identify the function of DEGs. The Fig. 1C showed that several terms were enriched, immune related functions such as neutrophil activation, neutrophil activation involved in immune response, neutrophil mediated immunity, neutrophil degranulation, regulation of leukocyte activation, T cell activation, positive regulation of cytokine production, lymphocyte differentiation and T cell differentiation were primarily enriched. KEGG analysis was conducted to identify the key pathways of DEGs. The Fig. 1D showed that human T-cell leukemia virus 1 infection, Epstein-Barr virus infection, Th17 cell differentiation, Th1 and Th2 cell differentiation and PD-L1 expression and PD-1 checkpoint pathway in cancer were significantly enriched. These pathways are immune-related pathways may play a crucial role in IA. In addition, the results of GSEA analysis (Fig. 1E) indicated that MAPK signaling pathway, NOD-like receptor signaling pathway and regulation of actin cytoskeleton were up-regulated, while Herpes simplex virus 1 infection, RNA transport and Spliceosome were down-regulated.

### Construction Of Co-expression Network

The WGCNA analysis was conducted on 2303 DEGs. Softpower 18 was chose to construct the co-expression network. The results were shown in Fig. 2A, we showed the all modules and the merged modules, which were merged because of similarity of modules. After modules were merged, a total of 8

modules including green, midnightblue, cyan, lightcyan, black, grey60, salmon and grey were obtained. The grey module was removed in analysis, because of these genes not belonging to any module. Correlations between modules and IA were shown in Fig. 2B. Correlation analysis showed that the black module was the mostly significant correlation with IA ( $r = 0.62$ ). As shown in Fig. 2C, the results of cluster also indicated that black module is the highest relationship with IA, followed by salmon ( $r = 0.53$ ) and grey60 ( $r = 0.52$ ). Meanwhile, the module expression patterns of black, salmon and grey60 were shown in Fig. 2D. In addition, Fig. 2E showed the relationship among module eigengenes.

## Go Analysis Was Applied To Analyze Black Module

We analyzed the black module, which was the highest relationship with IA, using GO analysis. The results showed that there several terms were same as the results of total DEGs GO results (Fig. 3A). However, there were still some immune-related terms were uniquely enriched, such as regulation of inflammatory response, myeloid cell differentiation, negative regulation of cytokine production, myeloid leukocyte differentiation and macrophage activation. Figure 3B showed gene expression of immune-related functions. The genes of immune-related functions were almost all up-regulated, while down-regulated genes were in the minority (Fig. 3C). As shown in Fig. 3D GO-GSEA analysis was further performed, the black module genes were mainly enriched in secretory granule, secretory vesicle, cytoplasmic vesicle part, intracellular vesicle and cytoplasmic vesicle. Finally, the cluster results were shown in Fig. 3E.

## KEGG Analysis Was Applied To Analyze Black Module

We also performed the KEGG analysis for black module. Histogram and bubble charts of pathway enrichment were shown in Fig. 4A. Immune-related pathways such as Human T-cell leukemia virus 1 infection, NOD-like receptor signaling pathway, TNF signaling pathway, Acute myeloid leukemia and Fc epsilon RI signaling pathway were significantly enriched. Overlapped top ten pathways were shown in Fig. 4B. The top ten pathways were shown in circle type (Fig. 4C), gene expression of these pathways was also presented. The heatmap of enriched pathways was shown in Fig. 4D. Almost all genes were up-regulated, while *FCER1A* and *HLA-DQA1* were significantly down-regulated. In order to identify which diseases were related to the black module, DOSE analysis was conducted. As was shown in Fig. 4E, Pneumonia, Lung diseases, Carotid Atherosclerosis, Periodontitis and Juvenile arthritis were significantly enriched. Remarkably, Pneumonia and Lung were the top two enriched diseases. They may be correlated with IA. In addition, the string database [21] was used to identify key genes, which may play an important role in IA. Eight genes were screened as key genes, including *MMP9*, *TLR8*, *TLR2*, *CYBB*, *ITGAX*, *ITGAM* and *MPO* (Fig. 4F).

### Hub genes in black module and ROC curves of biomarkers

We identified a total of 621 genes in black module using WGCNA in R. 87 genes ( $r > 0.6$ ) were significantly related with IA. Then, hub genes were identified by degree values, the results were shown in Fig. 5A. *TLR4*,

*TP53/PIG3*, *TMTC1*, *CYSTM1*, *FAR1*, *MKNK1*, *GAS7* and *ITGAM* were identified as the hub genes. The hub genes expression and statistical analysis between hematological patients with IA and normal samples were conducted (Fig. 5B). As was shown in Fig. 5C, the hub genes expression and statistical analysis between hematological patients with IA and non-IA. Finally, *TLR4*, *TP53/PIG3* and *TMTC1* were identified as potential biomarkers for IA, because they were significantly differential expression between hematological patients with IA and non-IA. Moreover, to explore if *TLR4*, *TP53/PIG3* and *TMTC1* could be outstanding biomarkers. ROC curve analysis was performed (Fig. 5D). The results revealed that the area under curve (AUC) for the three genes was beyond 0.7. At the optimal cut-off value of *TLR4* (cut-off value = 8.109), sensitivity was 78.3% and specificity was 72.7%. The results for *TP53/PIG3* (cut-off value = 4.470), sensitivity was 91.3% and specificity was 54.5%. In addition, for *TMTC1* (cut-off value = 2.898), sensitivity and specificity were 78.3% and 81.8%, respectively. Then, the three genes were validated if they were high expression in hematological patients with bacterial infections. Surprisingly, *TLR4* and *TP53/PIG3* were high expression in hematological patients with bacterial infections (Fig. 5E). However, the expression levels of *TP53/PIG3* were obviously higher in hematological patients with bacterial infections than hematological patients with IA.

