HIF-1 and macrophage activation signalling pathways are potential biomarkers of invasive aspergillosis

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Abstract. Invasive aspergillosis (IA) is a severe disease, the pathogenesis of which remains unclear. The present study aimed to determine the molecular mechanism of IA and to identify potential biomarkers using bioinformatics analysis. The GSE78000 dataset, which includes data from patients with IA and healthy individuals, was downloaded from Gene Expression Omnibus. Differentially expressed genes (DEGs) between the IA and control groups were identified with the 'affy' package in R software. The Gene Ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) databases were then used to analyse the function and pathway enrichment of DEGs. The protein-protein interaction network was analysed with the Search Tool for the Retrieval of Interacting Genes (STRING) website. In addition, DEGs were confirmed using reverse transcription-quantitative PCR and western blotting in samples with IA (n=6) and control samples (n=6) collected from the Department of Respiratory and Critical Care Medicine of the First Affiliated Hospital of Henan University of Science and Technology (Luoyang, China). The present study identified 735 DEGs, including 312 upregulated and 423 downregulated genes. Through GO and KEGG analyses of the DEGs, macrophage activation and hypoxia-inducible factor 1 (HIF-1) signalling pathways were revealed to be significantly upregulated and downregulated, respectively, in patients with IA compared with that of the healthy individuals. Subsequently, correlation analysis of macrophage activation and HIF-1 signalling pathways was revealed using correlation as a distance metric for hierarchical clustering correlation analysis. However, there was no protein-protein interaction between the macrophage activity regulation and HIF-1 signalling pathways based on STRING analysis. In summary, the present study identified candidate genes and associated molecules that may be associated to IA and revealed potential biomarkers and therapeutic targets for IA.

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Abbreviations: IA, invasive aspergillosis; DEGs, differentially expressed genes; GO, Gene Ontology; PPI, protein-protein interaction; HIF-1, hypoxia-inducible factor 1; KEGG, Kyoto Encyclopaedia of Genes and Genomes; BP, biological process; STRING, Search Tool for the Retrieval of Interacting Genes

Key words: correlation analysis, biomarkers, microarray, macrophage, IA, HIF-1

Introduction

Invasive aspergillosis (IA) is a common invasive fungal disease caused by *Aspergillus* infection, with 60% of all people with a fungal disease having an Aspergillus infection. Pathogenic *Aspergillus* species include *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus terreus* (1). *Aspergillus* is present widely in the environment, and it can also exist on the human epidermis, mucosa of the mouth, as well as other parts of the body. When the immune barriers of an individual are normal, *Aspergillus* generally does not cause disease. However, when an individual is immunocompromised, *Aspergillus* will multiply in large numbers in epidermal tissues, causing infection. The excessive use of antibiotics may lead to a reduction in the total number of normal bacterial flora, resulting in the uncontrolled reproduction of *Aspergillus*, which can also lead to IA (2).

The incidence of IA has been increasing with the wide application of broad-spectrum antibiotics, immunosuppressive agents and corticosteroids, the diagnosis and treatment of organs, stem cell transplantation and catheter technology, the increasing incidence of malignant tumours and acquired immunodeficiency syndrome (3). According to clinical statistics, IA is the second most common invasive fungal disease worldwide (4). Due to the difficulty in making an early diagnosis and a lack of effective treatment measures, worldwide mortality from IA is 30-95% (5-8), and mortality in intensive care units has been reported to be as high as 80% (9).

Because a lack of early diagnosis is the main reason for the severity and high mortality of IA, the identification and rapid screening of biomarkers is necessary for improving early diagnosis (10). Identification of the molecular mechanisms underlying the pathogenesis of IA is also required. Analysis of microarray-based

mRNA expression levels can be used to identify genetic risk factors and investigate the molecular pathobiology of IA (11).

Our previous study revealed that alveolar macrophages (AM) serve a role in the resistance to *Aspergillus* infection in human THP-1-derived macrophages (12). The level of the CD23 protein in macrophages directly affects the function of AM. PU.1 is critical for innate immunity against IA as it regulates important C-type lectin receptors (CLR) expression in human macrophages. CD23, encoded by the Fc fragment of the IgE receptor II gene was recently reported to be a novel CLR. CD23 is a low-affinity IgE receptor and serves key roles in the IgE-mediated immune response, regulating cell differentiation and inflammation (12). HIF-1 as a critical mediator of EtOH-mediated metabolic derangements in AM. FES proteins can positively regulate the PU.1 and CD23 proteins, and HIF-1 can negatively regulate FES proteins. However, an increase in HIF-1 levels, induced by IA, does not reduce the levels of CD23, indicating that the increase in HIF-1 levels may not reduce the ability of AM to resist fungal infection (13). It was further revealed that mice with IA may have deficiencies in glycolytic energy metabolism and AM activity, thus promoting the occurrence of the disease (14). The present study hypothesized that an increase in HIF-1 levels, following AI, may serve a role in overcoming the defects of the AM glycolysis pathway and improving the ability of AM to resist fungal infection. Therefore, in the present study, the association between macrophage activity and the HIF-1 signalling pathway was confirmed through GO and KEGG enrichment analyses. Both methods were used to jointly analyse clinical information and microarray data from patients with IA in order to identify genes associated with clinical features. These genes may have important clinical implications and may serve as diagnostic or prognostic biomarkers and therapeutic targets.

Materials and methods

Microarray data resources. Microarray data from the GSE78000 dataset, comparing the gene expression levels in blood samples from patients suffering from IA with that of patients without IA or healthy individuals (considered as the healthy group in the present study) (15), was obtained from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) to screen differentially expressed genes (DEGs), identify key genes involved in IA onset and deterioration.

Principal coordinates analysis (PCoA). PCoA is a visualization method that examines the similarities or differences of the data, and allows the observation of differences between individuals or groups (16). The original expression levels from the GSE78000 dataset were standardised using log (1+x). The dissimilarity indices of the standardized samples were calculated using the Bray Curtis method, using the 'vegdist' tool (https://github.com/vegandevs/vegan/) (17). The distance matrix of the dissimilarity index was calculated using the PCoA of the software package 'ape' (http://ape-package.ird.fr/). After sorting a series of eigenvalues and eigenvectors, the top 6 samples with were selected through PCoA analysis.

