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ORIGINAL RESEARCH

Identification of Biomarkers Associated with Oxidative Stress and Immune Cells in Acute Pancreatitis

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Purpose: Oxidative stress promotes disease progression by stimulating the humoral and cellular immune responses. However, the molecular mechanisms underlying oxidative stress and immune responses in acute pancreatitis (AP) have not been extensively studied.

Patients and Methods: We analyzed the GSE194331 dataset and oxidative stress-related genes (OSRGs). We identified differentially expressed immune cell-associated OSRGs (DE-ICA-OSRGs) by overlapping key module genes from weighted gene co-expression network analysis, OSRGs, and DEGs between AP and normal samples. Functional enrichment analysis was performed to investigate the functions of DE-ICA-OSRGs. We then filtered diagnostic genes using receiver operating characteristic curves and investigated their molecular mechanisms using single-gene set enrichment analysis (GSEA). We also explored the correlation between diagnostic genes and differential immune cells. Finally, we constructed a transcription factor-microRNA-messenger RNA (TF-miRNA-mRNA) network of biomarkers.

Results: In this study, three DE-ICA-OSRGs (ARG1, NME8 and VNN1) were filtered by overlapping key module genes, OSRGs and DEGs. Functional enrichment results revealed that DE-ICA-OSRGs were involved in the cellular response to reactive oxygen species and arginine biosynthesis. Latterly, a total of two diagnostic genes (ARG1 and VNN1) were derived and their expression was higher in the AP group than in the normal group. The single-gene GSEA enrichment results revealed that diagnostic genes were mainly enriched in macroautophagy and Toll-like receptor signaling pathways. Correlation analysis revealed that CD8 T cells, resting memory T CD4 cells, and resting NK cells were negatively correlated with ARG1, and neutrophils were positively correlated with ARG1, which was consistent with that of VNN1. The TF-miRNA-mRNA regulatory network included 11 miRNAs, 2 mRNAs, 10 transcription factors (TFs), and 26 pairs of regulatory relationships, like NFKB1-has-miR-2909-VNN1.

Conclusion: In this study, two immune cell oxidative stress-related AP diagnostic genes (ARG1 and VNN1) were screened to offer a new reference for the diagnosis of patients with AP.

Keywords: acute pancreatitis, oxidative stress, diagnosis, immunity

Introduction

Acute pancreatitis (AP) is a potentially life-threatening acute inflammatory disorder of the pancreas, and is a potentially life-threatening disease.[1] The clinical presentation of AP ranges from mild, which is found–70-80% of cases) to severe (associated with a high mortality rate from AP-related complications).[2,3] The mortality rate varies from 3% for acute edematous pancreatitis to 15% in cases with pancreatic necrosis.[4,5] AP should be suspected in patients with severe acute pain in the middle or left upper abdomen with posterior radiation. However, diagnosis requires biochemical or radiological evidence, such as computed tomography. Although much progress has been made in the understanding of AP, its pathogenesis is not fully understood. A history of alcohol intake, cholelithiasis, metabolic disorders, malignancy, or infection is associated with the incidence of AP. Additionally, AP will slowly progress to pancreatic cancer (PC), which has a poor prognosis with a 5-year survival rate of less than 0.07.[6,7] Currently, PDK inhibitors are commonly used to treat pancreatic cancer, while the development of novel PDK inhibitors is a research priority.[8–10] However, these traditional diagnostic methods have long

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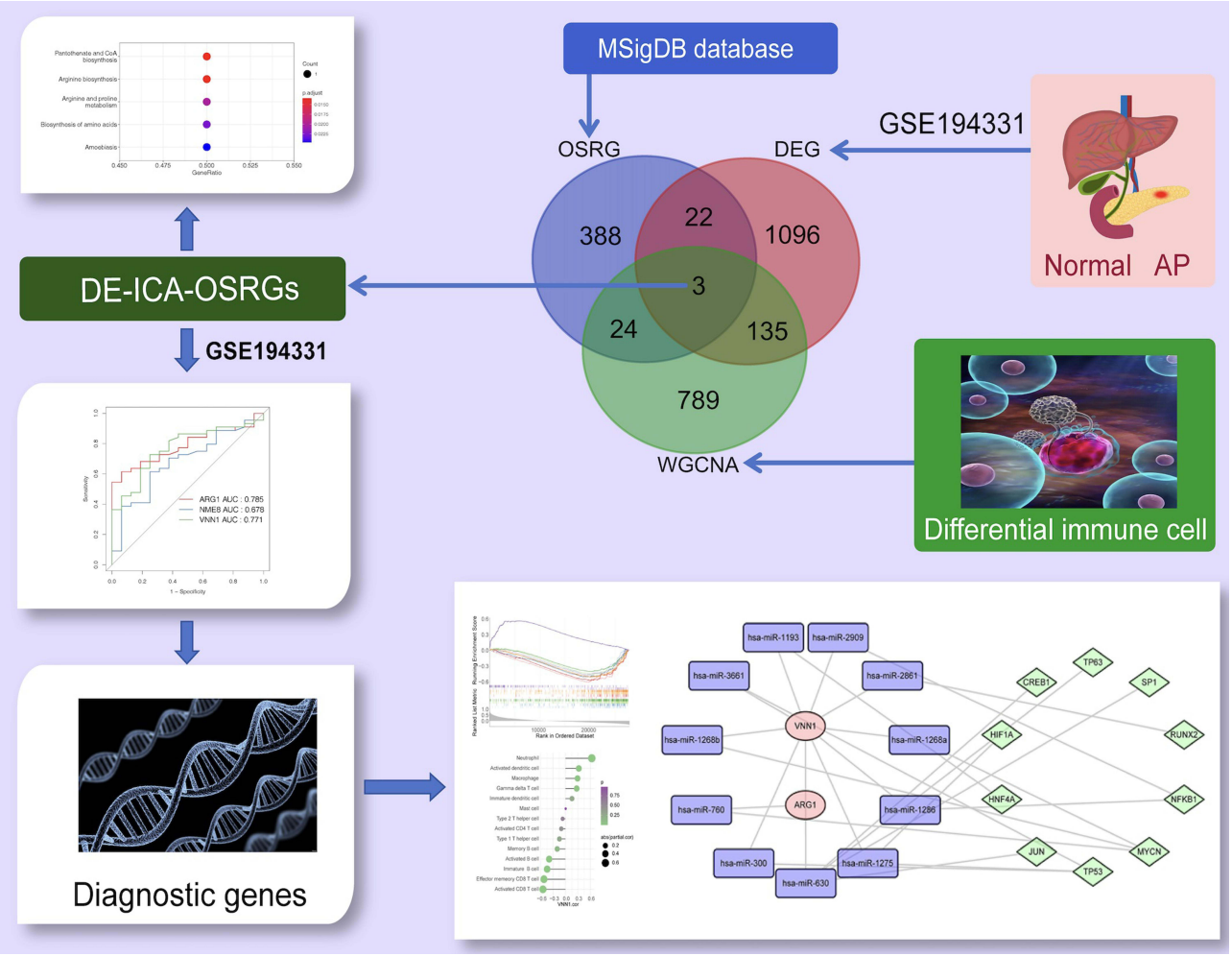
Graphical Abstract



detection cycles and complex evaluation systems and are unable to quickly assess the condition. Biomarkers as predictive indicators of individual factors have the advantage of simplicity.

