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Exploration of *CD2* and *CD3D* as potential immune-related biomarkers for IORT in breast cancer treatment

Shenglan Li, MD[a], Yubing Fu, MD[b], Huiying Zhang, MD[c,*]

Abstract

Breast cancer is a prevalent malignancy among women worldwide. Understanding its molecular mechanisms is crucial for prevention, early diagnosis, and treatment. Using a dataset of intraoperative radiotherapy (IORT) for breast cancer, we analyzed 21 breast tissue samples from patients who received IORT and 16 samples from those who did not. Principal component analysis was employed to reveal data structure, and differentially expressed genes (DEGs) were identified. We constructed a gene network using weighted gene co-expression network analysis and conducted functional enrichment analysis and gene set enrichment analysis. Immune infiltration analysis and protein-protein interaction network analysis were performed, resulting in gene expression heatmaps and Comparative Toxicogenomics Database analysis. Finally, regulatory microRNAs (miRNA) for core genes were predicted using miRNA prediction websites. A total of 2774 DEGs were identified. Principal component analysis demonstrated the differentiation between IORT and non-IORT samples. DEGs were enriched in key biological processes, such as T-cell receptor signaling, immunological synapse formation, and apoptosis. Gene set enrichment analysis validated the functional enrichment of DEGs. Weighted gene co-expression network analysis constructed 15 modules and identified hub genes. Protein-protein interaction network analysis revealed 4 core genes (*CD2*, *CD3D*, *CD3G*, and *CD3E*). miRNA prediction identified regulatory miRNAs for these core genes. Comparative Toxicogenomics Database analysis revealed that these core genes are associated with breast tumors and inflammation. Immune infiltration analysis showed a high proportion of Macrophages M0 and Macrophages M2 in the samples and revealed correlations between T cells and neutrophils. These findings suggest that the core genes may play key roles in the pathological changes and immune regulation of breast cancer tissues. *CD2* and *CD3D* may serve as potential immune-related biomarkers for IORT in the treatment of breast cancer, influencing tissue pathological changes in breast cancer patients by regulating immune responses and cell signaling pathways.

Abbreviations: BP = biological process, CTD = Comparative Toxicogenomics Database, DEGs = differentially expressed genes, FDR = false discovery rate, GO = gene ontology, GSEA = gene set enrichment analysis, IORT = intraoperative radiotherapy, KEGG = Kyoto Encyclopedia of Genes and Genomes, MM = module membership, PCA = principal component analysis, PPI = protein-protein interaction, STRING = Search Tool for the Retrieval of Interacting Genes, TCR = T-cell receptor, TOM = topological overlap matrix, WGCNA = weighted gene co-expression network analysis.

Keywords: breast cancer, *CD2*, *CD3D*, DEGs, IORT

1. Introduction

Breast cancer is one of the most common malignancies among women worldwide, with high incidence and mortality rates, posing a significant public health challenge.[1] Despite significant advances in early diagnosis and comprehensive treatment in recent years, breast cancer remains a major threat to women's health.[2] The etiology of breast cancer is complex and multifactorial, involving genetic factors, chromosomal abnormalities, and gene fusions.[3] Genetic mutations, such as those in the *BRCA1* and *BRCA2* genes, significantly increase the risk of developing breast cancer.[4,5] In addition, mutations in other genes like *TP53*, *PTEN*, and *PALB2* are also closely associated with the occurrence of breast cancer.[6–8] In-depth research into the molecular mechanisms of breast cancer is crucial for its prevention, early diagnosis, and treatment.

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The datasets generated during and/or analyzed during the current study are publicly available.

This study was conducted using publicly available datasets. All data were obtained from open-access databases in accordance with their respective data-use policies. No human participants or animals were directly involved in this study; therefore, ethical approval and informed consent were not applicable.

The data in this article are from public databases and are exempt from ethical review.

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Intraoperative radiotherapy (IORT) is a technique where a single high dose of radiation is directly delivered to the tumor bed during surgery.[9] IORT offers several advantages, including the direct elimination of residual cancer cells during surgery, reduced radiation exposure to normal tissues, and a shortened treatment cycle.[10] In the treatment of breast cancer, IORT can serve as an immediate adjuvant therapy following tumor resection, reducing the risk of local recurrence.[11] Compared to traditional postoperative radiotherapy, IORT offers notable benefits, including enhanced targeting, significant efficacy, and reduced side effects.

With the advancement of high-throughput sequencing technologies and bioinformatics analysis methods, we can delve deeply into the molecular characteristics of tumors at the genetic level.[12] Bioinformatics technologies provide robust tools for elucidating the molecular mechanisms of tumor development by integrating multi-layered data, including gene expression profiles, genomic variations, and protein-protein interaction (PPI) networks.[13] These applications not only help identify core genes closely related to tumors but also reveal the roles of these genes in specific biological processes (BPs) through enrichment and pathway analyses.[14]

Although the roles of *CD2* and *CD3D* have been reported in various tumors, their expression and biological functions in breast cancer patients undergoing IORT remain unclear. *CD2* and *CD3D* are T cell-related immune regulatory genes that play critical roles in immune responses. In breast cancer, T-cell dysfunction may lead to tumor immune evasion. Therefore, investigating the expression of *CD2* and *CD3D* and their impact on the immune microenvironment in IORT-treated breast cancer patients is of significant importance.

In this study, we utilized bioinformatics technologies to conduct an in-depth analysis of gene expression differences between IORT and non-IORT breast cancer samples, identifying core genes *CD2* and *CD3D*, and performing enrichment and pathway analyses. In addition, we explored the potential roles of *CD2* and *CD3D* in IORT-treated breast cancer patients using public datasets. Through this research, we aim to uncover the potential mechanisms of *CD2* and *CD3D* in IORT-treated breast cancer patients, providing theoretical support for the application of IORT in breast cancer treatment.

2. Methods

2.1. IORT dataset

In this study, the configuration file of the breast cancer IORT dataset GSE253650 was downloaded from the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/), generated from GPL24676. GSE253650 comprises 21 breast tissue samples from patients who underwent IORT and 16 breast tissue samples from those who did not undergo IORT. This dataset was utilized to explore the differential gene expression patterns between IORT and non-IORT samples in terms of tissue pathological changes and immune regulation in breast cancer patients.

2.2. Principal component analysis

Principal component analysis (PCA) was employed to reduce dimensionality and visualize the data samples, revealing the underlying structure of the data and identifying differential biological features. The analysis was conducted using the R software package "stats" (version 3.6.0; R Foundation for Statistical Computing, Vienna, Austria). Specifically, the expression profiles were first subjected to *z*-score normalization, followed by dimensionality reduction analysis using the "prcomp" function to obtain the reduced-dimensional matrix. This analysis was utilized to visualize the distribution and differences among different samples.

2.3. Differentially expressed genes analysis

The R package "limma" (Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia) was utilized for probe summarization and background correction of the matrix from GSE253650. The Benjamini-Hochberg method was employed to adjust the original *P*-values, and fold change was calculated using the false discovery rate (FDR). The cutoff criteria for differentially expressed genes (DEGs) were set as $P < .05$ and fold change >1.5. A volcano plot was generated to visualize the DEGs.