Identification of DEGs. Raw data for gene expression levels were read and processed using the 'affy' package (version 1.50.0) in the R software (version 4.0.1, https://www.r-project.org/) (18). Gene probes were annotated using the Affymetrix Human Genome U219 Array (accession no. GPL21464) as an annotation profile and unmatched probes were discarded. When multiple probes matched one gene symbol, the average values of the probes were calculated as the final expression level of the gene. DEGs from IA and control samples were screened using the Linear Models for Microarray (Limma) package (version 3.24.14) in R (19). Limma was used to analyse the data according to the expression levels by fitting linear models and to determine statistical significance with moderated t-statistics. The P-value was adjusted according to the false discovery rate (19). Genes with an adjusted value of $P<0.05$ and at least a 2-fold increase or decrease were considered DEGs.

Functional and pathway enrichment analyses. The biological functions of the DEGs were explored with GO (http://geneontology.org/) and KEGG (https://www.kegg.jp/) enrichment analyses (20,21) using the online tool Database for Annotation, Visualization and Integrated Discovery (version 6.8; https://david.ncifcrf.gov/home.jsp) (18,22). The GO terms included biological process (BP), cellular component and molecular function. KEGG provides a set of functionalities, including input by identifications and sequences, identification of frequent and statistically enriched pathways, a choice of four statistical tests and the option of multiple testing correction (23). Significant GO and KEGG pathways with threshold counts ≥ 2 and $P<0.05$ were selected for further analysis.

Correlation analysis of predicted target genes. A prerequisite for understanding cellular functions at the molecular level is determining the functional interactions among the various proteins in the cell (24). The correlation matrix was generated based on the results of hierarchical clustering of gene expression. Spearman's ρ statistic was used to estimate a rank-based measure of association with hierarchical clustering (Hclust)=0.05 and $P<0.05$ as the cut-off values.

Protein-protein interaction (PPI) network. A PPI network among co-expressed DEGs was constructed using the STRING database (version 11.0; http://string-db.org/) (25) and a PPI score (medium confidence) ≥ 0.4 was defined as the cut-off value (26,27).

IA specimens. Between January 2020 and May 2021, whole blood samples from 6 cases with IA (3 males, 3 females; age, 67±8 years) and 6 cases without IA (controls; 3 males, 3 females; age, 65±9 years) were collected from the Department of Respiratory and Critical Care Medicine of the First Affiliated Hospital of Henan University of Science and Technology (Luoyang, China). Patients that had a history of IA or had clinical or biochemical evidence of other comorbidities were excluded (Table SI). Patients with IA and controls provided written informed consent prior to using their blood samples in the present study. The current study was approved by the Ethics Committee of The First Affiliated Hospital of Henan University of Science and Technology (approval no. HUST2034532, Luoyang, China).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Complete RNA was extracted from whole blood with TRI reagent (Merck KGaA), and all mRNA was subjected to reverse transcription (RT) and qPCR using a PrimeScript® RT Master Mix (Perfect Real Time) kit (Takara Biotechnology

Table I. Sequence information for primers used in reverse transcription-quantitative PCR.

Gene name	NCBI gene identification number	Primer sequence (5'-3')
ARG1	383	F: GCTACTCTCAGGATTAGATATA
		R: CAAGGTTATTGCAACTGCTGTGT
CD177	57126	F: AGCATTCAGGGCTGCGTGGCCCA
		R: CACATCACGCTTCTCACGCGCAG
FES	2242	F: ACGTGTGGAGCTTTGGCATCTTG
		R: CACGGCATCAGGACACAGCTCTG
HMOX1	3162	F: CAACAAAGTGCAAGATTCTGCCC
		R: AGGACCCATCGGAGAAGCGGAGC
ICAM1	3383	F: CCGCAAGGTGACCGTGAATGTGC
		R: CGCTGGCGGTTATAGAGGTACGT
IL4R	3566	F: TGGGCAGTGGCATTGTCTACTCA
		R: ACAGCAAGGACTGGCCATGACAG
ITGAM	3684	F: ACCTCCTGATCGTGAGCACAGCT
		R: CGACAGAGCTGCCCACGATGAGC
MMP2	4313	F: TGGAGACAAATTCTGGAGATACA
		R: TGCAGGTCCACGACGGCATCCAG
PFKFB3	5209	F: ACGCCTGTCGCTTATGGCTGCCG
		R: GACACTATTGCGTCTCATGAGCG
TIMP1	7076	F: CTGGAACAGCCTGAGCTTAGCTC
		R: GTCCGTCCACAAGCAATGAGTGC
ALDOC	230	F: AGCCTCTGCACTCAATGCCTGGC
		R: GCAAGCCCATTCACCTCAGCCCG
BLK	640	F: CGCAACCTGGAGCGCGGCTACCG
		R: AAGTCCTCCAGCACCGACTGCAG
LDHB	3945	F: AAGGATATACCAACTGGGCTATT
		R: ATCCCCTTTACCATTGTTGACAC
RPS12	6206	F: ATCCAACTGTGATGAGCCTATGT
		R: CTACACAACTGCAACCAACCACT
RPL8	6132	F: GCATCAGGGAACTATGCCACCGT
		R: CACACCAACCACAGCTCTGTTGG
RPL35	11224	F: CACGTGCCATGCGCCGCCGGCTC
		R: TTGACCGCGTACTTCCGCAGCGG
RPS6	6194	F: TTCAGCGTCTTGTTACTCCACGT
		R: GCATATTCTGCAGCCTCTTCTTT
RPS16	6217	F: GTGTAGACATCCGTGTCCGTGTA
		R: TATTTCTGGTAATAGGCCACCAG
GAPDH	2597	F: CATCACTGCCACCCAGAAGACTG
		R: ATGCCAGTGAGCTTCCCGTTCAG

F, forward; R, reverse; ARG1, arginase 1; FES, FES proto-oncogene tyrosine kinase; HMOX1, heme oxygenase 1; ICAM1, intercellular adhesion molecule 1; IL4R, interleukin 4 receptor; ITGAM, integrin subunit alpha M; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3; TIMP1, TIMP metallopeptidase inhibitor 1; ALDOC, aldolase fructose-bisphosphate C; BLK, BLK proto-oncogene Src family tyrosine kinase; LDHB, lactate dehydrogenase B; RP, ribosomal protein.

Co., Ltd.) and SYBR Green Master Mix (Takara Biotechnology Co., Ltd.), respectively, according to the manufacturer's protocols. qPCR was performed on a Roche LightCycler® 480 II Real-Time System (Roche Diagnostics). The program was as follows: 2 min at 94˚C followed by 40 cycles of 30 sec at 94˚C and 20 sec at 60˚C. The results were analysed using the $2^{-\Delta\Delta Cq}$ method (28). The genes were amplified using specific primers, and GAPDH was used as the reference gene in qPCR (Table I).