## Discussion

*Aspergillus* species appear widely in our environment, most ubiquitously in soil, water and decaying plant matter [26]. There are more than 250 species, which were identified as the *Aspergillus* species, and infections caused by *Aspergillus* species are the primary cause of fatality in patients with chronic granulomatous disease [27, 28]. Moreover, there is the most dread of mortality (about 85%-100%) in compromised patients with central nervous system aspergillosis [29]. Due to IA has a rapidly progressive [30], diagnosis and treatment should be done as quickly as we can. Yet even galactomannan (GM), beta-D-glucan (BDG) and polymerase chain reaction (PCR) have been ubiquitously used to the diagnosis of IA [31, 32], clinical diagnosis is notoriously challenged, because of the low sensitivity and specificity of the conventional methods and the unspecific syndromes [33].

*Aspergillus* GM is the most frequently researched and used biomarker for the diagnosis of IA. Previous studies reported that GM serum had a sensitivity of 68–74%, while GM bronchoalveolar lavage (BAL) had a sensitivity of 55–87% [34, 35]. However, there are false-positives, caused by food, medications and other fungi. Moreover, after posaconazole prophylaxis as the standard of care especially for patients undergoing chemotherapy with acute myeloid leukemia, the sensitivity of GM is only about 23.07–35.7% [36, 37]. Previous studies also reported that BDG serum and BDG BAL had sensitivity of 67–84% and 71%, respectively [38, 39]. However, for the diagnosis of IA, the BDG test cannot be an efficient tool [40]. Furthermore, the BDG test cannot distinguish between *Aspergillus* and other fungi [41]. As for PCR, there remain disputes among experts. Indeed, there was lack of standardization in different tissues, and biomarkers were very rare.

In this study, we screened out new biomarkers for the diagnosis of IA. Firstly, we analyzed the gene expression profile between hematological patients with IA and normal samples. The results of Go and

KEGG analysis indicated that several immune-related functions or pathways, such as neutrophil activation, neutrophil activation involved in immune response, neutrophil mediated immunity, Th17 cell differentiation, Th1 and Th2 cell differentiation were significantly enriched. In addition, GSEA analysis also indicated that MAPK signaling pathway and NOD-like receptor signaling pathway were up-regulated. Then, WGCNA analysis was conducted to identify the modules of the relationship with IA. Black module was identified as the key module and GO, KEGG and DOSE analysis were performed to explore the gene functions of black module. GO and KEGG of black module were similar to the results of DEGs. This also revealed that these immune-related functions or pathways may play a crucial role in the response to the infections of *Aspergillus*. In the DOSE analysis, Pneumonia and Lung diseases were significantly enriched. That may be correlated with the infections of *Aspergillus*. Because inhale the spores of *Aspergillus* to the lung is the precondition. Then, we identified eight hub genes in black module. Hub genes were validated by the gene expression and were analyzed using ROC curve. Finally, *TLR4*, *TP53/PIG3* and *TMTC1* were identified as new biomarkers for the diagnosis of IA.

*TLR4* was generally considered that played an important role in the recognition of bacterial lipopolysaccharides. However, TLR4 receptor was also reported that can recognize the pathogenic fungi and active the immune responses [42, 43]. In a review also introduced that TLR4 can recognize *Aspergillus* [44]. In our analysis, *TLR4* was significant overexpression when hematological patients suffering from IA were compared with hematological patients with non-IA patients and normal samples. The overexpression of *TLR4* may be that immune systems of hematological patients can recognize the *Aspergillus*, although immune systems of hematological patients cannot clear the infections because of immunodeficiency. Besides, the overexpression of *TLR4* may also provide us with a new diagnostic biomarker. At the optimal cut-off value of *TLR4*, the sensitivity and specificity to diagnose hematological patients suffering from IA and non-IA patients were 78.3% and 72.7%, respectively. While, *TP53/PIG3*, an inducer of reactive oxygen species (ROS), can be activated by *TP53* and may also participate in apoptosis [45]. *TP53/PIG3* was also significant overexpression in hematological patients with IA. This may provide an indirect clue that the overexpression of *TP53/PIG3* induces ROS production to antifungal infections. In this study, *TP53/PIG3* may be a potential diagnostic biomarker, because it had 93.1% sensitivity and 54.5% specificity to diagnose IA. That was high enough sensitivity to diagnose IA. As for *TMTC1*, there were few researches and the functions of *TMTC1* remain obscure so far. *TMTC1* was up-regulated about 2.6 folds, after IA patients compared with hematological patients with non-IA or normal samples. It may play an important role in the response to *Aspergillus* infections and be used as a new diagnostic biomarker. In addition, *TMTC1* can diagnose patients with IA with 78.3% sensitivity and 81.8% specificity in this study. In order to identify if the three biomarkers were only high expression in IA, they were validated in hematological patients with bacterial infections. The results showed that *TLR4* and *TP53/PIG3* were high expression in hematological patients with bacterial infections. As for *TMTC1*, we cannot annotate the gene from the microarray data. Although *TP53/PIG3* was high expression in hematological patients with bacterial infections, the expression levels of *TP53/PIG3* were up-regulated nearly 4 times in these patients. That was only up-regulated about 2 times in IA patients. Therefore, we can diagnose the patients as potential IA when *TP53/PIG3* was up-regulated no more than 2 times.