2.4. Weighted gene co-expression network analysis

Initially, we computed the median absolute deviation for each gene using the gene expression matrix of GSE253650. We then removed the bottom 50% of genes with the smallest median absolute deviation values. Subsequently, we used the R package weighted gene co-expression network analysis (WGCNA) "goodSamplesGenes" (UCLA, Los Angeles) function to eliminate outlier genes and samples. Next, we employed WGCNA to construct a scale-free co-expression network. Specifically, Pearson correlation matrices and average linkage methods were applied to all pairs of genes. Then, a weighted adjacency matrix was constructed using the power function $A_{mn} = |C_{mn}|^{\beta}$, where C_{mn} represents the Pearson correlation between genes mmm and nnn, and A_{mn} represents the adjacency between genes mmm and nnn. Here, β is a soft-thresholding parameter that emphasizes strong correlations between genes while attenuating weak and negative correlations. Setting β to 10, we transformed the adjacency into a topological overlap matrix (TOM), which measures the network connectivity of a gene by summing its adjacencies with all other genes. The TOM-based dissimilarity was calculated as 1 – TOM1 – TOM1 – TOM. To classify genes with similar expression profiles into gene modules, hierarchical clustering based on TOM-based dissimilarity measures was performed with a minimum module size (number of genes) set to 30. The sensitivity was set to 3. In addition, to further analyze the modules, we calculated the dissimilarity of module characteristic genes, selected a cutting line for the module dendrogram, and merged some modules. Furthermore, we merged modules with a distance <0.25. Notably, the gray module was considered a set of genes that could not be assigned to any module.

2.5. Functional enrichment analysis

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses are computational methods for assessing gene function and biological pathways. In this study, the differential gene list selected from the Venn diagram was input into the KEGG REST API (https://www.kegg.jp/kegg/rest/keggapi.html) to obtain the latest KEGG Pathway gene annotations, which served as the background. Genes were mapped to the background set, and enrichment analysis was performed using the R package "clusterProfiler" (version 3.14.3; Bioconductor Core Team, Fred Hutchinson Cancer Research Center, Seattle) to obtain the results of gene set enrichment. In addition, GO annotations of genes from the R package "org.Hs.eg.db" (version 3.1.0; Bioconductor Core Team, Fred Hutchinson Cancer Research Center, Seattle) were used as the background. The minimum gene set was set to 5, and the maximum gene set was set to 5000. A P-value < .05 and an FDR < 0.25 were considered statistically significant criteria.

Moreover, the Metascape database provides comprehensive gene list annotation and analysis resources, which can be visualized and exported. We utilized the Metascape (http://metascape.org/gp/index.html) database for functional enrichment analysis of the aforementioned differential gene list and exported the results.

2.6. Gene set enrichment analysis

For gene set enrichment analysis (GSEA), we obtained the GSEA software (version 3.0; Broad Institute of MIT and Harvard, Cambridge) from the GSEA website (DOI:10.1073/pnas.0506580102, http://software.broadinstitute.org/gsea/index.jsp). Samples were divided into 2 groups based on IORT and non-IORT, and the c2.cp.kegg.v7.4.symbols.gmt subset was downloaded from the Molecular Signatures Database (DOI:10.1093/bioinformatics/btr260, http://www.gsea-msigdb.org/gsea/downloads.jsp) to assess relevant pathways and molecular mechanisms. GSEA was conducted based on gene expression profiles and phenotype grouping, with a minimum gene set of 5, a maximum gene set of 5000, 1000 permutations, and statistical significance defined as a P-value < .05 and an FDR < 0.25. In addition, GO and KEGG analyses were performed on the entire genome as per GSEA guidelines.

2.7. Immune infiltration analysis

CIBERSORT (http://CIBERSORT.stanford.edu/) is a widely used computational method for estimating immune cell infiltration, with the LM22 gene signature defining 22 immune cell subtypes. We applied an integrated bioinformatics approach using the CIBERSORT package to analyze the gene expression matrix of GSE253650. Linear support vector regression was employed to deconvolute the expression matrix of immune cell subtypes, estimating immune cell abundance. Samples with a confidence score of $P < .05$ were selected based on the cutoff criterion.

2.8. Construction and analysis of PPI network

The Search Tool for the Retrieval of Interacting Genes (STRING) database (http://string-db.org/) aims to collect, score, and integrate all publicly available PPI information sources and supplement them with predicted interactions. In this study, the differential gene list was input into the STRING database to construct a predicted PPI network for core genes (confidence score > 0.4). Cytoscape software was utilized for biological network analysis and 2-dimensional visualization. The PPI network formed by the STRING database was visualized, and core genes were predicted using Cytoscape software. Initially, the PPI network was imported into Cytoscape, and the top 10 genes with the best correlation were calculated using 5 algorithms (MCC, MNC, DMNC, Degree, and Closeness), and their intersection was obtained. Finally, the core gene list was exported after visualization.

2.9. Gene expression heatmap

We utilized the R package heatmap to generate heatmaps depicting the expression levels of core genes identified in the PPI network across samples from GSE253650. These heatmaps visually represent the expression differences of core genes between IORT and non-IORT samples.

2.10. Comparative Toxicogenomics Database analysis

Comparative Toxicogenomics Database (CTD) integrates vast amounts of data on interactions between chemicals, genes, phenotypes, and diseases, providing significant convenience for research on disease-related environmental exposure factors and potential drug mechanisms. Core genes were input into the CTD website to identify the most relevant diseases associated with these core genes. In addition, radar plots depicting the expression differences of each gene were created using Excel.

2.11. miRNA analysis

We utilized microRNA (miRNA) prediction websites to search for miRNA information targeting the core genes. In our study, we employed the online database DIANA-TarBase (dianalab.e-ce.uth.gr) to screen for miRNAs regulating the core genes.

3. Results

3.1. Analysis of DEGs

In this study, using the predefined cutoff values, we identified DEGs in the IORT dataset GSE253650, resulting in a total of 2774 DEGs (Fig. 1A).

3.2. PCA

We conducted dimensionality reduction analysis on the IORT data samples to guide subsequent differential analysis. PCA and uniform manifold approximation and projection were employed as 2 different dimensionality reduction methods for analysis and visualization, revealing the potential structure of the data. The results of dataset GSE253650 showed the distribution and characteristic differences among samples, indicating differentiation between IORT and non-IORT samples. While some samples exhibited good differentiation, demonstrating differences between IORT and non-IORT samples (Fig. 1B, C).

3.3. Functional enrichment analysis

3.3.1. Functional enrichment analysis of DEGs. We performed GO and KEGG analyses on the identified DEGs. According to the GO analysis results, in the BP category, DEGs were mainly enriched in cell adhesion, apoptosis process, and inflammatory response (Fig. 2A). In the cellular component category, they were primarily enriched in cell skeleton, endoplasmic reticulum, and Golgi apparatus (Fig. 2B). In the molecular function category, they were mainly concentrated in signal receptor activity, DNA binding, and cytokine receptor activity (Fig. 2C). In the KEGG analysis, DEGs were mainly enriched in T-cell receptor (TCR) signaling pathway, Toll-like receptor signaling pathway, apoptosis, p53 signaling pathway, and cancer pathways (Fig. 2D).