Western blot assay. Whole blood from healthy patients and patients with IA was extracted using a Whole Blood Protein Extraction kit (EX1200, Beijing Solarbio Science & Technology Co., Ltd.) and quantified via the bicinchoninic acid assay (Pierce; Thermo Fisher Scientific, Inc.). A total of 24 μg protein sample was loaded per lane and then electrophoresed on 10% SDS-PAGE gels and transferred to PVDF membranes (MilliporeSigma) using electrophoresis systems (Tanon

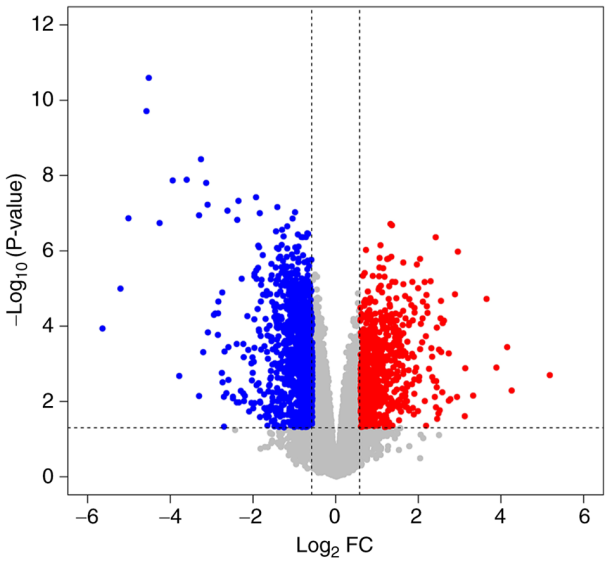

Figure 1. Volcano plot demonstrating the differentially expressed genes in the GSE78000 dataset. The x-axis indicates log_2FC, and the y-axis indicates -lg_{10} (P-value). Red dots indicate upregulated genes, blue dots indicate downregulated genes and grey dots indicate genes without a significant difference in expression levels. FC, fold change.

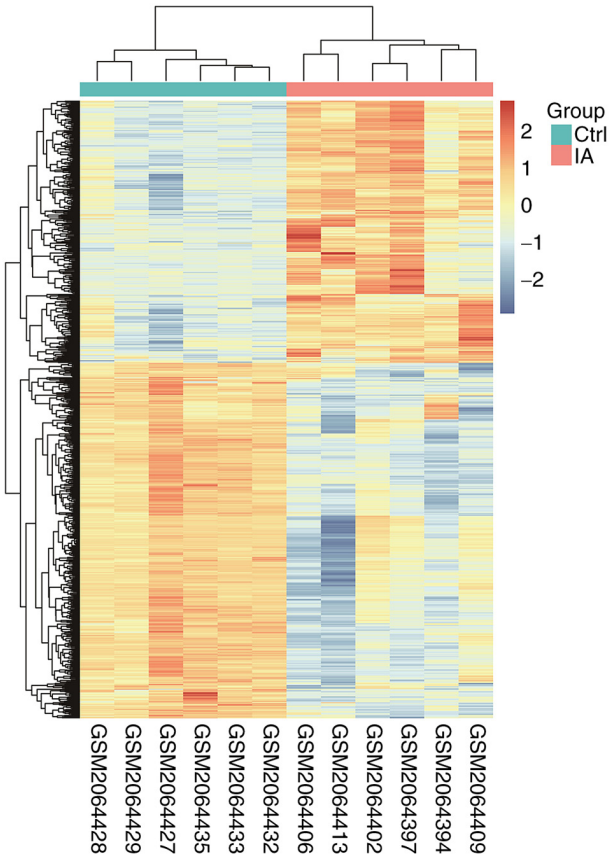

Figure 2. Heatmap demonstrating the differentially expressed genes in the GSE78000 dataset. Blue represents a lower expression level, red represents a higher expression level and white indicates that there was no difference in the expression level among the genes. Each column represents one subject in the dataset, and each row represents one gene. The gradual change in colour ranging from blue to red represents the process of downregulation to upregulation as number of standard deviations from mean expression. IA, invasive aspergillosis.

VE-180 and Tanon VE-186, respectively; Tanon Science and Technology Co., Ltd.). The PVDF membranes were blocked with 5% (w/v) skimmed milk powder at room temperature for 2 h and incubated at 4˚C overnight with the following primary antibodies: IL4 (1:1,000; cat. no. ab34277; Abcam), ITGAM (1:500; cat. no. ab133357; Abcam), MMP2 (1:1,000; cat no. ab181286; Abcam), GAPDH (1:5,000; cat. no. ab181602; Abcam) and RPL8 (1:1,000; cat. no. ab169538; Abcam). The membranes were then washed five times with 1X PBS-5% Tween 20 and incubated with HRP-labelled goat anti-rabbit IgG (1:10,000; cat. no. ab205718; Abcam) and anti-rat IgG (1:10,000; cat. no. ab205720; Abcam) secondary antibodies at room temperature for 1 h. Blots were subsequently visualized using an enhanced chemiluminescence detection kit (MilliporeSigma) according to the manufacturer's protocols. A ChemiDoc MP (Bio-Rad Laboratories, Inc.) scanning system was used to assess the immunoreactive protein bands.

Statistical analysis. The data are presented as the mean ± SEM. Statistical analysis was performed using GraphPad Prism 6.05 software (GraphPad Software; Dotmatics). The data were analysed using paired Student's t-tests as appropriate. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Identification of DEGs in IA. The GSE78000 dataset was downloaded from the GEO database and included 45 samples in total. Subsequently, the samples of 23 patients with IA and 9 healthy individuals were selected for primary analysis and unclassified samples were excluded. According to the results of PCoA analysis, 12 blood samples (6 patients with IA matched with 6 patients without IA or healthy individuals) were selected for further analysis (Fig. S1 and Table SII). The Limma package was then used to identify DEGs by comparing samples with IA with matched control samples. The Limma package identified 312 upregulated and 423 downregulated genes (a total of 735 genes; Table SIII). The expression levels and distribution status of all DEGs in the GSE78000 dataset are presented in a volcano plot (Fig. 1) and a heatmap (Fig. 2).