## Conclusions

Our studies screened out three new diagnostic biomarkers. More importantly, *TLR4*, *TP53I3/PIG3* and *TMTC1* can combine with other biomarkers to increase the sensitivity and specificity. In addition, *TP53I3/PIG3* may also be used to distinguish bacterial and *Aspergillus* species infections. However, in order to detect IA more exaction, there are still many questions that need to be addressed and more researches in exploring new diagnostic biomarkers should be done.

## Abbreviations

IA: Invasive aspergillosis;; WGCNA: Weighted gene co-expression network analysis;; *S. aureus*: *Staphylococcus aureus*;; *E.coli*: *Escherichia coli*;; HM: hematologic malignancies;; HSCT: hematopoietic stem cell transplant;; *A. fumigatus*: *Aspergillus fumigatus*;; DEGs: differentially expressed genes;; GO: gene ontology;; KEGG: Kyoto encyclopedia of genes and genomes;; GSEA: gene set enrichment analysis;; NCBI: National Center for Biotechnology Information;; DOSE: Disease Ontology Semantic enrichment;; ROC: receiver operating characteristic;; FC: fold change;; AUC: area under curve;; GM: galactomannan;; BDG: beta-D-glucan;; PCR: polymerase chain reaction;; ROS: reactive oxygen species.

## Declarations

### Acknowledgments

Not applicable

### Authors contributions

RL and JC designed the research activity planning. WD drafted the manuscript and participated in bioinformatics analysis. YM, PL, CH, YZ, KZ, XL and JS participated in bioinformatics analysis and revised the manuscript.

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### Availability of data and materials

The data that support the findings of this study are openly available in GEO at <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE78000>.

### Ethics approval and consent to participate

Not applicable

## Consent for publication

Not applicable

## Competing interests

The authors declare that they have no competing interests.