Oxidative stress is associated with several models of acute and chronic pancreatitis and has been shown to induce apoptosis and necrosis in pancreatic acinar cells. The pathogenesis of AP is still unclear, while the oxidative stress pathway is recognized as a classical underlying mechanism in AP.[11] Oxidative stress in AP leads to pathological calcium signaling and mitochondrial dysfunction, leading to pancreatic acinar cell necrosis and AP.[12] In addition, oxidative stress may amplify the inflammatory cytokine response once AP occurs.[13] At present, genes related to oxidative stress have been diagnosed as biomarkers in a variety of inflammatory diseases, but are unknown in AP. Various immune cells begin to infiltrate the pancreatic tissue within minutes of the onset of AP, which is closely related to the prognosis and severity of AP.[14] Numerous studies have shown that immune cells alleviate AP through genetic interventions and pharmacology, bringing hope for the treatment of AP.[15,16] As an emerging therapy, immunotherapy has great prospects. However, owing to the complexity of the underlying immune mechanisms of AP, a great deal of work is required to elucidate the regulatory networks. Oxidative stress has been shown to promote the progression of non-alcoholic steatohepatitis by stimulating humoral and cellular immune responses.[17] In the arterial wall, expanded oxidative stress can increase the accumulation of modified lipoproteins, change macrophage metabolism, and cause atherosclerotic immune cell infiltration.[18] It is unknown in the AP whether oxidative stress modulates the immune response to play a corresponding role.

Based on the transcriptome data of patients with AP in a public database, this study screened two diagnostic genes related to oxidative stress through a series of bioinformatics methods, including gene differential expression analysis and weighted gene co-expression network analysis (WGCNA), and evaluated the diagnostic value of the screened diagnostic genes, which provided a new biomarker for the diagnosis of this disease. In addition, we explored the correlation between these diagnostic genes and immune function as well as the related pathways and biological processes enriched by them, providing a theoretical basis for the Discussion of the molecular mechanism of AP based on oxidative stress.

Materials and Methods

Source of Data

The GSE194331 dataset, including RNA-seq data from 87 AP and 32 normal samples, was sourced from the Gene Expression Omnibus (GEO) database. Next, the normal and AP groups were classified randomly into training and validation cohorts at a 1:1 ratio (training cohort: 16 healthy controls (HC) and 44 AP samples; validation cohort: 16 HC and 43 AP samples). Subsequently, 437 oxidative stress-related genes (OSRGs) (Supplementary Table 1) were sourced from the MSigDB database.

Identification of Differentially Expressed Genes (DEGs)

First, DEGs between the AP and normal groups were selected using the DESeq2 package (v 1.32.0)[19] in the training cohort with an adjusted P value < 0.05 and $|\log_2$Fold Change (FC)$|$ > 1. The results of the differential analysis are illustrated using a volcano map and a heatmap. The heatmap only illustrates the expression of the top 100 DEGs.

Immuno-Microenvironmental Analysis

The proportion of 22 immune cell subtypes was computed for each sample using the CIBERSORT algorithm (v 1.03)[20] in the training cohort. Correlation analysis of the ratio of immune cells was performed. Subsequently, the differential immune cells between the AP and normal groups were compared and a box-plot was plotted.

Filtering for Key Module Genes by WGCNA

A co-expression network was constructed using WGCNA (v. 1.69)[21] on the training cohort. First, the samples were clustered, and outliers were removed to ensure the accuracy of the analysis. The optimal soft threshold (β) was chosen to approximate the scale-free distribution of the network. A cluster dendrogram was obtained by calculating the adjacency and similarity. The modules were partitioned using a dynamic tree cutting algorithm. Next, we evaluated the correlation between each module and the differential immune cells and selected the module with the highest absolute value of correlation coefficient with each differential immune cell as the key module (non-MEgrey, $P < 0.05$, and $|cor| \geq 0.9$). Finally, the genes in the key modules with $|GS| > 0.4$ and $|MM| > 0.8$ were identified as key module genes for follow-up analysis.

Screening for Differentially Expressed-Immune Cell Associated (ICA)-OSRGs (DE-ICA-OSRGs) and Functional Enrichment Analysis

The DE-ICA-OSRGs were screened for overlapping OSRGs, key module genes, and DEGs. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses of DE-ICA-OSRGs were performed using the clusterProfiler package (v 4.4.3).[22] A significant P analysis result was defined as an adjusted P-value < 0.05.

Screening and Expression Validation of Diagnostic Genes

To explore the ability of DE-ICA-OSRGs to distinguish between the normal and AP groups, we plotted the receiver operating characteristic (ROC) curves for these genes. The AUC values of the ROC curve were computed using the pROC package (v. 1.16.2)[23] to screen for the diagnostic genes. Finally, the expression levels of the diagnostic genes between the normal and AP groups were compared between the training and validation cohorts.

Single-Gene GSEA Analysis

Single-gene GSEA was performed to identify the enriched regulatory pathways and biological functions of each diagnostic gene with a Normalized Enrichment Score (NES)| > 1, adjusted *P* value < 0.05, and q < 0.25. The top 10 results for GO and KEGG significance were visualized separately.

Immuno-Infiltration and Correlation Analysis

To investigate immune cell infiltration in the AP and normal samples, we determined the infiltration of each immune element in all samples using the ssGSEA algorithm. Heatmaps and box plots of the differences in immune elements between the AP and normal samples were plotted to demonstrate the infiltration results. Finally, the correlation between each diagnostic gene and the differential immune elements was analyzed using the Spearman method. We also computed the correlation between differential immune cells and each diagnostic gene.

Construction of a TF-miRNA-mRNA Network of Transcription Factors (TFs)

miRNAs corresponding to diagnostic genes were predicted using the miRWalk online tool. Subsequently, the TFs targeting the miRNAs were predicted using TransmiR v2.0 database.[24] Finally, and the TF-miRNA-mRNA regulatory network was obtained based on the miRNA-TF regulatory network and miRNA-mRNA regulatory network.

Statistical Analysis

All bioinformatics analyses were performed using R language. Pearson's correlation analysis was used to conduct correlation analysis.