3.3.2. GSEA. In addition, we conducted GSEA on the entire genome to identify potential enrichments among non-DEGs and validate the results of DEGs. The intersection of enriched terms with GO and KEGG enrichment terms is shown. The enrichment results of the IORT dataset GSE253650 matrix revealed that DEGs were mainly enriched in the apoptosis process, inflammatory response, Golgi apparatus, cytokine receptor activity, TCR signaling pathway, apoptosis, and p53 signaling pathway (Fig. 2E–H).

3.3.3. Metascape enrichment analysis. In the enrichment items of Metascape, the results of GO enrichment items included positive regulation of inflammatory response, immune response, p53 transcriptional gene network, and cell adhesion (Fig. 3A). We also output enrichment networks colored by enrichment terms and P-values (Fig. 3B, C), visually representing the associations and confidence of various enrichment items. The enrichment results of Metascape supported the results of GO and KEGG enrichment.

3.4. WGCNA

The selection of the soft-thresholding power is a crucial step in WGCNA. We performed network topology analysis to determine the soft-thresholding power. In our WGCNA, we set the soft-thresholding power to 10 (Fig. 4A). A hierarchical clustering tree of all genes was constructed, resulting in 15 modules (Fig. 4B), and the interactions between significant modules were analyzed (Fig. 4C). Subsequently, a heatmap

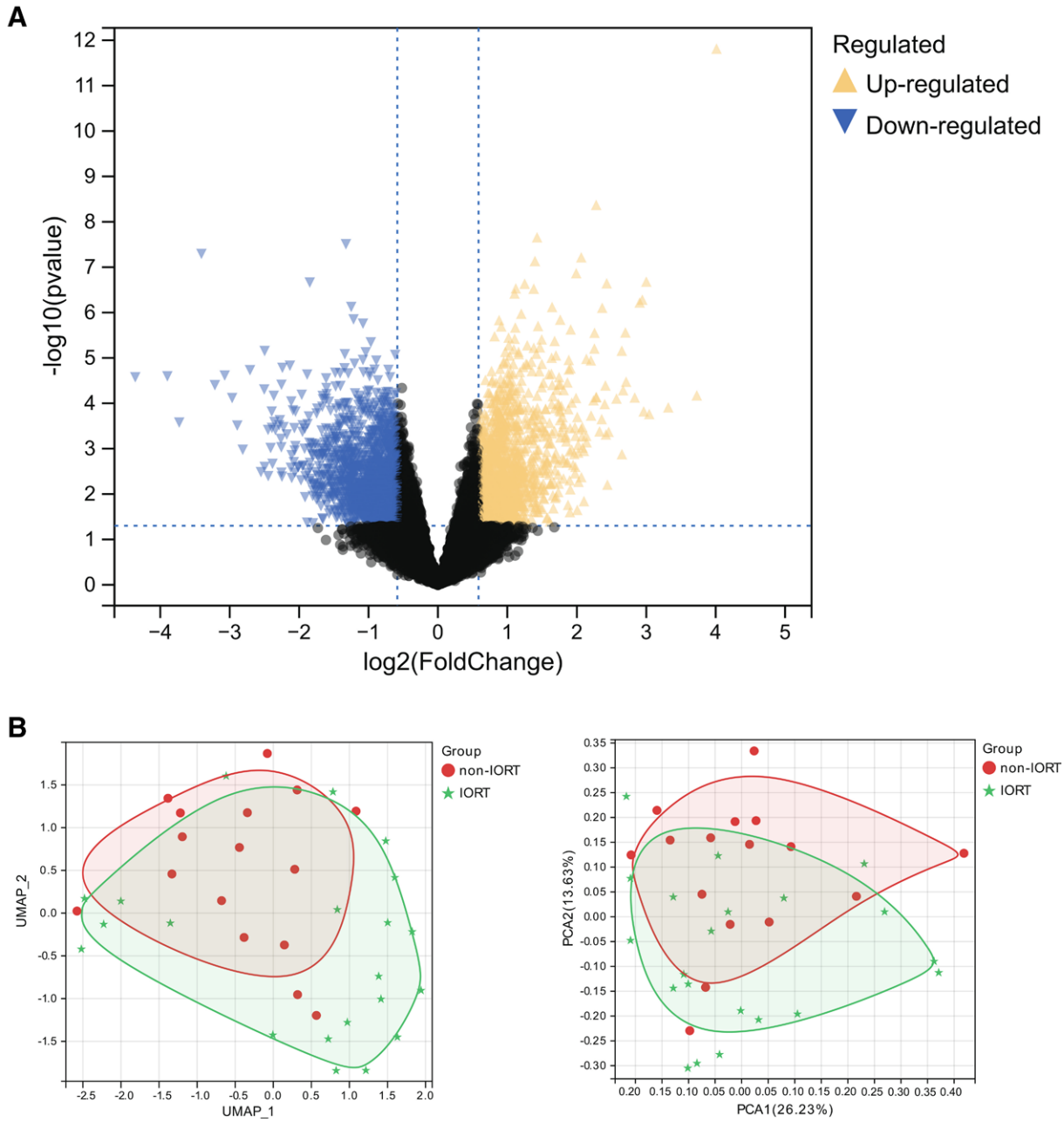

Figure 1. (A) Resulting in a total of 2774 DEGs. (B, C) PCA. DEGs = differentially expressed genes, IORT = intraoperative radiotherapy, PCA = principal component analysis.

of module-trait correlations was generated (Fig. 5A). We calculated the correlation between module eigengenes and gene expression to obtain module membership (MM), and genes with high connectivity in clinically significant modules (|MM| > 0.8) were identified as hub genes. A scatter plot of gene significance versus MM for hub genes was generated (Fig. 5B). We constructed a Venn diagram using WGCNA and DEGs to identify overlapping genes (Fig. 5C) for subsequent construction of the PPI network.

3.5. Construction and analysis of PPI network

We input the overlapping genes identified by WGCNA and DEGs into the STRING online database to construct the PPI network. The network was visualized using Cytoscape software, and core genes were analyzed (Fig. 6A). Subsequently, using 5 algorithms (MCC, MNC, DMNC, Degree, and Closeness), we identified hub genes (Fig. 6B–F) and generated a Venn diagram to obtain the intersection as core genes (Fig. 6G). Ultimately, we identified 4 core genes (*CD2*, *CD3D*, *CD3G*, and *CD3E*).

3.6. Heatmap of core gene expression

We visualized the expression levels of core genes in the IORT dataset GSE253650 matrix and generated a heatmap (Fig. 7). We observed that core genes (*CD2*, *CD3D*, *CD3G*, and *CD3E*) were highly expressed in IORT samples and lowly expressed in non-IORT samples. Based on these results, we hypothesized that core genes (*CD2*, *CD3D*, *CD3G*, and *CD3E*) may play important roles in IORT.