## References

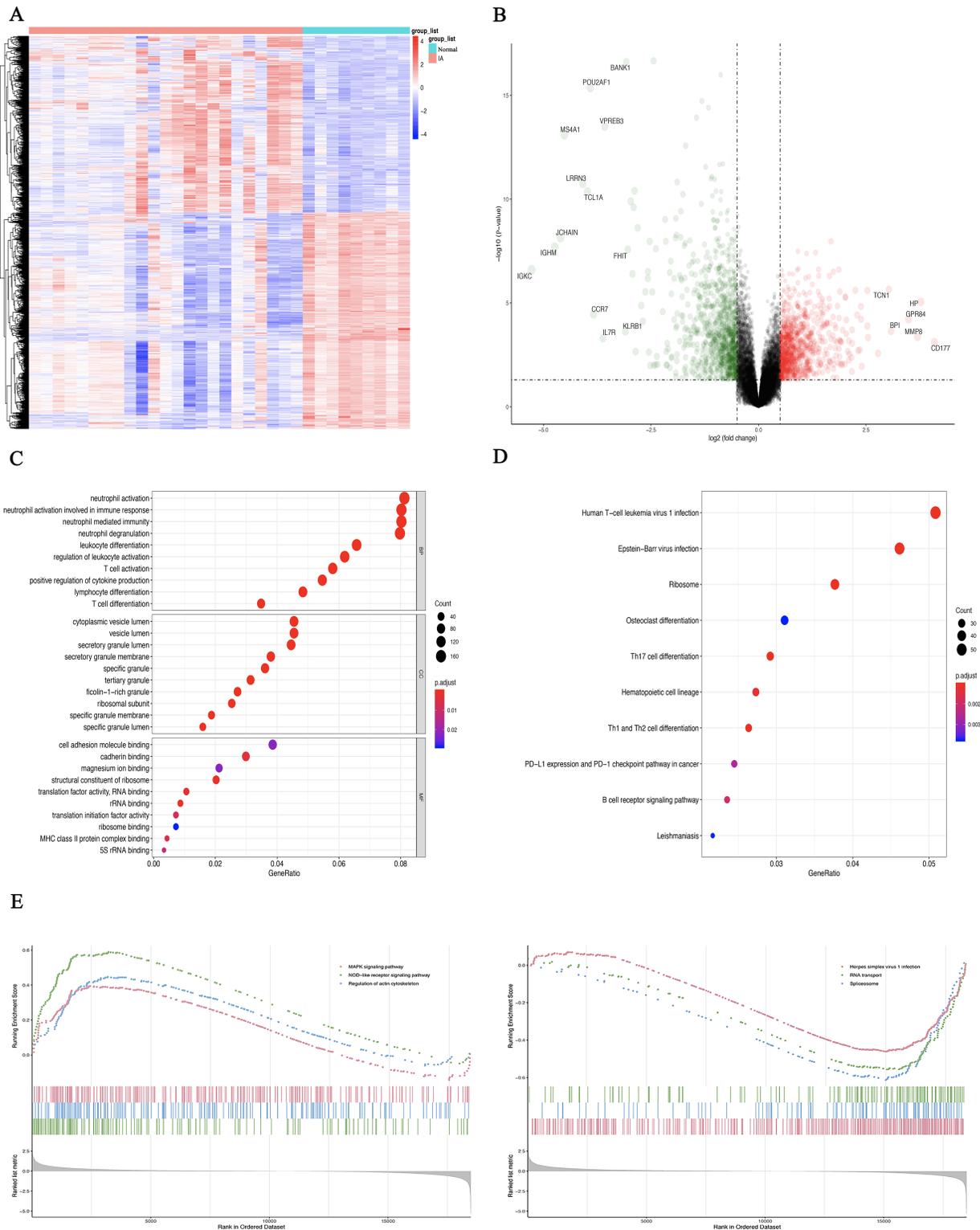
1. Flückiger U, Marchetti O, Bille J, Eggimann P, Zimmerli S, Imhof A, Garbino J, Ruef C, Pittet D, Täuber M. Treatment options of invasive fungal infections in adults. *Swiss Med Wkly*. 2006;136(29–30):447–63.
2. Marr KA, Carter RA, Boeckh M, Martin P, Corey L. Invasive aspergillosis in allogeneic stem cell transplant recipients: changes in epidemiology and risk factors. *Blood*. 2002;100(13):4358–66.
3. Rudramurthy SM, Paul RA, Chakrabarti A, Mouton JW, Meis JF. Invasive aspergillosis by *Aspergillus flavus*: epidemiology, diagnosis, antifungal resistance, and management. *Journal of Fungi*. 2019;5(3):55.
4. Kontoyiannis DP, Marr KA, Park BJ, Alexander BD, Anaissie EJ, Walsh TJ, Ito J, Andes DR, Baddley JW, Brown JM. Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001–2006: overview of the Transplant-Associated Infection Surveillance Network (TRANSNET) Database. *Clin Infect Dis*. 2010;50(8):1091–100.
5. Nicolle M-C, Bénet T, Thiebaut A, Bienvenu A-L, Voirin N, Duclos A, Sobh M, Cannas G, Thomas X, Nicolini F-E: **Invasive aspergillosis in patients with hematologic malignancies: incidence and description of 127 cases enrolled in a single institution prospective survey from 2004 to 2009.** *haematologica* 2011, **96**(11):1685–1691.
6. Pagano L, Caira M, Picardi M, Candoni A, Melillo L, Fianchi L, Offidani M, Nosari A. Invasive aspergillosis in patients with acute leukemia: update on morbidity and mortality—SEIFEM-C Report. *Clin Infect Dis*. 2007;44(11):1524–5.
7. Morrison BE, Park SJ, Mooney JM, Mehrad B. Chemokine-mediated recruitment of NK cells is a critical host defense mechanism in invasive aspergillosis. *J Clin Investig*. 2003;112(12):1862–70.
8. Schaffner A, Douglas H, Braude A. Selective protection against conidia by mononuclear and against mycelia by polymorphonuclear phagocytes in resistance to *Aspergillus*: observations on these two lines of defense in vivo and in vitro with human and mouse phagocytes. *J Clin Investig*. 1982;69(3):617–31.
9. Stevens DA. Th1/Th2 in aspergillosis. *Med Mycol*. 2006;44(Supplement\_1):229–35.
10. Chakrabarti A, Chatterjee SS, Das A, Shivaprakash M. Invasive aspergillosis in developing countries. *Med Mycol*. 2011;49(Supplement\_1):35–47.