Results

Identification of DEGs and Immune Infiltration Analysis

In total, 1256 DEGs, including 1076 upregulated and 180 downregulated genes, were identified between the AP and normal groups (Figure 1A and Supplementary Table 2). The heatmap illustrates the expression of the top 100 DEGs between AP and HC samples (Figure 1B). Bars represent the proportion of 22 immune cells in each sample (Figure 1C). The Results illustrated that resting memory CD4 T cells had the highest positive association with neutrophils, whereas gamma delta T cells had the highest negative association with follicular helper T cells (Figure 1D and E). In total, six immune cell types (CD8 + T cells, resting mast cells, resting NK cells, neutrophils, resting memory CD4 + T cells, and activated NK cells) were significantly different between the AP and normal groups (Figure 1F).

Identification of Key Module Genes

To probe for genes associated with differential immune cells, we performed WGCNA analysis. The sample clustering results showed that there were no outliers (Figure 2A). With a soft threshold equal to 22 ($R^2 = 0.80$ (red line) and average connectivity close to 0, the interactions between genes maximally conformed to a scale-free distribution (Figure 2B). Seventeen modules were identified (Figure 2C). Of these, MEgreenyellow CD8 T cells (Cor = −0.52, $P = 2\times10^{-5}$; CD8 T cells: Cor = −0.7, $P = 6\times10^{-10}$; resting NK cells: Cor = −0.41, $P = 0.001$; Neutrophils: Cor = 0.9, $P = 3\times10^{-22}$) and MEturquoise (CD8 T cells: Cor = −0.59, $P = 7\times10^{-7}$; CD8 T cells: Cor = −0.81, $P = 4\times10^{-15}$; resting NK cells: Cor = −0.46, $P = 2\times10^{-4}$ and Neutrophils: Cor = 0.9, $P = 2\times10^{-22}$) showed the highest correlation with each differential immune cell type (Figure 2D). Therefore, these two modules were considered key modules. Finally, 951 genes in the two key modules were defined as key module genes for subsequent analysis (Figure 2E and F).

Screening and Functional Enrichment of DE-ICA-OSRGs

Three DE-ICA-OSRGs (ARG1, NME8, and VNN1) were filtered by overlapping 437 OSRGs, 951 key module genes, and 1256 DEGs (Figure 3A). The results of the enrichment analysis indicated that DE-ICA-OSRGs had 223 GO entries and 5 KEGG pathways. DE-ICA-OSRGs were mainly enriched in GO entries such as cellular responses to reactive and

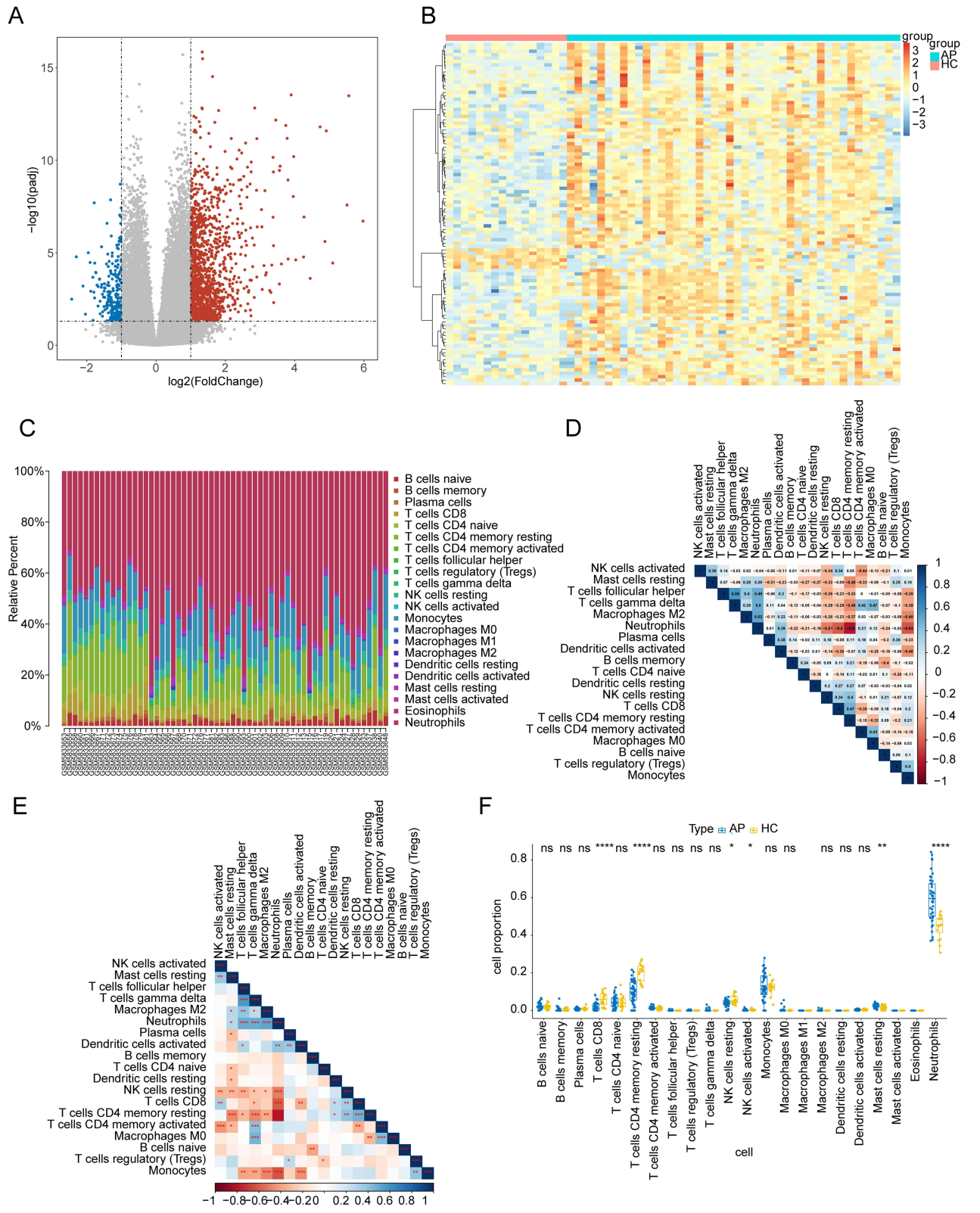


Figure 1 Identification of differentially expressed genes (DEGs) and immune infiltration analysis. (**A**) The volcano plot of DEGs between the acute pancreatitis (AP) and normal groups. (**B**) The heatmap of the top 100 DEGs between the AP and healthy control (HC) samples. (**C**) The composition of 22 kinds of immune cells. (**D**) The heatmap of the correlation between immune cells. The numbers in the square represent correlations. (**E**) The heatmap of the correlation between immune cells. (**F**) Box plot of the proportion of immune cells between the AP and normal groups.* P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001.
Abbreviation: ns, no significance.