Functional and pathway enrichment analyses of DEGs. After obtaining the DEGs, GO and KEGG enrichment analyses were performed to examine the classification of the DEGs. The GO analysis revealed that the BP terms included 'regulation of macrophage activation', MF terms included 'cytokine receptor activity' and CC terms included 'immunological synapse' (Fig. 3 and Table SIV). This finding demonstrated that the occurrence of IA was associated with abnormal immune function. At the same time, a previous study reported that the HIF-1 signalling pathway plays a role in antifungal immunity (13); thus, the potential implication of the HIF-1 signalling pathway in IA was further investigated. It was revealed that the significantly enriched KEGG pathways of the DEGs included the HIF-1 signalling pathway (Fig. 4 and Table SV). The present study demonstrated that there are 18 major signalling molecules involved in the macrophage activation signalling pathway and HIF-1 signalling pathway. Among

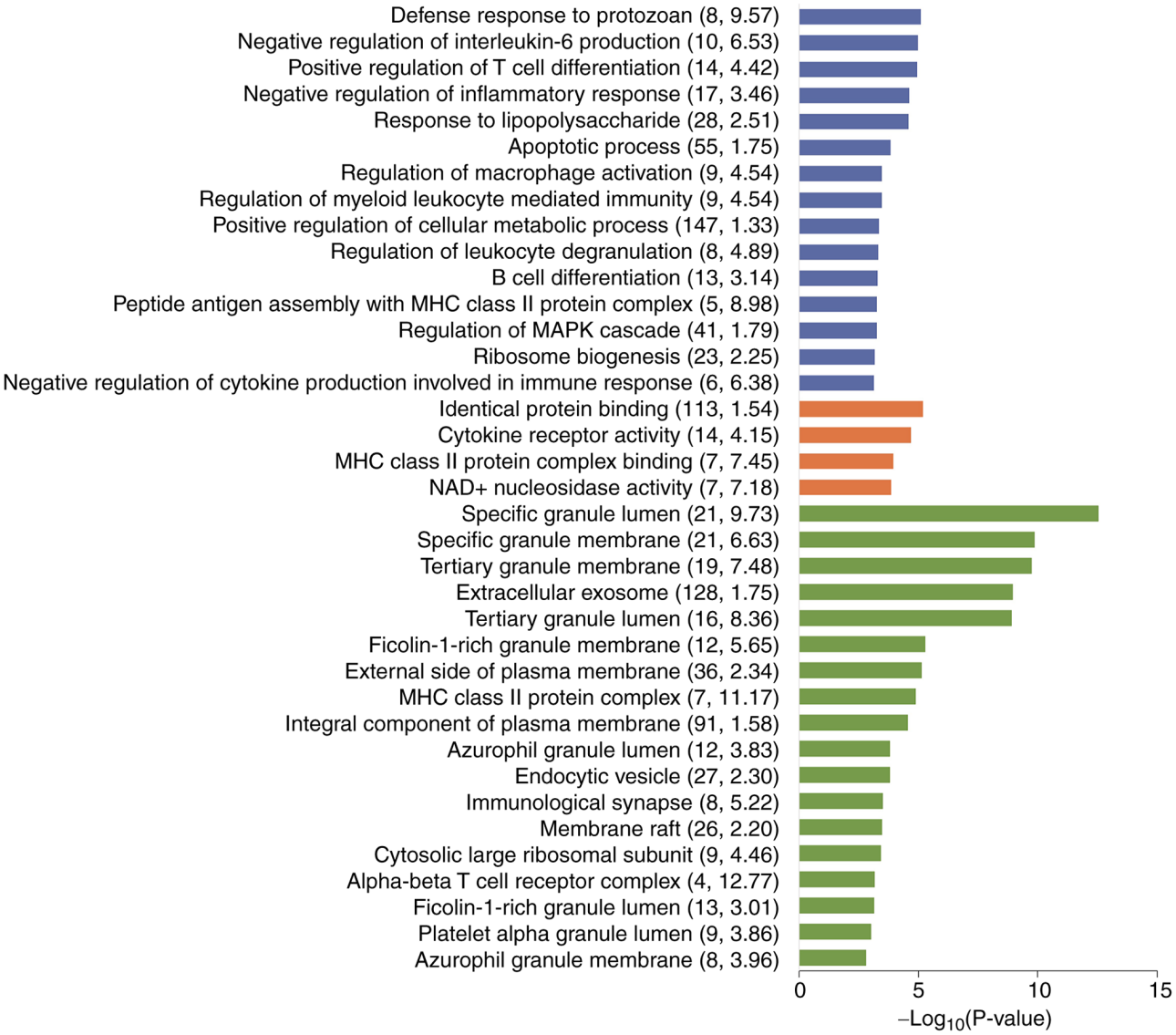

Figure 3. GO functional annotation of DEGs. GO enrichment analysis of the 37 most significantly enriched terms of the shared DEGs in the GSE78000 dataset. The x-axis indicates -lg$_{10}$ (P-value), and the y-axis indicates the 37 most significantly enriched GO pathways. The number in brackets represent number of genes and enrichment factor, respectively. Blue bars represent 'biological processes', orange bars represent 'molecular functions' and green bars represents 'cellular components'. GO, Gene Ontology; DEGs, differentially expressed genes.

them, the expression of ICAM1, MMP2, TIMP1, ARG1, CD177, IL4R, FES, HMOX1, ITGAM and PFKFB3 were upregulated and the expression of RPS12, ALDOC, LDHB, BLK, RPS6, RPS16, RPL8 and RPL35 were downregulated in IA group compared with control groups (Fig. 5A).

Correlation analysis of macrophage activation and HIF-1 signalling pathways. In the aforementioned experiments, the expression levels of the signalling pathways moleucles regulating macrophage activity and HIF-1 were significantly different between the IA and control samples. To clarify whether correlation occurred between the macrophage activation and HIF-1 signalling pathways, Hclust correlation analysis was used to predict co-expressed interactions (Fig. 5B). With HClust=0.05 as the cut-off for a significant difference, the 18 genes were revealed to be correlated with each other. Subsequently, the expression levels of them were compared between the control and IA groups (Fig. 6A). Compared with the control group, the expression levels of all 10 genes in the macrophage activity regulation signalling pathway were significantly higher (P<0.01) in the IA group. The expression levels of the eight genes in the HIF-1 signalling pathway were decreased significantly in the IA group compared with the control group. Except for RPS6 (P<0.05), the other seven genes exhibited a significant difference of P<0.01 (Fig. 6A). Furthermore, the protein levels of IL4, ITGAM and MMP2 were upregulated, while the RPL8 protein level was downregulated in patients with IA compared with healthy controls (Fig. 6B). The aforementioned results demonstrated the correlation between the two types of genes.