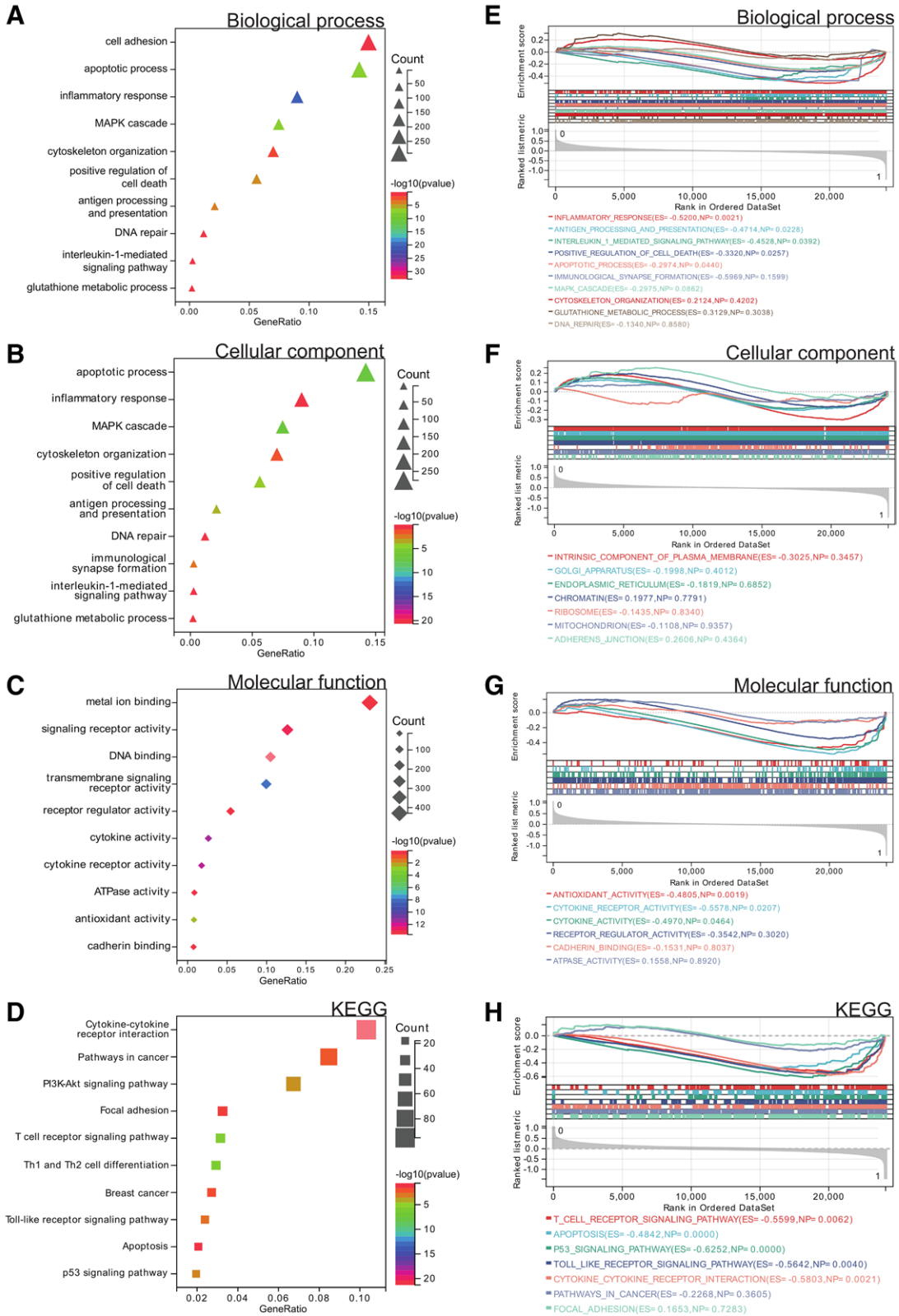

Figure 2. Functional enrichment analysis. (A) BP. (B) CC. (C) MF. (D) KEGG analysis. (E–H) GSEA. BP = biological process, CC = cellular component, GSEA = gene set enrichment analysis, KEGG = Kyoto Encyclopedia of Genes and Genomes, MF = molecular function.

3.7. Prediction and functional annotation of miRNAs associated with hub genes

In this study, we input the hub gene list into miRNA prediction websites to identify relevant miRNAs, enhancing our understanding of gene expression regulation (Table 1).

Using the DIANA-TarBase website, we predicted that the related miRNAs for *CD2* gene are hsa-miR-106a-5p, hsa-miR-106b-5p, and hsa-miR-129-2-3p; for *CD3D* gene are hsa-miR-34a-5p and hsa-miR-26a-5p; for *CD3G1* gene are hsa-miR-132-3p, hsa-miR-182-5p, and hsa-miR-196a-3p; and for *CD3E* gene are hsa-miR-103a-3p, hsa-miR-107, and hsa-miR-15a-5p.