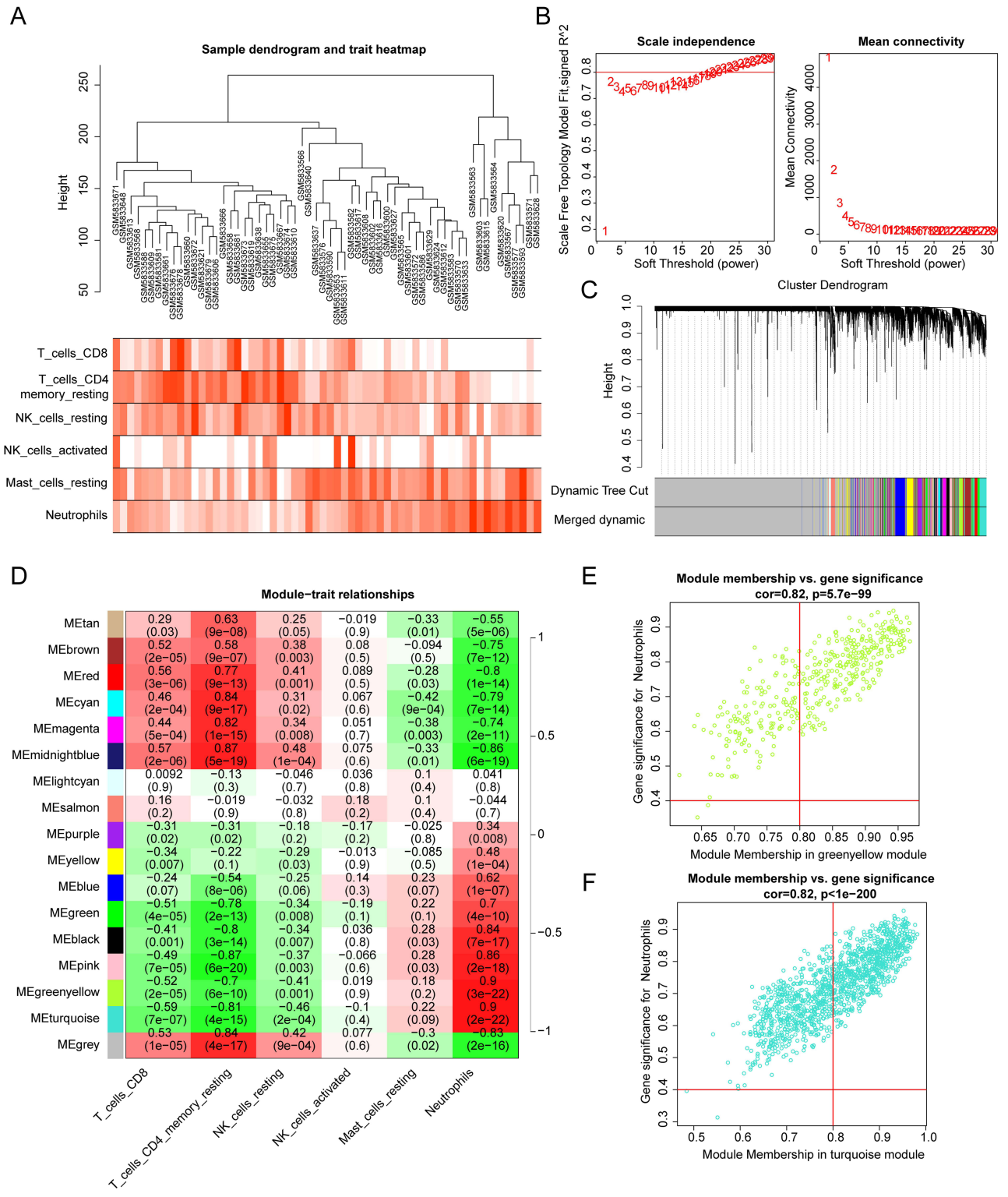


Figure 2 Weighted gene co-expression network analysis (WGCNA). (**A**) Immune cell sample clustering and phenotypic information. (**B**) Screening for suitable soft thresholds and the soft threshold is selected as 22. (**C**) The cluster dendrogram with the gene modules and module merging. (**D**)The correlation between gene modules and immune cell fraction. (**E**) The correlation with each differential immune cells shown in scatter plot in greenyellow modules. (**F**) The correlation with each differential immune cells shown in scatter plot in turquoise modules.