Gene expression levels represent the level of RNA expression, and they cannot account for the final functionality at the protein level. To further investigate the relationship between macrophage activation and HIF-1 signalling pathways, PPI analysis was employed. Strong interactions were revealed among the 12 proteins of the macrophage activity regulation signalling pathway, and

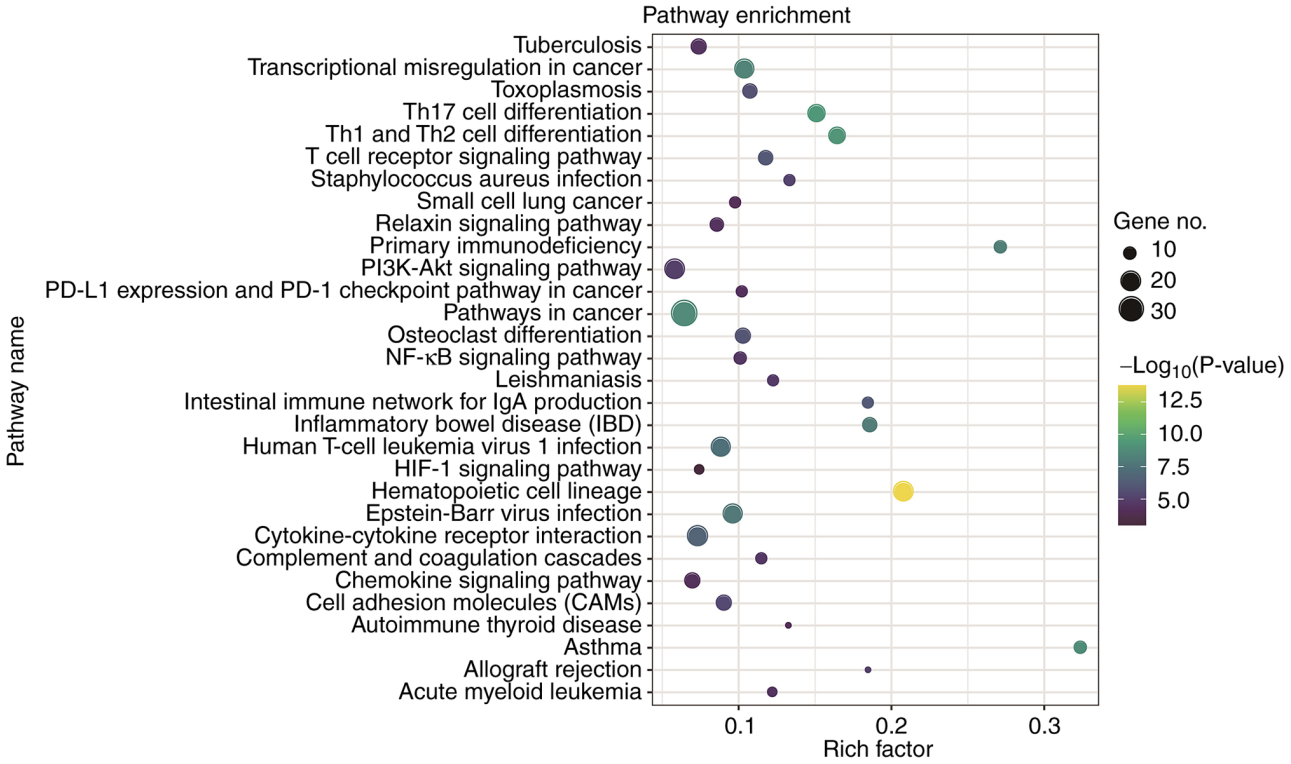

Figure 4. Pathway enrichment of DEGs using KEGG analysis. KEGG enrichment analysis of the top 30 enriched pathways of the shared DEGs. The x-axis indicates the rich factor, and the y-axis indicates the top 30 enriched KEGG pathways. KEGG, Kyoto Encyclopaedia of Genes and Genomes; DEGs, differentially expressed genes.

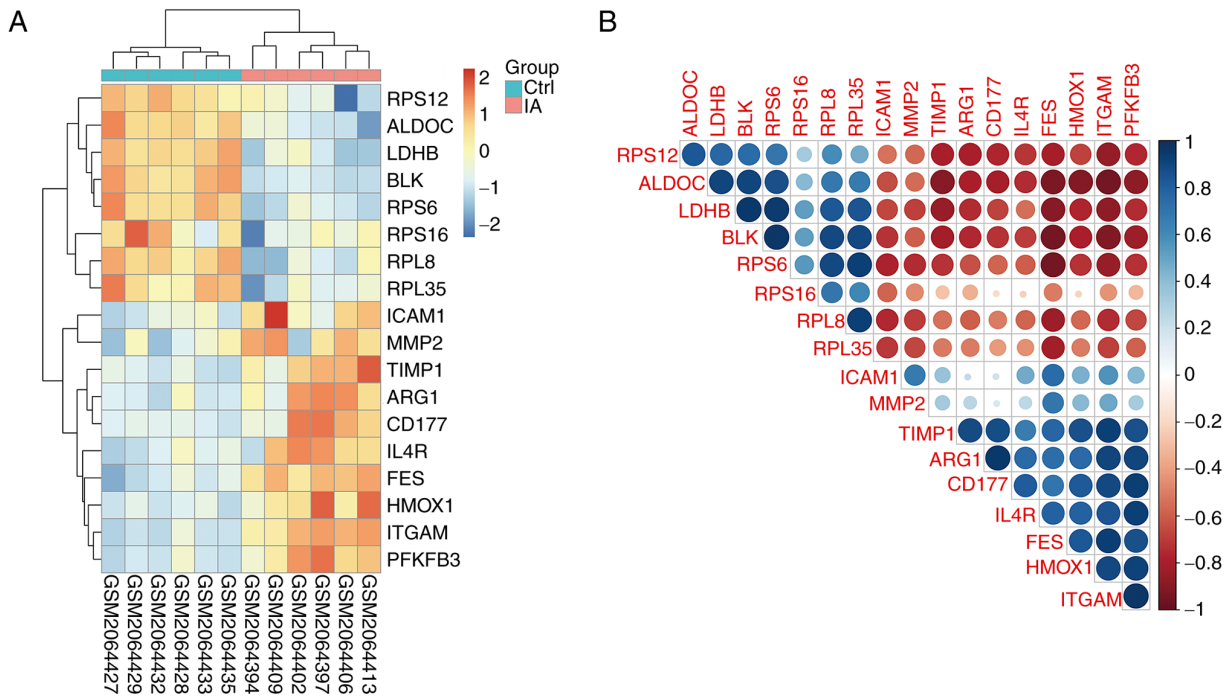

Figure 5. Correlations between macrophage activity and hypoxia-inducible factor 1 signalling pathway-associated genes in blood samples from patients with IA and controls. (A) Differences in the gene expression levels between healthy controls and patients with IA. The colour scale from blue to red represents the process of downregulation to upregulation as number of standard deviations from mean expression. (B) Correlations of gene expression levels in blood samples from patients with IA and controls. The correlation coefficient ranges from -1 (red colour) to +1 (blue colour). The red colour represents negative correlations. The blue colour represents positive correlations. Larger circles indicate greater correlation. IA, invasive aspergillosis.