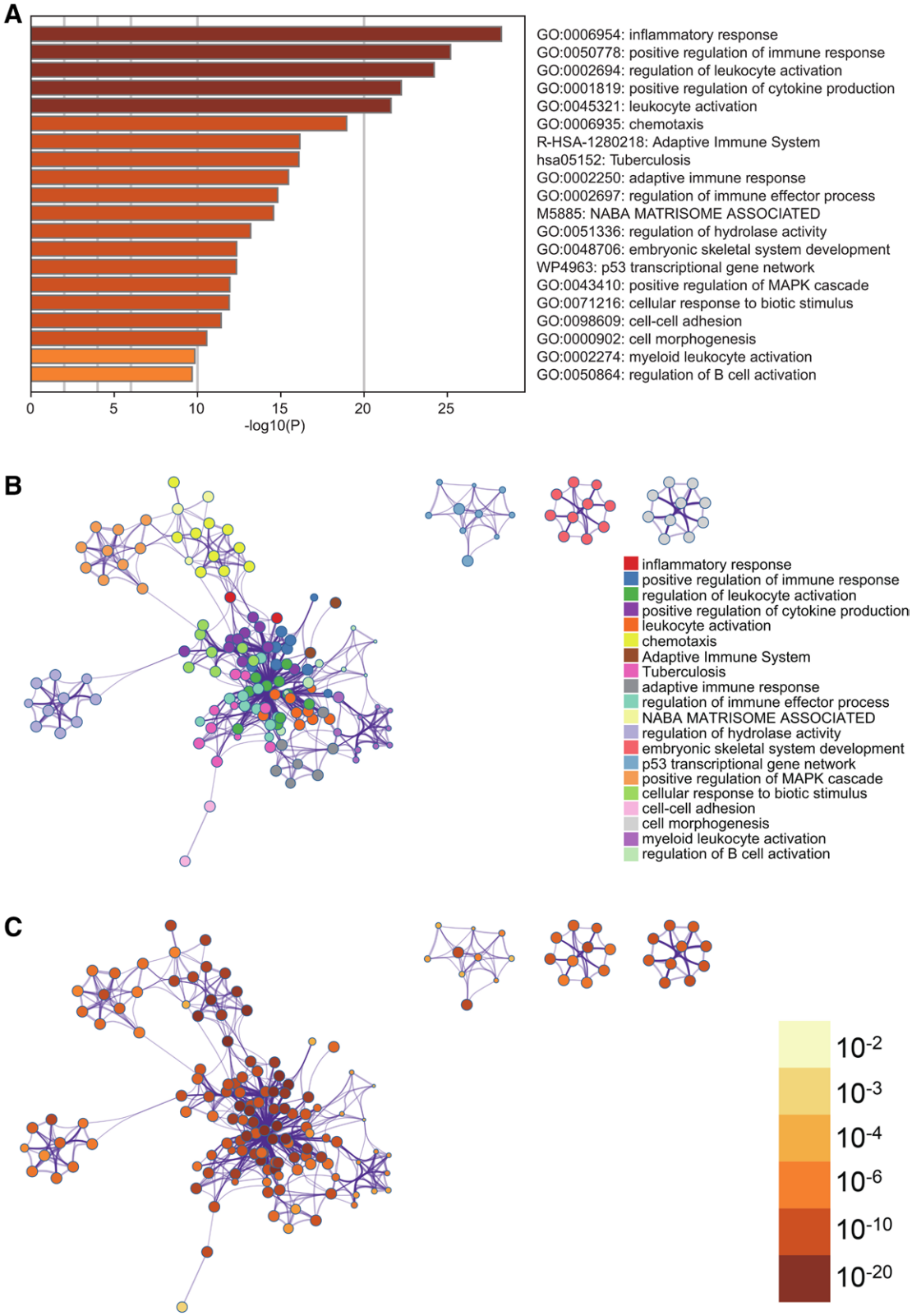

Figure 3. Metascape enrichment analysis. (A) The results of GO enrichment items included positive regulation of inflammatory response, immune response, p53 transcriptional gene network, and cell adhesion. (B, C) Outputted enrichment networks colored by enrichment terms and *P*-values. GO = gene ontology.

3.8. CTD analysis

In this study, we input the hub gene list into the CTD website to search for diseases related to the core genes, improving our understanding of the association between genes and diseases. We found that core genes (*CD2*, *CD3D*, *CD3G*, and *CD3E*) are associated with breast neoplasms, inflammation, necrosis, postoperative pain, and skin ulcers (Fig. 8). Based on the results of the above analysis, we selected *CD2* and *CD3D* as the target genes for this study, speculating that they may play important roles in the histopathological changes and immune regulation of IORT in breast cancer patients.

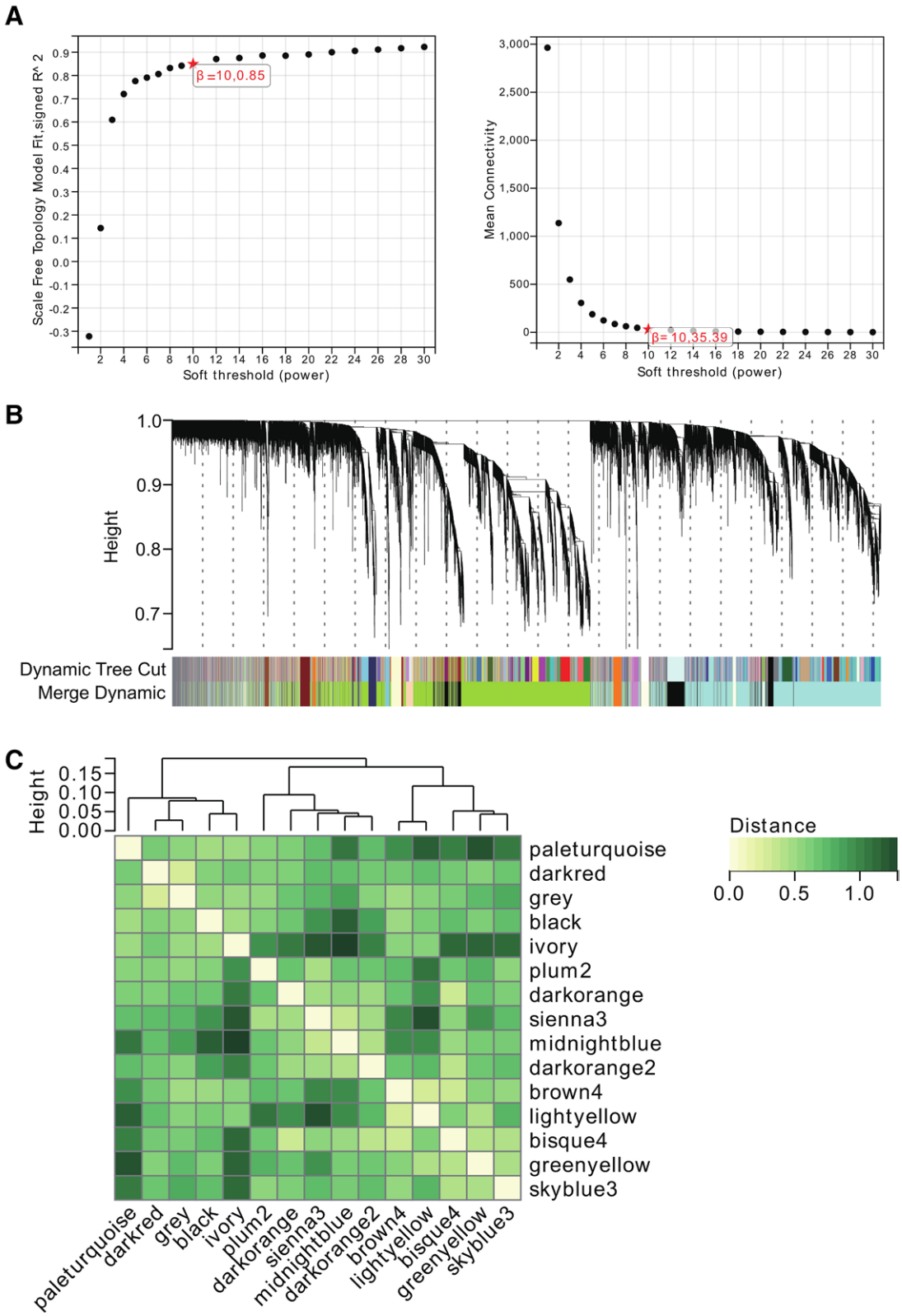

Figure 4. WGCNA. (A) Set the soft-thresholding power to 10. (B) A hierarchical clustering tree of all genes was constructed, resulting in 15 modules. (C) The interactions between significant modules were analyzed. WGCNA = weighted gene co-expression network analysis.