11. Shankar S, Mahadevan A, Sundaram C, Sarkar C, Chacko G, Lanjewar D, Santosh V, Yasha T, Radhakrishnan V. Pathobiology of fungal infections of the central nervous system with special reference to the Indian scenario. *Neurol India*. 2007;55(3):198.
12. Guegan H, Robert-Gangneux F, Camus C, Belaz S, Marchand T, Baldeyrou M, Gangneux J-P. Improving the diagnosis of invasive aspergillosis by the detection of *Aspergillus* in broncho-alveolar lavage fluid: comparison of non-culture-based assays. *J Infect*. 2018;76(2):196–205.
13. Dix A, Czakai K, Springer J, Fliesser M, Bonin M, Guthke R, Schmitt AL, Einsele H, Linde J, Löffler J. Genome-Wide Expression Profiling Reveals S100B as Biomarker for Invasive Aspergillosis. *Frontiers in microbiology*. 2016;7:320.
14. Ahn SH, Tsalik EL, Cyr DD, Zhang Y, van Velkinburgh JC, Langley RJ, Glickman SW, Cairns CB, Zaas AK, Rivers EP. Gene expression-based classifiers identify *Staphylococcus aureus* infection in mice and humans. *PLoS One*. 2013;8(1):e48979.
15. Davis S, Meltzer PS. GEOquery: a bridge between the Gene Expression Omnibus (GEO) and BioConductor. *Bioinformatics*. 2007;23(14):1846–7.
16. Smyth GK: **Limma: linear models for microarray data**. In: *Bioinformatics and computational biology solutions using R and Bioconductor*. Springer; 2005: 397–420.
17. Wickham H: **ggplot2: elegant graphics for data analysis**: Springer; 2016.
18. Kolde R. Pheatmap: pretty heatmaps. R package version. 2012;61(926):915.
19. Yu G, Wang L-G, Han Y, He Q-Y. clusterProfiler: an R package for comparing biological themes among gene clusters. *Omics: a journal of integrative biology*. 2012;16(5):284–7.
20. Yu G, Wang L-G, Yan G-R, He Q-Y. DOSE: an R/Bioconductor package for disease ontology semantic and enrichment analysis. *Bioinformatics*. 2014;31(4):608–9.
21. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. *BMC Bioinform*. 2008;9(1):559.
22. Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, Santos A, Doncheva NT, Roth A, Bork P. **The STRING database in 2017: quality-controlled protein–protein association networks, made broadly accessible**. *Nucleic acids research* 2016:gkw937.
23. Zhang X, Feng H, Li Z, Li D, Liu S, Huang H, Li M. Application of weighted gene co-expression network analysis to identify key modules and hub genes in oral squamous cell carcinoma tumorigenesis. *OncoTargets therapy*. 2018;11:6001.
24. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome research*. 2003;13(11):2498–504.
25. Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez J-C, Müller M. pROC: an open-source package for R and S + to analyze and compare ROC curves. *BMC Bioinform*. 2011;12(1):77.
26. Saxena S, Kukreti S. **EFFECT OF ASPERGILLUS SPECIES ON SKIN INFECTION**. *International Journal of Medical and Biomedical Studies* 2018.

27. Seyedmousavi S, Lionakis M, Parta M, Peterson S, Kwon-Chung K. **Emerging Aspergillus species almost exclusively associated with primary immunodeficiencies.** In: *Open forum infectious diseases: 2018*. Oxford University Press US: ofy213.
28. King J, Henriët SS, Warris A. Aspergillosis in chronic granulomatous disease. *Journal of Fungi*. 2016;2(2):15.
29. Kumar D, Nepal P, Singh S, Ramanathan S, Khanna M, Sheoran R, Bansal SK, Patil S. CNS aspergilloma mimicking tumors: Review of CNS aspergillus infection imaging characteristics in the immunocompetent population. *Journal of Neuroradiology*. 2018;45(3):169–76.
30. Singh N, Paterson DL. Aspergillus infections in transplant recipients. *Clin Microbiol Rev*. 2005;18(1):44–69.
31. Hoenigl M, Prattes J, Spiess B, Wagner J, Pruellner F, Raggam R, Posch V, Duettmann W, Hoenigl K, Wölfler A. Performance of galactomannan, beta-d-glucan, Aspergillus lateral-flow device, conventional culture, and PCR tests with bronchoalveolar lavage fluid for diagnosis of invasive pulmonary aspergillosis. *J Clin Microbiol*. 2014;52(6):2039–45.
32. Buchheidt D, Reinwald M, Hoenigl M, Hofmann W-K, Spiess B, Boch T. The evolving landscape of new diagnostic tests for invasive aspergillosis in hematology patients: strengths and weaknesses. *Curr Opin Infect Dis*. 2017;30(6):539–44.
33. Ullmann AJ, Aguado JM, Arikan-Akdagli S, Denning DW, Groll AH, Lagrou K, Lass-Flörl C, Lewis RE, Muñoz P, Verweij PE. Diagnosis and management of Aspergillus diseases: executive summary of the 2017 ESCMID-ECMM-ERS guideline. *Clinical microbiology infection*. 2018;24:e1–38.
34. Pfeiffer CD, Fine JP, Safdar N. Diagnosis of invasive aspergillosis using a galactomannan assay: a meta-analysis. *Clin Infect Dis*. 2006;42(10):1417–727.
35. Affolter K, Tamm M, Jahn K, Halter J, Passweg J, Hirsch HH, Stolz D. Galactomannan in bronchoalveolar lavage for diagnosing invasive fungal disease. *Am J Respir Crit Care Med*. 2014;190(3):309–17.
36. Aslan M, Oz Y, Aksit F, Akay OM. Potential of polymerase chain reaction and galactomannan for the diagnosis of invasive aspergillosis in patients with febrile neutropenia. *Mycoses*. 2015;58(6):343–9.
37. Metan G, Keklik M, Dinç G, Pala Ç, Yıldırım A, Saraymen B, Köker MY, Kaynar L, Eser B, Cetin M. Performance of galactomannan antigen, beta-d-glucan, and Aspergillus-lateral-flow device for the diagnosis of invasive aspergillosis. *Indian Journal of Hematology Blood Transfusion*. 2017;33(1):87–92.
38. Karageorgopoulos DE, Vouloumanou EK, Ntziora F, Michalopoulos A, Rafailidis PI, Falagas ME.  $\beta$ -D-glucan assay for the diagnosis of invasive fungal infections: a meta-analysis. *Clin Infect Dis*. 2011;52(6):750–70.
39. Rose SR, Vallabhajosyula S, Velez MG, Fedorko DP, VanRaden MJ, Gea-Banacloche JC, Lionakis MS. The utility of bronchoalveolar lavage beta-D-glucan testing for the diagnosis of invasive fungal infections. *Journal of infection*. 2014;69(3):278–83.