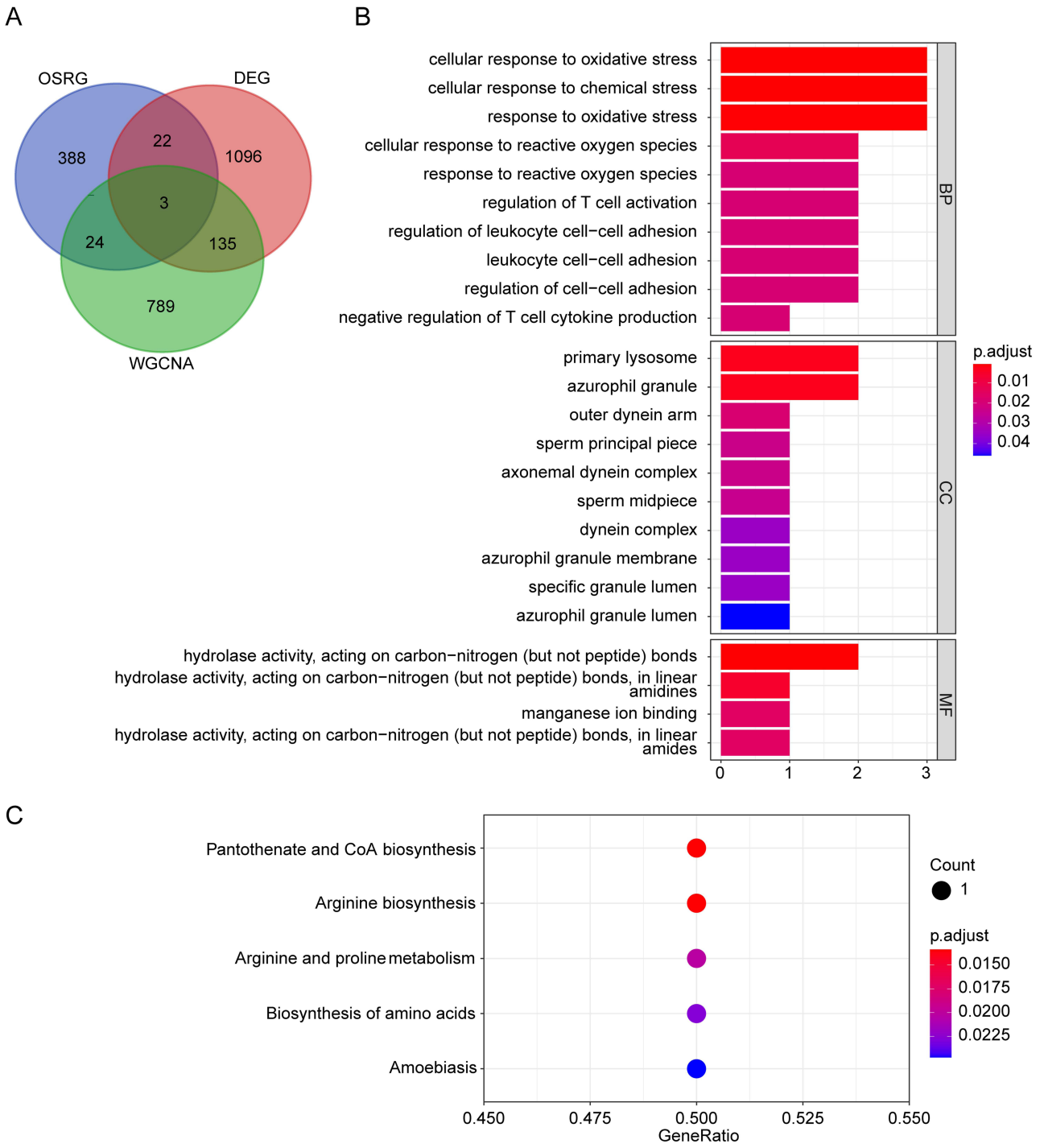


Figure 3 Screening and functional enrichment of differentially expressed-immune cell associated-oxidative stress related genes (DE-ICA-OSRGs). (**A**) The Venn map between the 437 OSRGs, 951 key module genes and 1256 DEGs. (**B**) Gene Ontology (GO) enrichment analysis of three DE-ICA-OSRGs. (**C**) Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of three DE-ICA-OSRGs.

responses to oxidative stress (Figure 3B and Supplementary Table 3). KEGG enrichment results included arginine biosynthesis, pantothenate, and CoA biosynthesis (Figure 3C and Supplementary Table 4).

Identification and Expression of Diagnostic Genes

The results of the ROC curves for the training cohort demonstrated decent diagnostic performance of the ARG1 and VNN1 genes, with AUC values greater than 0.7, while NME8 was poorly diagnosed (Figure 4A). The diagnostic effectiveness of

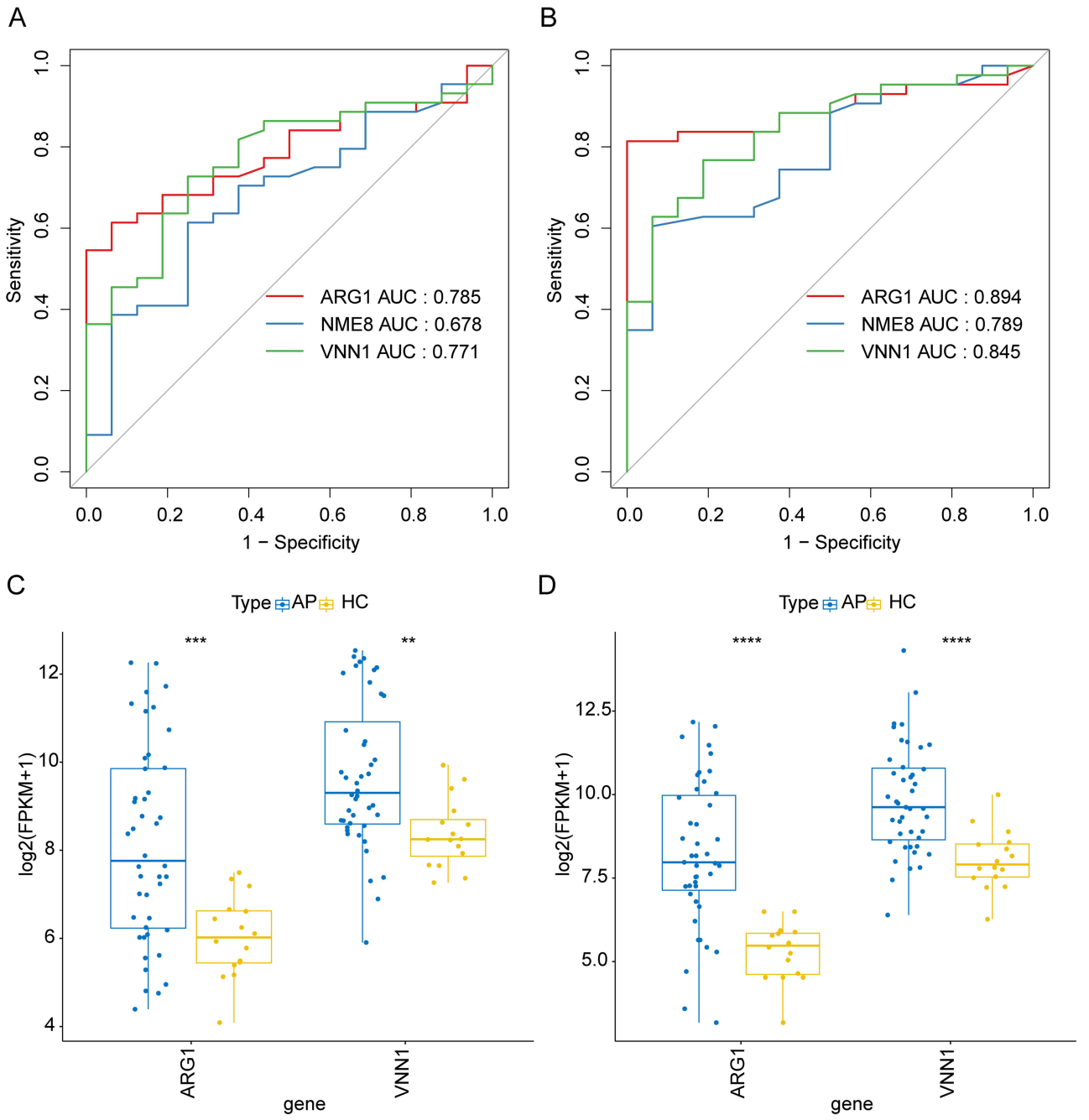


Figure 4 Identification and expression of diagnostic genes. Receiver operating characteristic (ROC) curves for three diagnostic genes in predicting the immunotherapy response. (**A**) Training cohort. (**B**) Validation cohort. The expression of ARG1 and VNN1 between AP and normal group. (**C**) Training cohort. (**D**) Validation cohort. **P < 0.01, ***P < 0.001, ****P < 0.0001.

Abbreviation: ns, no significance.

ARG1 and VNN1 was demonstrated in the validation cohort (Figure 4B). Hence, ARG1 and VNN1 were selected as diagnostic genes for this study. The expression of ARG1 and VNN1 in the AP group was significantly higher than that in the normal group in the training cohort, and the expression trend in the validation cohort was consistent (Figure 4C and D).