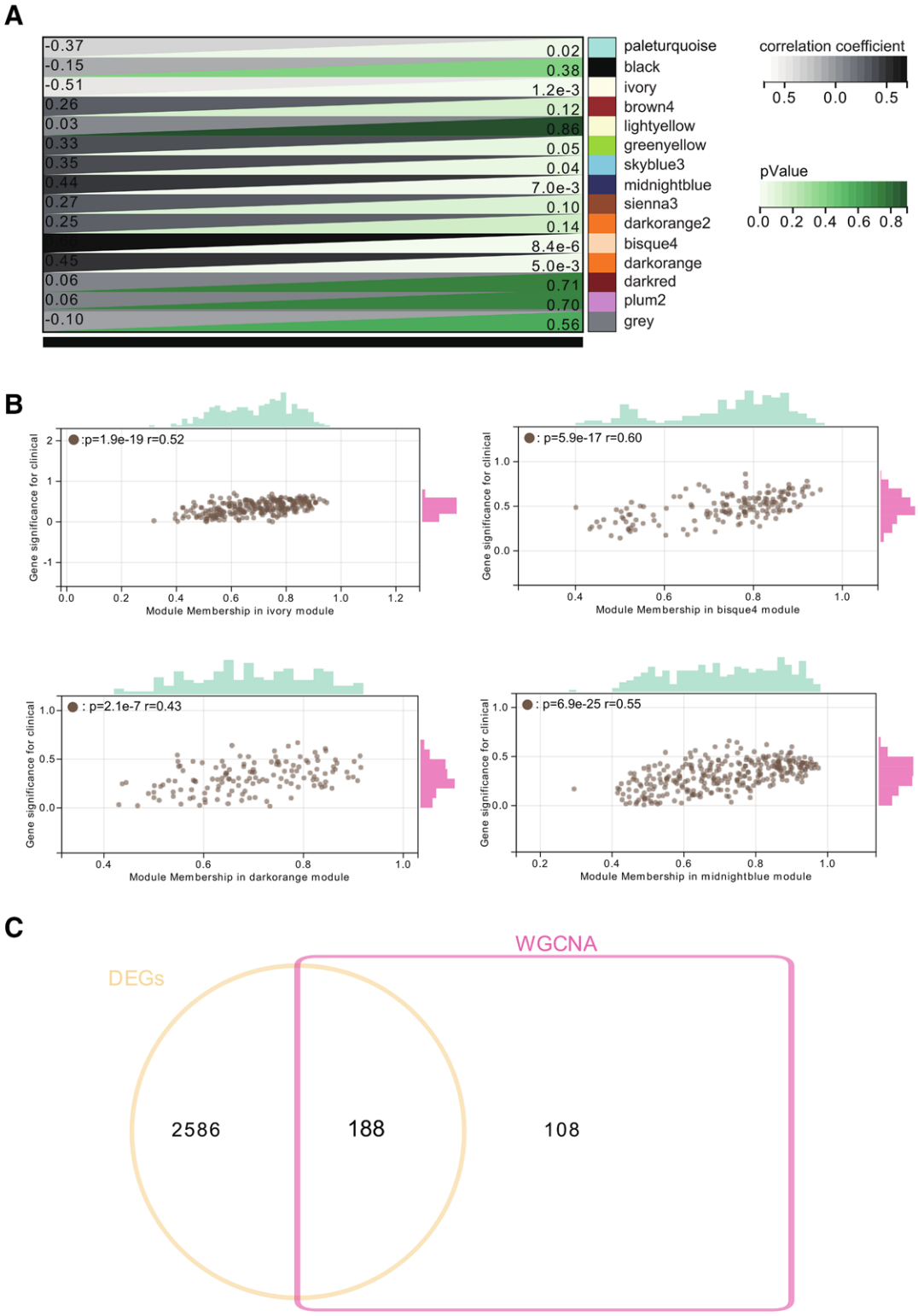

Figure 5. WGCNA. (A) A heatmap of module-trait correlations was generated. (B) A scatter plot of GS versus MM for hub genes was generated. GS = gene significance, MM = module membership, WGCNA = weighted gene co-expression network analysis.

3.9. Immune infiltration analysis

We used the CIBERSORT package to analyze the matrix of the IORT dataset GSE253650. At a 95% confidence level, we obtained the proportion of immune cells in the whole gene expression matrix. The results suggested that Macrophages M0 and Macrophages M2 are relatively high in the samples (Fig. 9A). We also obtained a heatmap of immune cell expression in the dataset, which showed that Macrophages M2 are highly expressed in IORT samples and lowly expressed in non-IORT samples (Fig. 9B). Furthermore, we conducted correlation

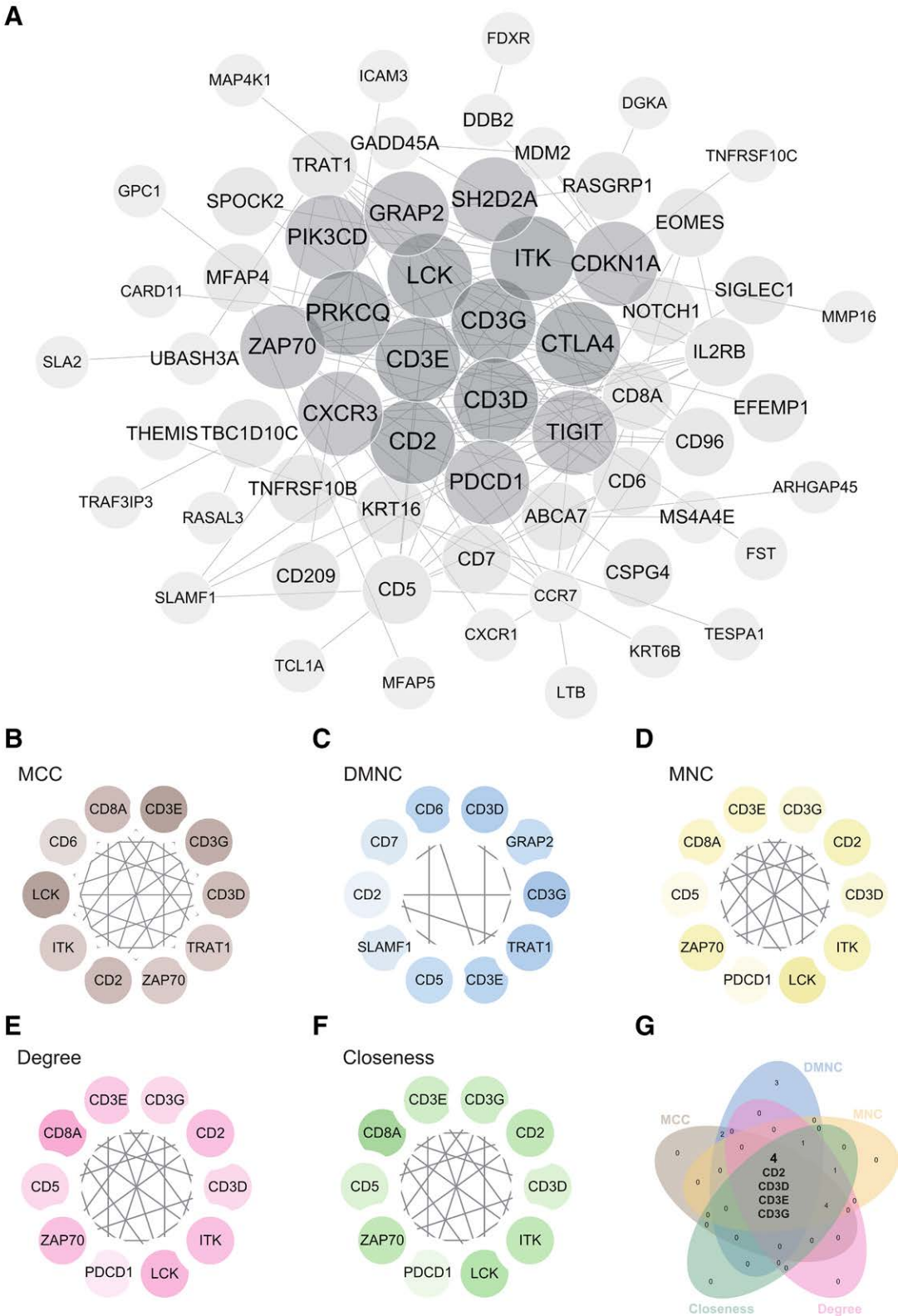

Figure 6. Construction and analysis of PPI network. (A) PPI network. (B–F) Five algorithms (MCC, MNC, DMNC, Degree, and Closeness) identified hub genes. (G) A Venn diagram to obtain the intersection as core genes. PPI = protein-protein interaction.

analysis of infiltrating immune cells and obtained a co-expression pattern of immune cell components. The results showed that when T cells CD4 memory activated expression is high, the expression of neutrophils also increases. There is a strong positive correlation between T cells CD4 memory activated and neutrophils, suggesting that they may play a significant role in the histopathological changes and immune regulation of IORT in breast cancer patients (Fig. 9C).