40. Badiee P, Alborzi A, Karimi M, Pourabbas B, Haddadi P, Mardaneh J, Moieni M. Diagnostic potential of nested PCR, galactomannan EIA, and beta-D-glucan for invasive aspergillosis in pediatric patients. *The Journal of Infection in Developing Countries*. 2012;6(04):352–7.
41. Johnson G, Ferrini A, Dolan SK, Nolan T, Agrawal S, Doyle S, Bustin SA. Biomarkers for invasive aspergillosis: the challenges continue. *Biomark Med*. 2014;8(3):429–51.
42. Rødland EK, Ager-Wick E, Halvorsen B, Müller F, Frøland SS. Toll like receptor 5 (TLR5) may be involved in the immunological response to *Aspergillus fumigatus* in vitro. *Med Mycol*. 2011;49(4):375–9.
43. Lin Y-T, Verma A, Hodgkinson P. C: Toll-like receptors and human disease: lessons from single nucleotide polymorphisms. *Curr Genom*. 2012;13(8):633–45.
44. Lass-Flörl C, Roilides E, Löffler J, Wilflingseder D, Romani L. Minireview: host defence in invasive aspergillosis. *Mycoses*. 2013;56(4):403–13.
45. Zhang W, Luo J, Chen F, Yang F, Song W, Zhu A, Guan X. BRCA1 regulates PIG3-mediated apoptosis in a p53-dependent manner. *Oncotarget*. 2015;6(10):7608.