Single-Gene GSEA of Diagnostic Genes

Single-gene GSEA was performed to explore the regulatory pathways and molecular functions of the diagnostic genes. ARG1 was mainly enriched in GO terms such as ribonucleoprotein complex biogenesis, rRNA metabolic process, and gocc azurophil granules (Figure 5A and Supplementary Table 5); VNN1 was enriched for macroautophagy and negative regulation of

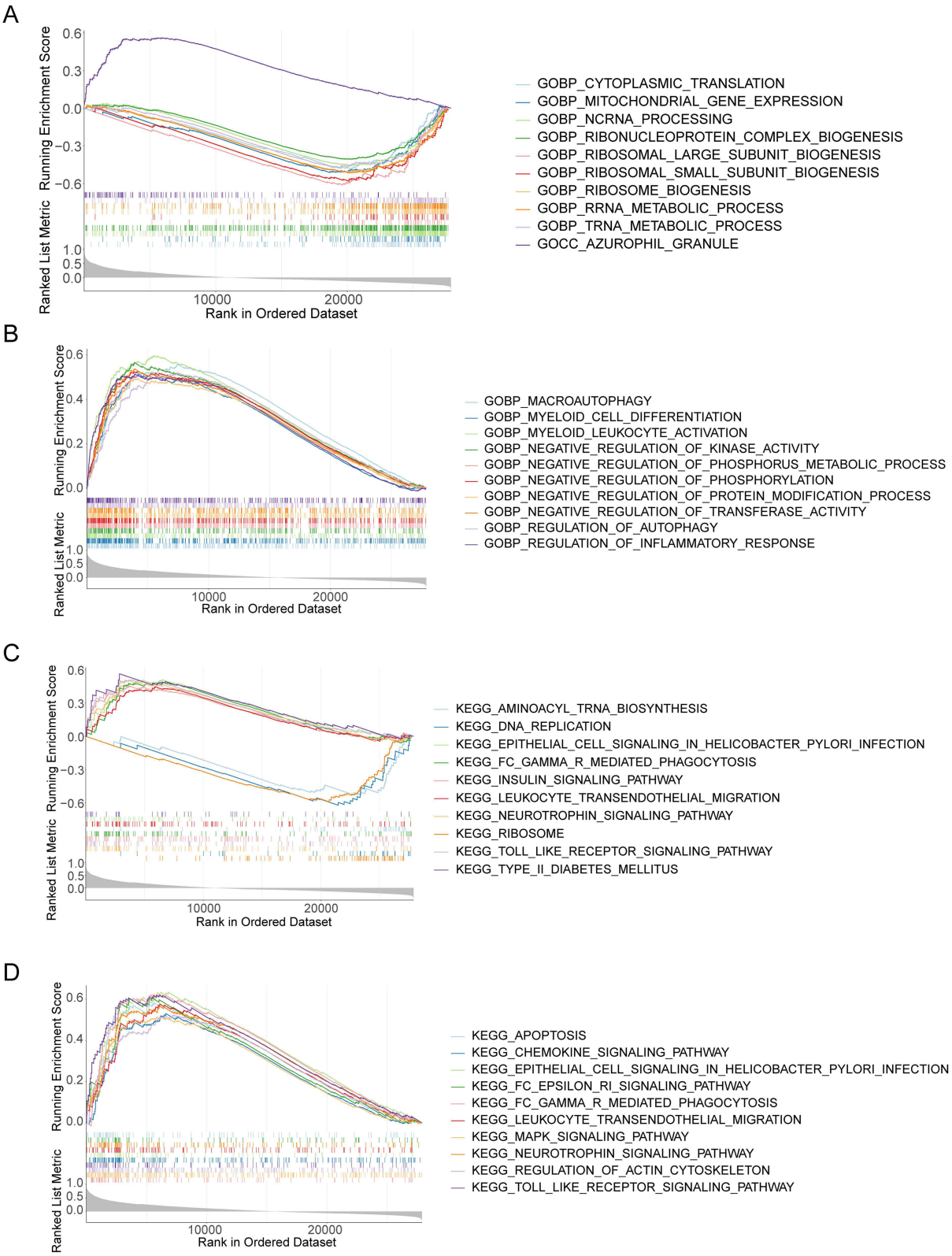


Figure 5 Gene Set Enrichment Analysis (GSEA) of diagnostic genes. (**A**) GO enrichment analysis of ARG1. (**B**) GO enrichment analysis of VNN1. (**C**) KEGG enrichment analysis of ARG1. (**D**) KEGG enrichment analysis of VNN1.

phosphorus metabolic process (Figure 5B and Supplementary Table 6). KEGG enrichment results included Toll like receptor signaling pathway and FC gamma R-mediated phagocytosis (Figure 5C and D, Supplementary Tables 7 and 8).

Correlation of Differential Immune Cells with Two Diagnostic Genes

The infiltration of immune elements in AP and normal tissues is shown in Figure 6A and B. The results of differential analysis revealed the existence of 14 differential immune elements (activated B cells, macrophages, etc). in AP and