there were also interactions among the eight proteins of the HIF-1 signalling pathway. However, there was no PPI between the macrophage activity regulation and HIF-1 signalling pathways (Fig. 7).

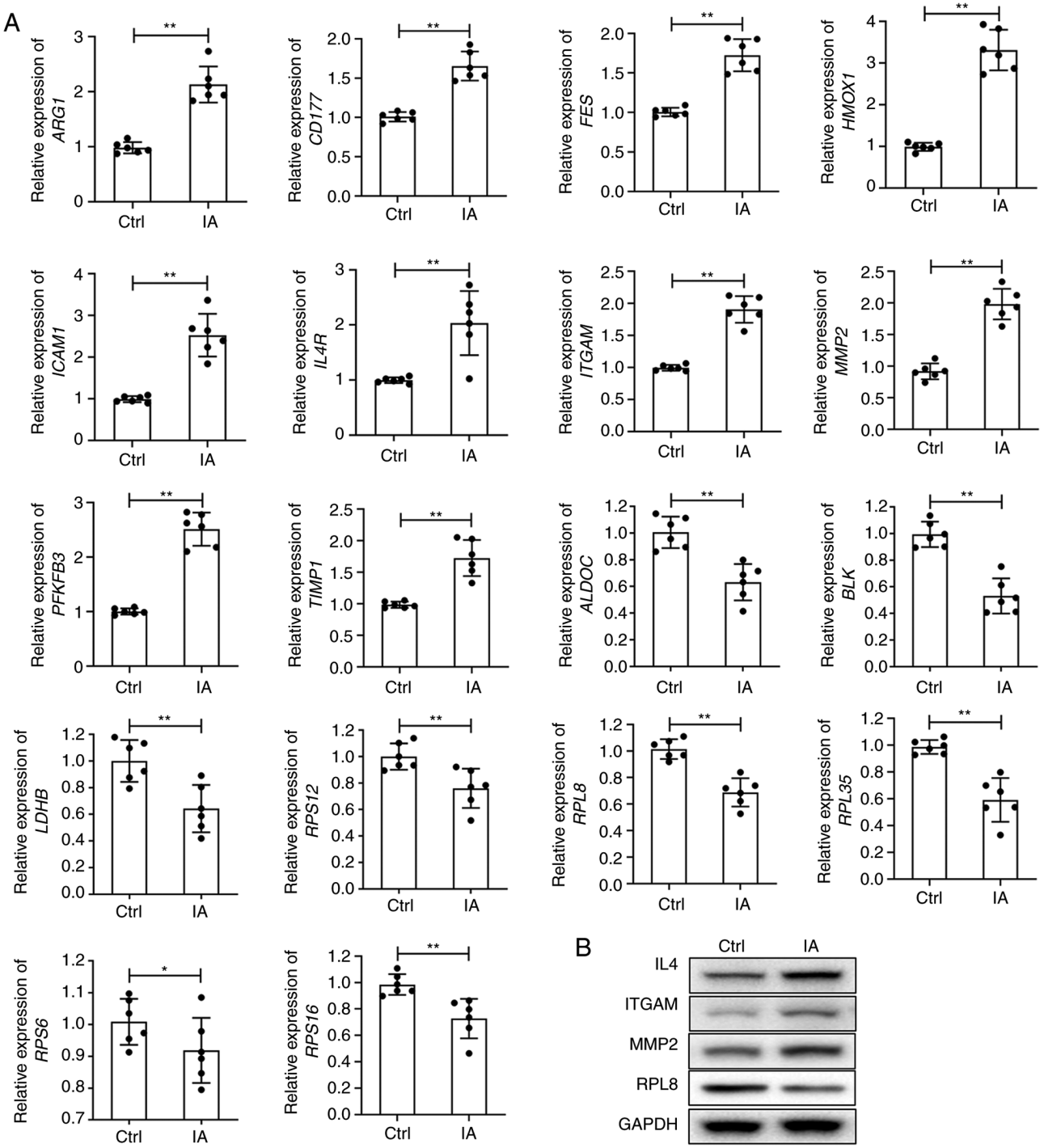

Figure 6. Expression levels of genes associated to the regulation of macrophage activity and the HIF-1 signalling pathway. (A) Reverse transcription-quantitative PCR analysis of the mRNA expression levels of genes regulating the macrophage activity signalling pathway and the HIF-1 signalling pathway in healthy controls and patients with IA. (B) The protein expression levels of IL4, ITGAM, MMP2 and RPL8 as well as a GAPDH control were determined through western blotting. *P<0.05 and **P<0.01 vs. the control group (n=5). HIF-1, hypoxia-inducible factor 1; Ctrl, control; IA, invasive aspergillosis.

Discussion

Galactomannan assay, PCR, β-D-glucan testing and biopsy are the primary methods of diagnosing aspergillosis. The lack of clinical statistical data and a diagnostic consensus are the main reasons for the severity and high mortality of IA; however, identification and rapid screening of simple biomarkers would allow for an early diagnosis. Understanding the molecular mechanisms underlying the pathogenesis of IA is required. Although a number of prognostic models have been proposed, the majority of the models are based on clinical parameters and lack accuracy. Therefore, the pre-diagnosis of IA needs to be accurate, and improved IA-specific biomarkers are required. Improved biomarkers will provide more accurate clinical information that could enhance decision-making for patient management (29).