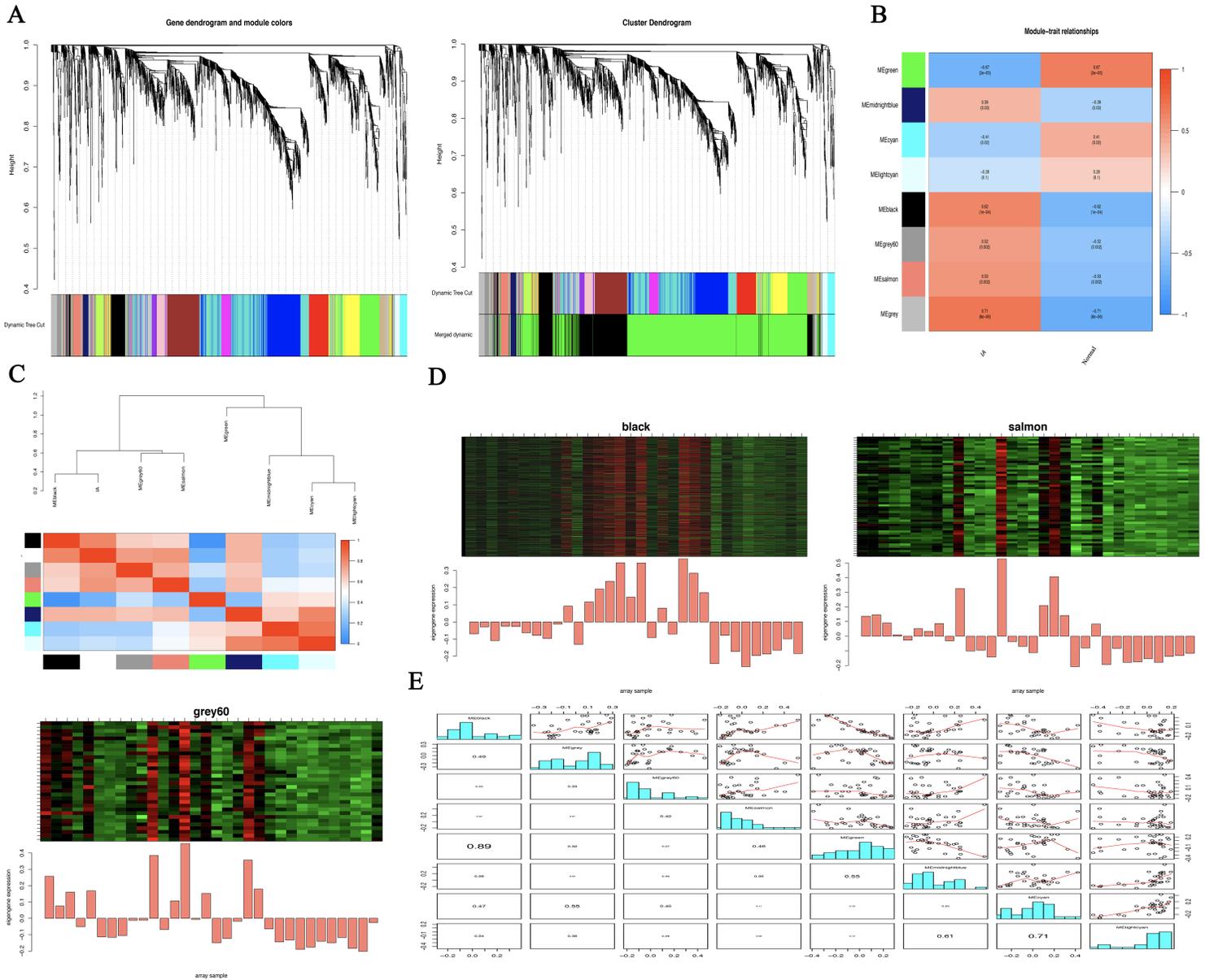
## Figures



**Figure 1**

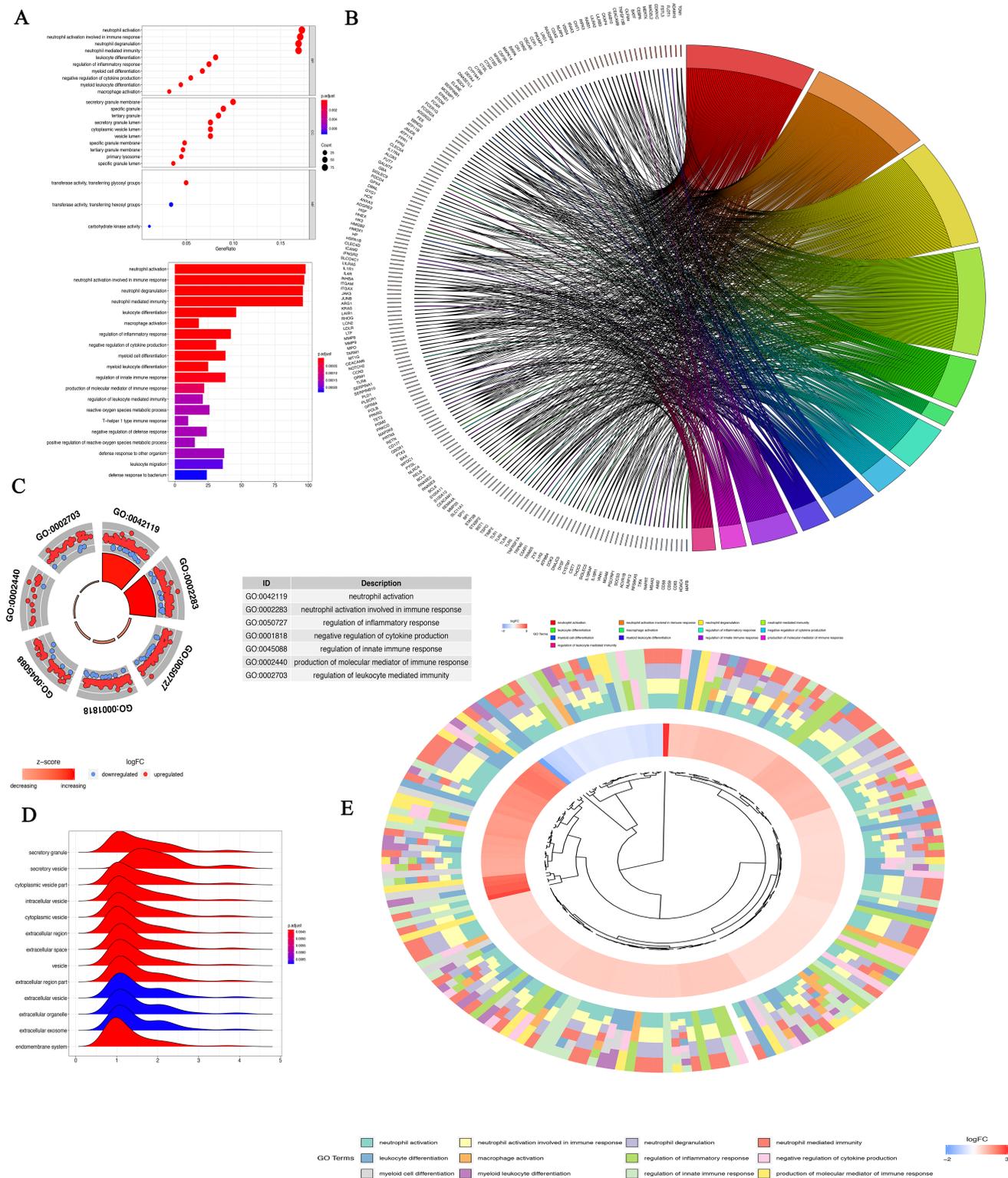
IA-induced DEGs. (A) The expression of DEGs was displayed by heatmap. (B) Volcano plot of DEGs. The abscissa represents  $\log_2$  (fold change), and the ordinate represents  $-\log_{10}$  p-values. Red plots represented up-regulated genes, green plots represented down-regulated genes. (C) DEGs were analyzed by GO. Node size is based on the numbers of genes, the color intensity corresponds to the adjust p-value.

(D) KEGG analysis was applied to DEGs. Top 10 of pathway enrichment was displayed by gene numbers and adjust p-value. (E) GSEA was conducted to analyze the signaling pathways of DEGs.