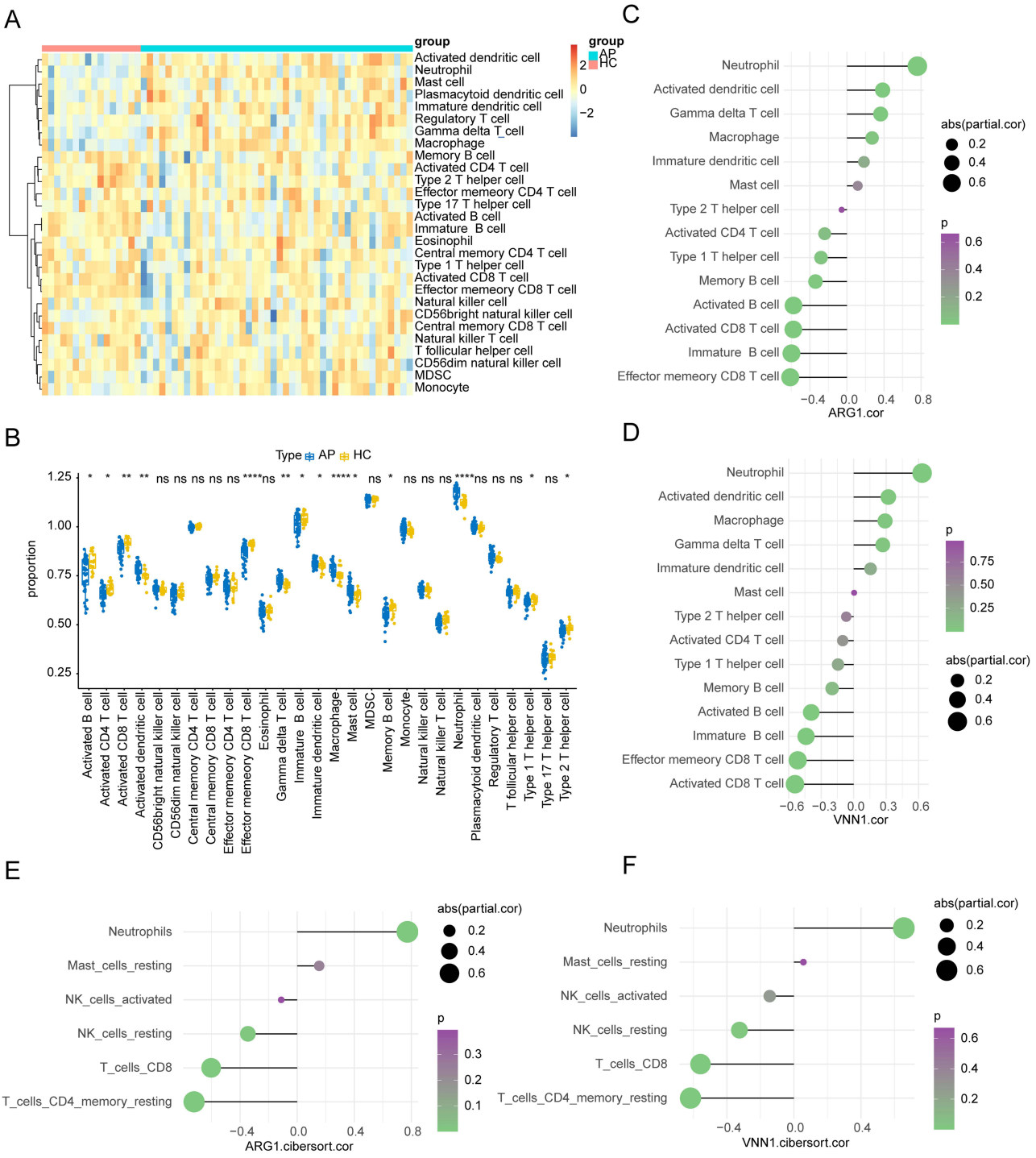


Figure 6 Correlation of differential immune cells with two diagnostic genes. (**A**) The heatmap of immune elements between AP and normal groups. (**B**) Box plot of the proportion of immune elements between the AP and normal groups. (**C**) Correlation analysis of ARG1 and immune cells. (**D**) Correlation analysis of VNN1 and immune cells. (**E**) Correlation analysis of ARG1 and differential immune cells by the CIBERSORT algorithm. (**F**) Correlation analysis of VNN1 and differential immune cells by the CIBERSORT algorithm.*P < 0.05, **P < 0.01, **** P < 0.0001.
Abbreviation: ns, no significance.

normal samples (Figure 6B). The results of the correlation analysis indicated that ARG1 and VNN1 were negatively correlated with effector memory CD8 T cells and positively correlated with neutrophil count (Figure 6C and D). Furthermore, the results of the analysis of the association with differential immune cells recognized by the CIBERSORT algorithm indicated that ARG1 and VNN1 were negatively associated with CD8 T cells, resting memory T CD4 cells, and resting NK cells, and positively correlated with neutrophils (Figure 6E and F).

TF-miRNA-mRNA Network of Diagnostic Genes

A total of 465 miRNAs, corresponding to the two diagnostic genes, were identified after prediction. Subsequently, 10 TFs corresponding to miRNAs were identified. Subsequently, a TF-miRNA-mRNA regulatory network containing 23 nodes (11 miRNAs (hsa-miR-2909, hsa-miR-630, etc)., two mRNAs (ARG1 and VNN1), and 10 TFs (CREB1, HIF1A, HNF4A, JUN, MYCN, NFKB1, RUNX2, SP1, TP53, and TP63)) with 26 edges was created (Figure 7).

Discussion

AP is a sterile inflammation of the pancreas which evokes a systemic inflammatory response syndrome with various severity.[25] Many studies have shown that oxidative stress plays a main role in the pathogenesis of pancreatic tissue injury in a murine model of AP.[26,27] In AP, it is unknown whether oxidative stress modulates the immune response to play a corresponding role. In this study, two diagnostic genes related to oxidative stress were screened using a series of bioinformatics methods, including gene differential expression analysis and WGCNA, and their diagnostic value was evaluated.

Several studies have shown that many genes are associated with macrophages and CD8 T cells in the development of AP.[28,29] In our study, two diagnostic genes, arginase 1 (ARG1) and Vanin1 (VNN1), were obtained, and their expression was higher in the AP group than in the normal group. ARG1 is not only a marker of M2 macrophages, but also a metabolic enzyme mainly located in the cytoplasm.[30,31] Research has shown that arginine is necessary for the activation and proliferation of CD8 T cells.[32,33] However, the pancreatic islets β Cell specific CD8 T cells can disrupt the production of pancreatic islets β Cells that pose a threat to the immune environment of the pancreas.[34] In a study, the ARG1 and reactive oxygen species (ROS) expressions were both enhanced in the severe acute pancreatitis (SAP) group.[35] Macrophages are the most common immune cells in pancreatic injury, and upregulation of ARG1 may indicate an increase in M2 macrophages.[36] M2 macrophages are highly common infiltrating immune cells in the matrix of pancreatic cancer, and their role in AP needs further experimental analysis.[37] Another study showed that deficiency of S100A9 in the pancreatic duct alleviate AP by targeting VNN1 mediated