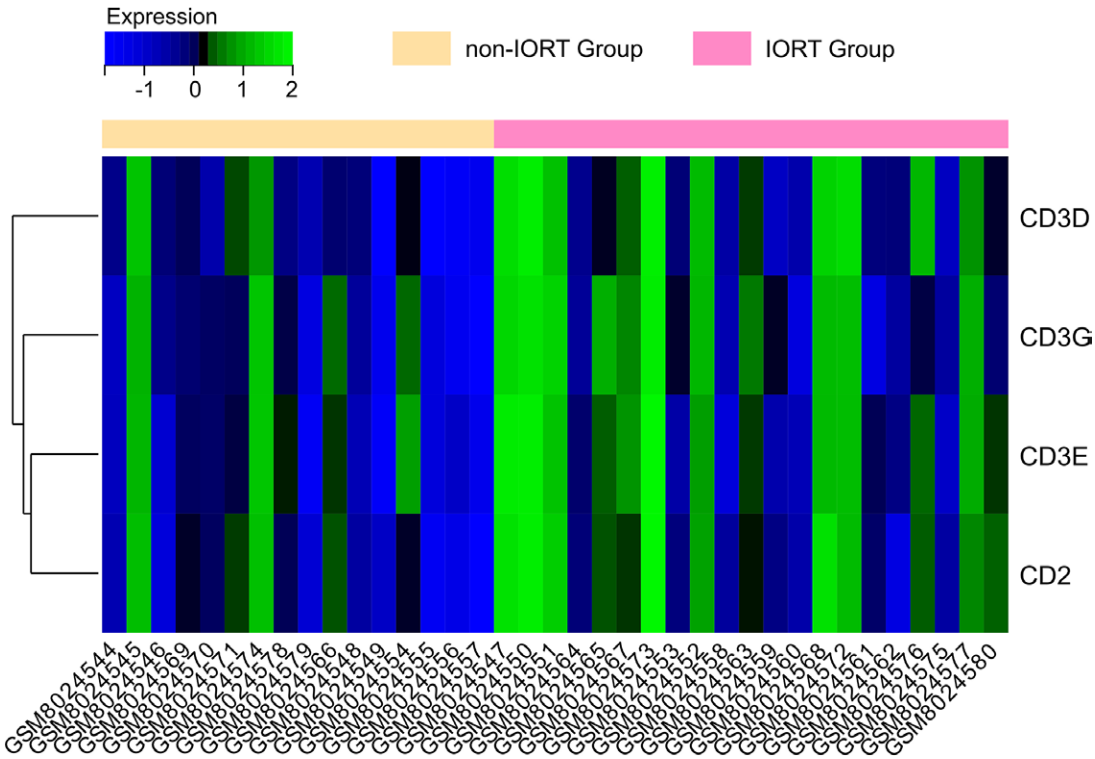

Figure 7. Heatmap of core gene expression. The expression levels of core genes in the IORT dataset GSE253650 matrix and generated a heatmap. IORT = intraoperative radiotherapy.

Table 1

A summary of miRNAs that regulate hub genes.

Gene	miRNA		
CD2	hsa-miR-106a-5p	hsa-miR-106b-5p	hsa-miR-129-2-3p
CD3D	hsa-miR-34a-5p	hsa-miR-26a-5p	
CD3G1	hsa-miR-132-3p	hsa-miR-182-5p	hsa-miR-196a-3p
CD3E	hsa-miR-103a-3p	hsa-miR-107	hsa-miR-15a-5p

miRNA = microRNA.

4. Discussion

Breast cancer is one of the most common malignant tumors in women worldwide and is a leading cause of cancer-related death in women, with breast cancer alone accounting for 30% of all new cancer diagnoses in women.[15] The incidence of breast cancer continues to rise globally, particularly in developed and developing countries. According to data from the International Agency for Research on Cancer, breast cancer has the highest incidence rate among women globally, accounting for 24.2% of all cancer cases in women.[1] Clinical manifestations of breast cancer vary, including breast masses, nipple discharge, and skin changes. In severe cases, metastatic lesions can occur, with common sites of metastasis including the bones, lungs, liver, and brain.[16–18] Breast cancer can be classified into various subtypes based on its morphological and molecular characteristics, including ductal carcinoma, lobular carcinoma, and medullary carcinoma.[16] These subtypes exhibit significant differences in biological behavior and treatment response, increasing the complexity and challenges of treatment. Despite advancements in breast cancer treatment modalities, many patients still face the risk of recurrence and metastasis after treatment.[19] Therefore, in-depth exploration of the molecular mechanisms of breast cancer is of paramount importance for the development of new targeted treatment strategies and improvement of patient prognosis.

IORT is a technique that delivers a single high dose of radiation therapy directly to the tumor bed during surgery and has garnered widespread attention in the treatment of breast cancer in recent years.[20] The application of IORT not only improves local control rates but also reduces the risk of postoperative recurrence.[21] However, IORT also has some drawbacks, such as the potential for local tissue damage and increased risk of postoperative complications.[22] Practical limitations of IORT include technical complexity and high equipment requirements. Nevertheless, the prospects for the application of IORT in the treatment of breast cancer remain promising, particularly in patients at high risk of local recurrence.[23] Despite the significant clinical efficacy of IORT, further research is needed to fully understand its molecular mechanisms and its impact on tumors and surrounding tissues. The main results of this study indicate that *CD2* and *CD3D* are highly expressed in IORT samples, while they are lowly expressed in non-IORT samples, suggesting that these 2 genes may play an important role in the process of IORT treatment.