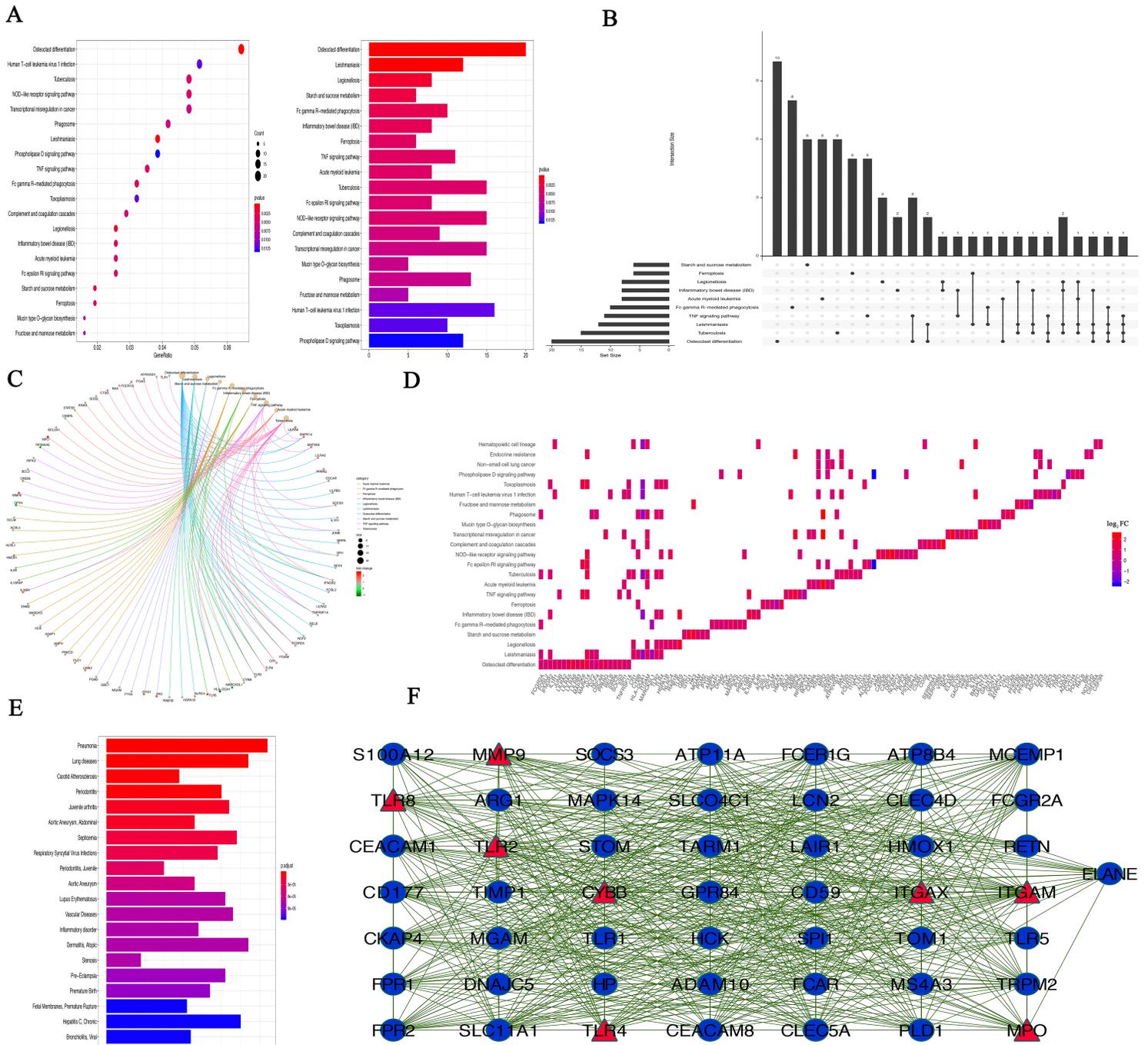
**Figure 2**

WGCNA was performed to identify the key modules. (A) The cluster dendrogram of module eigengenes. (B) The correlations between modules and IA were shown. (C) The results of cluster between module eigengenes and IA. (D) The module expression pattern. The heatmap represents the expression of module genes, the bar charts represent the eigengene profiles. (E) Relationship between module eigengenes.



**Figure 3**

GO analysis was applied to the genes of black module. (A) Bubble and Histogram charts of the GO results were displayed. (B) The gene expression of immune-related functions was displayed. The color intensity corresponds to the degree of genes expression. (C) Circular chart was displayed to show the rough expression of immune-related pathways. (D) GO-GSEA was conducted to analyze the genes of black module. (E) The results of GO were clustered.



**Figure 4**

KEGG pathway enrichment of black module. (A) Bubble and Histogram charts of the KEGG results were displayed. Top 20 of pathway enrichment was displayed by gene numbers and p-value. (B) Overlapped top ten pathways were displayed. (C-D) The gene expression of the significantly enriched pathways. (E) DOSE analysis was conducted to identify the correlation between diseases and IA. (F) Protein-protein interaction networks of the black module. The triangles represent the genes of black module with the highest degree.



TP53I3/PIG3 and TMTC1. (E) The expression of TLR4 and TP53I3/PIG3 between hematological patients suffering from bacterial infections and non-bacterial infections.