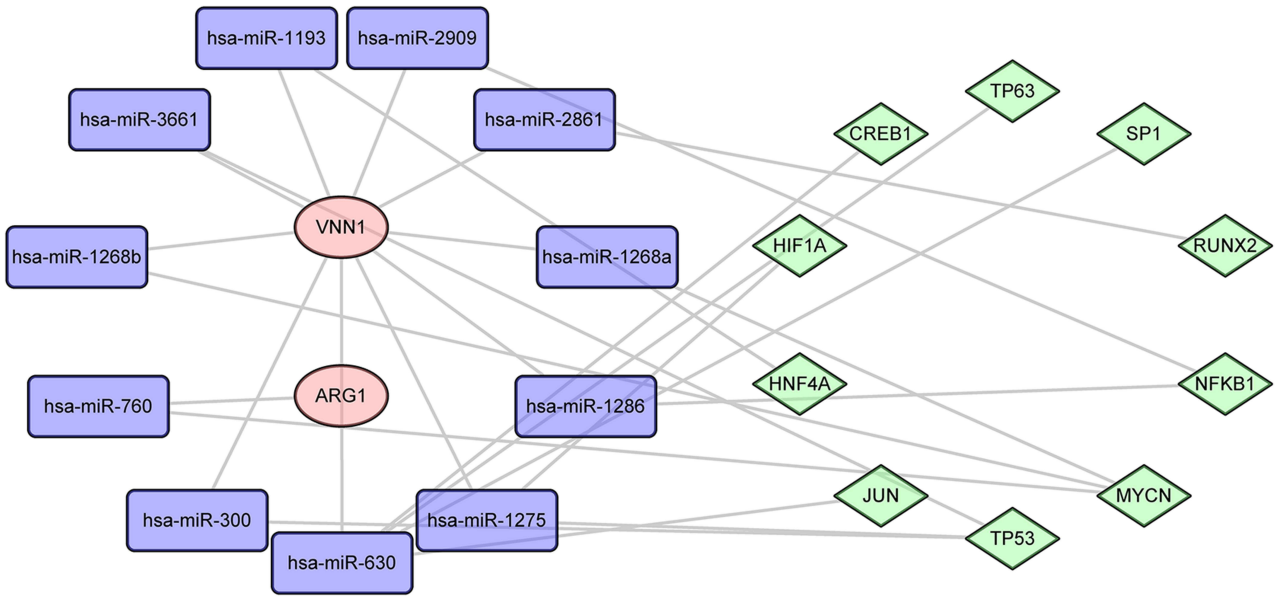


Figure 7 Construction of transcription factor-microRNA-messenger RNA (TF-miRNA-mRNA) regulatory network. Pink indicates diagnostic genes (mRNAs), purple indicates miRNA, green indicates TFs.

ROS release, which provides evidence for S100A9 as a prospective therapeutic target for AP.[38] Several reports have emphasized the crucial importance of VNN1 in pro-oxidative tissue injury through various mechanisms.[39,40] Another study observed that elevated VNN1 levels could directly up-regulate ROS generation in the islet microenvironment.[41] This is consistent with our research results.

ARG1 was mainly concentrated in mitochondrial gene expression and the Toll-like receptor signaling pathway. VNN1 was enriched in apoptosis and the mitogen-activated protein kinase(MAPK) signaling pathway. One study demonstrated the primary molecular function of estrogen-related receptor(ERR)γ as a transcriptional regulator of the mitochondrial gene expression program required for pancreatic acinar cell organelle homeostasis and function.[42] Therefore, the influence of ARG1 on mitochondrial gene expression may cause changes in reactive oxygen species, thereby affecting pancreatitis. Toll-like receptors (Tlr) signaling pathway may ship an important role in ap that attenuates organ damage and pancreatic neutrophil accumulation when Tlr2 is knocked down.[43] Another study showed that the Toll-like receptor 9/MyD88/TRAF6/NF-κB signaling pathway plays an important role in intestinal mucosal barrier injury in SAP.[44] Previous studies have verified that the injury of non-pancreatic organs in AP is primarily caused by apoptosis.[45] However, one study detected an increased expression of Bcl-2 associated X-protein (BAX) and a decrease in the B-cell lymphoma 2 protein (Bcl-2) after SAP induction.[46] Bcl-2 is an essential anti-apoptotic protein that is negatively regulated by BAX. During the progression of SAP, the p38 MAPK signaling pathway is involved in the regulation of NF-κB activation, which plays a crucial role in the inflammatory cascade.[47] Therefore, ARG1 and VNN1 may affect the development of AP through the above pathways.

There is growing evidence that the interactions between oxidative stress and cytokines are associated with the development of AP, leading to uncontrolled amplification of the inflammatory cascade and multiple organ dysfunction syndrome (MODS). SAP was a pancreatic and systemic infection disease caused by waterfall inflammatory response triggered by damage and necrosis of pancreatic acinar cells.[35] Correlation analysis showed that ARG1 and VNN1 were negatively correlated with efficient memory CD8 T cells, CD8 T cells, resting memory T CD4 cells and resting NK cells, and positively correlated with neutrophils. Previous human studies have reported that neutrophils and macrophages infiltrate the pancreas and are positively associated with inflammatory injury.[48] Neutrophil infiltration in the pancreas occurs in the early stage of AP, followed by macrophage recruitment.[49] In mice with acute pancreatitis, neutrophil depletion reduced the severity of pancreatitis and pancreatitis-associated lung injury.[50] Significant depletion of peripheral blood CD4+ and CD8+ subpopulations of T lymphocytes in the course of acute pancreatitis has previously been reported.[51] In VNN1 knockout mice, and reduced inflammation and apoptosis could be observed.[39] Also in a previous study, the expression of RVAN1, which is associated with oxidative stress, was upregulated in AP mice.[52] This is consistent with our findings that there was an upregulation of ARG1 and VNN1 expression and a high degree of neutrophil infiltration in the AP group, which was in contrast to CD8 T cells.

However, the genes in the present study were based only on bioinformatic analysis. Specific clinical trials were not verified in this study by PCR and other methods, besides, changes in AP progression when diagnostic genes are knocked out have also not been explored. The functions and mechanisms of these genes require further exploration through in vivo and in vitro experiments. Simultaneously, we will continue to focus on the role of these genes.

Conclusion

In this study, we examined the diagnostic genes for oxidative stress and immune-related AP, evaluated their value of diagnostic genes, and analyzed the pathways or biological functions to which diagnostic genes were enriched. However, the underlying molecular mechanisms must be studied in detail. Finally, two immune cell oxidative stress-related AP diagnostic genes (ARG1 and VNN1) were screened to be associated with CD8 T cells and neutrophils to provide a new reference for the diagnosis of patients with AP.

Abbreviations

AP, acute pancreatitis; OSRGs, oxidative stress-related genes; DE-ICA-OSRGs, differentially expressed immune cell-associated oxidative stress-related genes; WGCNA, weighted gene co-expression network analysis; DEGs, differentially expressed genes; ROC, receiver operating characteristic; GSEA, gene set enrichment analysis; TF-miRNA-mRNA,

transcription factor-microRNA-messenger RNA; GEO, Gene Expression Omnibus; HC, healthy control; FC, fold change; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; SIRS, systemic inflammatory response syndrome; ARG1, arginase 1; VNN1, Vanin1; ROS, reactive oxygen species; ASP, acute severe pancreatitis; MODS, multiple organ dysfunction syndrome; NES, Normalized Enrichment Score.

Data Sharing Statement

The datasets used and analyzed in the current study are available from the GEO database (https://www.ncbi.nlm.nih.gov/gds) [GSE194331], MSigDB database (https://www.gsea-msigdb.org/gsea/msigdb), and TransmiR v2.0 database (https://www.cuilab.cn/transmir).

Ethics Approval and Informed Consent

The authors are accountable for all aspects of this work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. As this was a retrospective study based on a public database, it has been exempted from ethical approval by the Human Biomedical Research Ethics Committee of Dongying People's Hospital (Approval No. 2024003).

Acknowledgments

Jie Song and Jie Gao collected the data.

Author Contributions

All authors made a significant contribution to the work reported, whether in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas, took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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