The *CD2* gene encodes the CD2 protein, a cell surface protein mainly expressed in T cells and natural killer cells, playing a crucial role in T-cell activation and immune response.[24] *CD2* enhances the interaction between T cells and target cells by binding to its ligand CD58, promoting the formation of immune synapses and signal transduction.[25,26] Studies

Figure 8. CTD analysis. Core genes (*CD2*, *CD3D*, *CD3G*, and *CD3E*) are associated with breast neoplasms, inflammation, necrosis, postoperative pain, and skin ulcers. CTD = Comparative Toxicogenomics Database.

have shown that *CD2* plays an important role in various immune-related diseases, including autoimmune diseases and infectious diseases.[27–30] In addition, *CD2* has been found to be associated with certain types of cancers, including leukemia and lymphoma.[31,32] In breast cancer research, the expression level of *CD2* is closely related to patient prognosis. High expression of *CD2* in tumor tissues of breast cancer patients is associated with better prognosis.[33] *CD2* may play a key role in immune surveillance and immune evasion in breast cancer by promoting T cell-mediated immune responses.[34] These

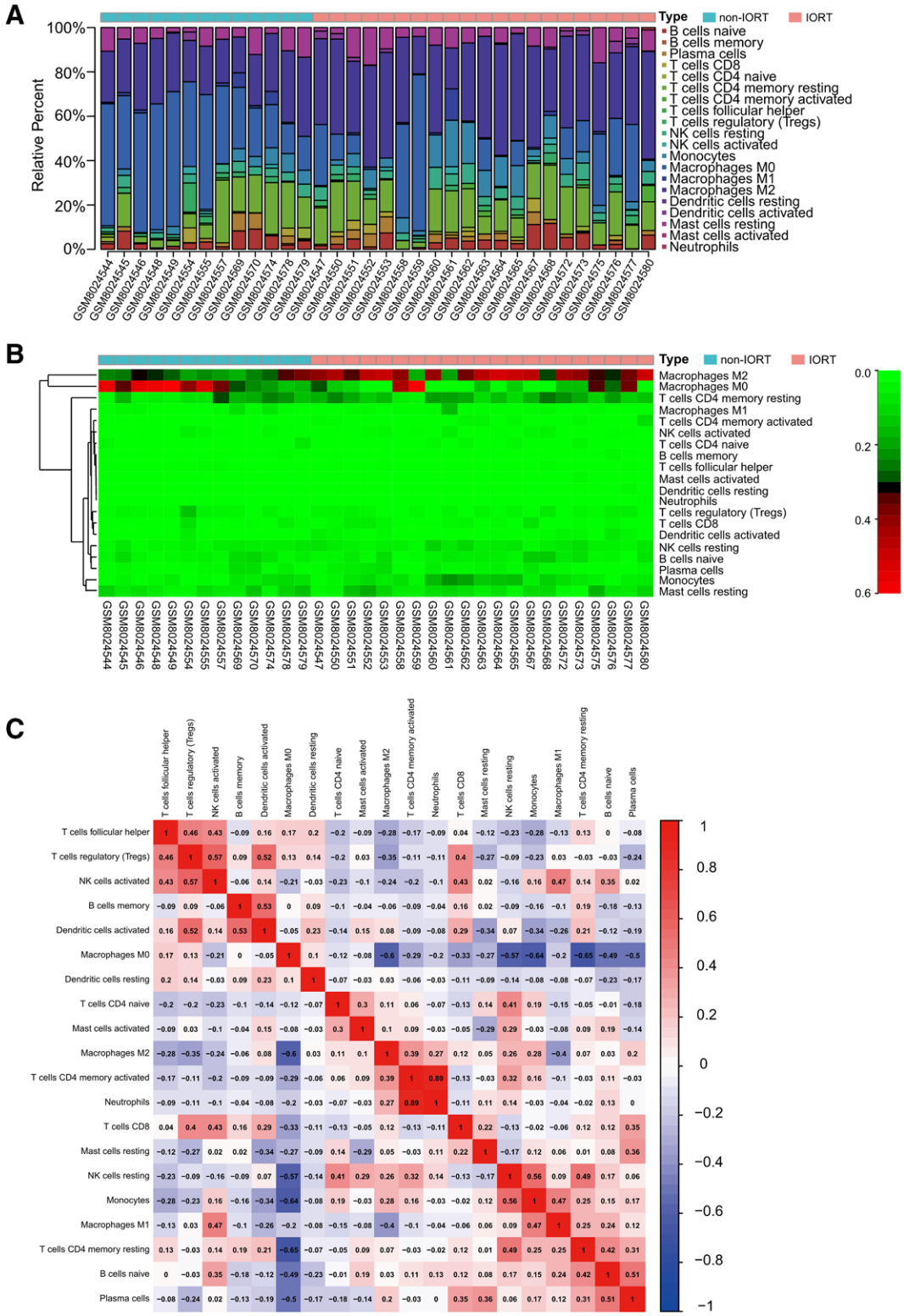

Figure 9. Immune infiltration analysis. (A) Macrophages M0 and Macrophages M2 are relatively high in the samples. (B) Macrophages M2 are highly expressed in IORT samples and lowly expressed in non-IORT samples. (C) Conducted correlation analysis of infiltrating immune cells and obtained a co-expression pattern of immune cell components. IORT = intraoperative radiotherapy.

findings suggest that the role of *CD2* in breast cancer extends beyond tumor cells themselves and involves complex immune regulatory networks. Therefore, we speculate that *CD2* may serve as an immune biomarker in the process of IORT treatment for breast cancer, potentially reflecting enhanced local immune responses, inhibiting residual tumor cells, and preventing recurrence.

The *CD3D* gene encodes the CD3D protein, a subunit of the TCR complex and a key component of T-cell activation and signal transduction.[35] *CD3D*, along with other *CD3* subunits such as *CD3E* and *CD3G*, forms the TCR complex, initiating T-cell immune responses by mediating antigen recognition and signal transduction.[36] Research indicates that *CD3D* plays an important role in immune-related diseases and cancer.[37] In breast cancer research, evidence suggests that high expression of *CD3D* is associated with increased tumor-infiltrating lymphocytes,[38] which often indicate better treatment response and prognosis.[39] Furthermore, the expression level of *CD3D* is closely related to the survival rate of breast cancer patients.[40] *CD3D* may enhance antitumor immune effects by regulating immune responses in the tumor microenvironment. These findings suggest that the role of *CD3D* in breast cancer also involves complex immune regulatory networks. Therefore, we speculate that *CD3D* may serve as an immune biomarker in the process of IORT treatment for breast cancer by enhancing T-cell activation and function, promoting the clearance of tumor cells.

The literature review above aligns with our results, further supporting our hypothesis. The main findings of this study indicate that *CD2* and *CD3D* are highly expressed in the IORT samples compared to the non-IORT samples. Based on this finding, we speculate that IORT may enhance local immune responses and thus improve treatment outcomes by regulating the expression of these genes. In addition, we conducted gene enrichment analysis and pathway analysis to uncover the potential functional mechanisms of *CD2* and *CD3D* in breast cancer. The results indicate that these genes are mainly involved in key BPs such as TCR signaling, immune synapse formation, and apoptosis, which are consistent with previous studies and further support the significant role of *CD2* and *CD3D* in immune regulation in breast cancer.

Although this study conducted rigorous bioinformatics analysis, there are still some limitations. Firstly, the sample size of our study is limited, which may affect the generalizability of the results. Secondly, we did not perform gene overexpression or knockout animal experiments to further validate their functions. In addition, our study mainly relied on public datasets, lacking validation from clinical samples. Therefore, future research should explore these aspects in depth. Validation of the specific mechanisms of action of *CD2* and *CD3D* in IORT treatment is essential.

5. Conclusion

This study, through bioinformatics analysis, has revealed the potential roles of *CD2* and *CD3D* in IORT treatment of breast cancer using public datasets. These results provide new insights into the molecular mechanisms of IORT in breast cancer treatment and lay a theoretical foundation for the development of new immune-related biomarkers for breast cancer treatment. By gaining a deeper understanding of the roles of these genes in IORT treatment, we hope to improve the efficacy of breast cancer treatment and enhance patient prognosis.

Author contributions

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