Bioinformatics analysis was performed to identify the correlation analysis modules associated to the diagnosis of IA. The macrophage activity regulation and HIF-1 signalling

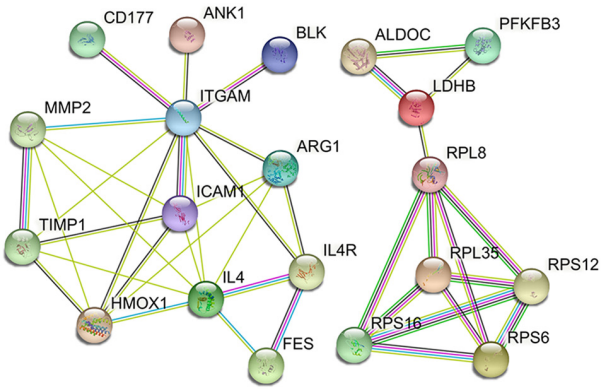

Figure 7. PPI network of the signalling molecules regulating macrophage activity and HIF-1 signalling pathways. The PPI network was analysed with The Search Tool for the Retrieval of Interacting Genes website. The 12 nodes on the left represent the signalling molecules regulating the macrophage activity signalling pathways, and the eight nodes on the right represent the eight signalling molecules in the HIF-1 signalling pathways; the line between two nodes indicates that there was a PPI between the two molecules. PPI, protein-protein interaction; HIF-1, hypoxia-inducible factor 1.

pathways were revealed to be significantly upregulated and downregulated, respectively, in patients with IA compared with that in healthy individuals. For further analysis of the correlation between macrophage activation and HIF-1 signalling pathways, blood samples were collected from allogeneic haematopoietic stem cell transplant recipients and patients receiving myelosuppressive chemotherapy (15). Therefore, the patients with IA were immunocompromised individuals. For patients with immunosuppression, increased macrophage activity may be a feedback mechanism. However, in the present study, the control group included patients with immunosuppression but without IA, and the macrophage activity in these individuals was not increased, thereby excluding the possibility that increased macrophage activity in the IA group was a compensatory response to immunosuppression. This suggests that the increased macrophage activity in patients with IA was an innate immune response to *Aspergillus* infection, which is consistent with the experimental results of previous studies (30-32). Tan *et al* (33) reported that macrophages exposed to lysyl oxidase like 4 *in vitro* can cause an immunosuppressive phenotype, activate the expression of the programmed cell death ligand 1 and inhibit the function of $CD8^+$ T cells. Fecher *et al* (34) reported that after infection with *Histoplasma capsulatum*, the increased activity of transcription factor cAMP response element-binding protein in HIF-1α-knockout mice further increased the production of IL-10 in macrophages (34). In the present study, the macrophage activation pathway was upregulated in the IA group compared with the control group. There are numerous molecules that regulate macrophage activity, of which HIF-1 has attracted attention. For example, Fecher *et al* (34) demonstrated that HIF-1α could promote macrophages to prevent fungal growth by inhibiting the production of IL-10 by macrophages. Additionally, studies have demonstrated that in a mouse model of *Aspergillus fumigatus* infection, mTOR-mediated HIF-1α activation is necessary for macrophage glycolysis activation and its role in controlling the growth of *Aspergillus fumigatus* (30,35). In addition, ω-alkynyl arachidonic acid polarizes macrophages to the M2 type by interfering with HIF-1α and pyruvate kinase (36). These previous studies have demonstrated that HIF-1α was necessary in the polarization of macrophages to the M1 type. Therefore, clarifying the expression profiles of the HIF-1 signalling pathway in patients with IA is required to understand the molecular mechanism underlying this process. The present study revealed that the HIF-1 signalling pathway was significantly downregulated in patients with IA compared with that of controls. This suggested that the activation of macrophages in patients with IA may be considered an M2-type activation. Therefore, although the activity of the macrophages in patients with IA was increased compared with that in the control group, the patients were immunosuppressed and could not eliminate the *Aspergillus* infection. Further experimental research is needed to confirm this hypothesis. At the same time, the present study results may indicate that the root cause of *Aspergillus* infection was the inhibition of HIF-1 expression in the patients with IA. Therefore, it is necessary to clarify the molecular biological mechanism causing HIF-1 inhibition with further in-depth research on this topic.

In the PPI analysis, there was no interaction between the macrophage activity regulation and HIF-1 signalling pathways. This indicated that the regulation of macrophage function by HIF-1 was not due to a direct interaction, but was mediated by intermediate signalling molecules, and future research in this field should focus on identifying these molecular signals.

In conclusion, a comprehensive bioinformatic analysis of the gene expression profiles of blood samples from patients with IA and patients without IA or healthy individuals was conducted, and 735 DEGs were identified. There were 18 co-expressed genes belonging to macrophage activation and HIF-1 signalling pathways. The present study indicated that downregulation of the HIF-1 signalling pathway and upregulation of macrophage activity may be the reason for *Aspergillus* infection and could be used as biomarkers for the prediction and diagnosis of IA.

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Availability of data and materials

The bioinformatics datasets generated and/or analysed during the current study are available in the GEO repository (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE78000). Other datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YM, YZ and MW conceived and designed the study. YH, FC and JQ analysed and interpreted the data. MW and YM drafted the manuscript. All authors have read and approved the final version of the manuscript. YM and MW confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Patients with IA and controls provided written informed consent prior to using their blood samples in the present study. The current study was approved by the Ethics Committee of The First Affiliated Hospital of Henan University of Science and Technology (approval no. HUST2034532; Luoyang, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Park HS, Jun SC, Han KH, Hong SB and Yu JH: Diversity, application, and synthetic biology of industrially important aspergillus fungi. Adv Appl Microbiol 100: 161-202, 2017.
2. Becker KL, Ifrim DC, Quintin J, Netea MG and van de Veerdonk FL: Antifungal innate immunity: Recognition and inflammatory networks. Semin Immunopathol 37: 107-116, 2015.
3. Takakura S: Zygomycosis. Nihon Rinsho 66: 2356-2361, 2008 (In Japanese).
4. Cadena J, Thompson GR III and Patterson TF: Invasive aspergillosis: Current strategies for diagnosis and management. Infect Dis Clin North Am 30: 125-142, 2016.
5. Cadena J, Thompson GR III and Patterson TF: Aspergillosis: Epidemiology, diagnosis, and treatment. Infect Dis Clin North Am 35: 415-434, 2021.
6. Latge JP and Chamilos G: Aspergillus fumigatus and aspergillosis in 2019. Clin Microbiol Rev 33: e00140-18, 2019.
7. Oren I and Goldstein N: Invasive pulmonary aspergillosis. Curr Opin Pulm Med 8: 195-200, 2002.
8. Darling BA and Milder EA: Invasive aspergillosis. Pediatr Rev 39: 476-478, 2018.
9. Verma N, Singh S, Syal A, Pradhan P, Singh M and Singh M: Invasive aspergillosis is a critical determinant of mortality in cirrhosis: A systematic review with meta-analysis. Med Mycol 59: 1092-1100, 2021.
10. Arvanitis M and Mylonakis E: Diagnosis of invasive aspergillosis: Recent developments and ongoing challenges. Eur J Clin Invest 45: 646-652, 2015.
11. Dhesi Z, Herbst S and Armstrong-James D: Transcript profiling of the murine immune response to invasive aspergillosis. Methods Mol Biol 845: 435-444, 2012.
12. Wang M, Zhang M, Qiu J, Liu C, Lou Y, Wang T, Zhang Y and Mao Y: PU.1-CD23 signaling mediates pulmonary innate immunity against Aspergillus fumigatus infection by driving inflammatory response. BMC Immunol 24: 4, 2023.
13. Fliesser M, Morton CO, Bonin M, Ebel F, Hünniger K, Kurzai O, Einsele H and Löffler J: Hypoxia-inducible factor 1α modulates metabolic activity and cytokine release in anti-Aspergillus fumigatus immune responses initiated by human dendritic cells. Int J Med Microbiol 305: 865-873, 2015.
14. Morris NL, Michael DN, Crotty KM, Chang SS and Yeligar SM: Alcohol-Induced glycolytic shift in alveolar macrophages is mediated by hypoxia-inducible factor-1 Alpha. Front Immunol 13: 865492, 2022.
15. Dix A, Czakai K, Springer J, Fliesser M, Bonin M, Guthke R, Schmitt AL, Einsele H, Linde J and Löffler J: Genome-Wide expression profiling reveals S100B as biomarker for invasive aspergillosis. Front Microbiol 7: 320, 2016.
16. Wang Y, Sun FZ, Lin W and Zhang SQ: AC-PCoA: Adjustment for confounding factors using principal coordinate analysis. PLoS Comput Biol 18: e1010184, 2022.
17. Delbeke H, Casteels I and Joossens M: DNA extraction protocol impacts ocular surface microbiome profile. Front Microbiol 14: 1128917, 2023.
18. Zhan Z, Chen Y, Duan Y, Li L, Mew K, Hu P, Ren H and Peng M: Identification of key genes, pathways and potential therapeutic agents for liver fibrosis using an integrated bioinformatics analysis. PeerJ 7: e6645, 2019.
19. Haldermans P, Shkedy Z, Van Sanden S, Burzykowski T and Aerts M: Using linear mixed models for normalization of cDNA microarrays. Stat Appl Genet Mol Biol 6: Article 19, 2007.
20. Ding J and Zhang Y: Analysis of key GO terms and KEGG pathways associated with carcinogenic chemicals. Comb Chem High Throughput Screen: Dec 18, 2017 (Epub ahead of print).
21. Chen L, Zhang YH, Lu G, Huang T and Cai YD: Analysis of cancer-related lncRNAs using gene ontology and KEGG pathways. Artif Intell Med 76: 27-36, 2017.
22. Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC and Lempicki RA: DAVID: Database for annotation, visualization, and integrated discovery. Genome Biol 4: P3, 2003.
23. Goutzelas Y, Kontou P, Mamuris Z, Bagos P and Sarafidou T: Meta-analysis of gene expression data in adipose tissue reveals new obesity associated genes. Gene 818: 146223, 2022.
24. Wallach T, Schellenberg K, Maier B, Kalathur RK, Porras P, Wanker EE, Futschik ME and Kramer A: Dynamic circadian protein-protein interaction networks predict temporal organization of cellular functions. PLoS Genet 9: e1003398, 2013.
25. Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, Doncheva NT, Legeay M, Fang T, Bork P, *et al*: The STRING database in 2021: Customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Res 49(D1): D605-D612, 2021.
26. Liu J, Zhou S, Li S, Jiang Y, Wan Y, Ma X and Cheng W: Eleven genes associated with progression and prognosis of endometrial cancer (EC) identified by comprehensive bioinformatics analysis. Cancer Cell Int 19: 136, 2019.
27. Chen Y, Li H, Lai L, Feng Q and Shen J: Identification of common differentially expressed genes and potential therapeutic targets in ulcerative colitis and rheumatoid arthritis. Front Genet 11: 572194, 2020.
28. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
29. Johnson G, Ferrini A, Dolan SK, Nolan T, Agrawal S, Doyle S and Bustin SA: Biomarkers for invasive aspergillosis: The challenges continue. Biomark Med 8: 429-451, 2014.
30. Margalit A and Kavanagh K: The innate immune response to aspergillus fumigatus at the alveolar surface. FEMS Microbiol Rev 39: 670-687, 2015.
31. Seifert M, Nairz M, Schroll A, Schrettl M, Haas H and Weiss G: Effects of the aspergillus fumigatus siderophore systems on the regulation of macrophage immune effector pathways and iron homeostasis. Immunobiology 213: 767-778, 2008.
32. Heinekamp T, Schmidt H, Lapp K, Pahtz V, Shopova I, Koster-Eiserfunke N, Kruger T, Kniemeyer O and Brakhage AA: Interference of aspergillus fumigatus with the immune response. Semin Immunopathol 37: 141-152, 2015.
33. Tan HY, Wang N, Zhang C, Chan YT, Yuen MF and Feng Y: Lysyl Oxidase-Like 4 Fosters an immunosuppressive microenvironment during hepatocarcinogenesis. Hepatology 73: 2326-2341, 2021.
34. Fecher RA, Horwath MC, Friedrich D, Rupp J and Deepe GS Jr: Inverse correlation between IL-10 and HIF-1alpha in macrophages infected with histoplasma capsulatum. J Immunol 197: 565-579, 2016.
35. Goncalves SM, Duarte-Oliveira C, Campos CF, Aimanianda V, Ter Horst R, Leite L, Mercier T, Pereira P, Fernandez-Garcia M, Antunes D, *et al*: Phagosomal removal of fungal melanin reprograms macrophage metabolism to promote antifungal immunity. Nat Commun 11: 2282, 2020.
36. Cheng Y, Feng Y, Xia Z, Li X and Rong J: ω-Alkynyl arachidonic acid promotes anti-inflammatory macrophage M2 polarization against acute myocardial infarction via regulating the cross-talk between PKM2, HIF-1α and iNOS. Biochim Biophys Acta Mol Cell Biol Lipids 1862: 1595-1605, 2017.