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PDIA6 acts as a prognostic biomarker and immune regulator in HNSCC with novel insights into EBV associated nasopharyngeal carcinoma

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Abstract

Background

Head and neck squamous cell carcinoma (HNSCC) is a heterogeneous malignancy with poor prognosis. The role of protein disulfide isomerase A6 (*PDIA6*) in HNSCC, particularly in Epstein-Barr virus (EBV)-associated nasopharyngeal carcinoma (NPC), remains unclear. The present study aimed to investigate the expression, prognostic value and biological functions of *PDIA6* in HNSCC.

Materials and Methods

PDIA6 expression was analyzed using The Cancer Genome Atlas (TCGA), Clinical Proteomic Tumor Analysis Consortium (CPTAC) and Gene Expression Omnibus (GEO) datasets. Bioinformatics was used to evaluate prognosis, pathways, immune infiltration and chemokine profiles.

Results

PDIA6 was significantly upregulated in HNSCC at the mRNA and protein levels. High PDIA6 expression correlated with advanced T stage, lymph node metastasis and poor survival, and was identified as an independent prognostic biomarker. PDIA6-related genes were enriched in endoplasmic reticulum (ER) homeostasis, ER stress and the PI3K-Akt pathway. PDIA6 expression was positively associated with immune cell infiltration and immunosuppressive markers.

Conclusion

PDIA6 is an independent prognostic factor in HNSCC that maintains ER homeostasis and promotes an immunosuppressive microenvironment. It may link EBV-induced ER stress, immune evasion and therapeutic resistance in NPC, providing insights for HNSCC prognostic stratification and precision therapy.

Introduction

HNSCC is among the most common malignant tumors worldwide and accounts for a substantial proportion of cancer-related mortality (1). Despite advances in multidisciplinary treatment, the long-term prognosis of HNSCC remains unsatisfactory, especially for patients with recurrent or metastatic disease (2,3). HNSCC includes several clinicopathologically distinct malignancies, among which NPC exhibits unique epidemiological, etiological and clinical features (4,5). NPC is highly prevalent in southern China and Southeast Asia, is closely associated with EBV infection, and is characterized by early lymph node metastasis, high invasiveness and notable sensitivity to radiotherapy (6,7). However, the molecular

heterogeneity and subtype-specific pathogenesis of HNSCC have hindered the development of reliable prognostic biomarkers and targeted therapeutic strategies (8,9).

PDIA6 is a member of the protein disulfide isomerase family, which is predominantly located in the ER and serves key roles in protein folding, redox homeostasis and the ER stress response (10,11). Accumulating evidence has demonstrated that PDIA6 is aberrantly expressed in various malignant tumors and contributes to cancer cell proliferation, metastasis, drug resistance and immune evasion (12–14). In HNSCC, several ER stress-related molecules have been reported to drive tumor progression and shape the tumor microenvironment (TME) (15,16). Nevertheless, the expression pattern, clinical significance and underlying biological functions of PDIA6 in HNSCC, particularly in the EBV-associated NPC subtype, have not been systematically elucidated.

Accordingly, the present study performed a comprehensive bioinformatic analysis to investigate the expression profile, prognostic value and molecular mechanisms of PDIA6 in HNSCC. By integrating the distinctive biological characteristics of NPC, the potential roles of PDIA6 in EBV-induced carcinogenesis, ER stress activation, immune suppression and therapeutic resistance were further explored. This study may provide novel insights into the molecular pathogenesis of HNSCC and facilitate the development of subtype-specific prognostic and therapeutic strategies for HNSCC patients, particularly those with NPC.

Materials and methods

Data sources

A total of 580 patients with head and neck squamous cell carcinoma (HNSCC) were enrolled in this study, including 517 cases from The Cancer Genome Atlas (TCGA) HNSCC cohort (https://portal.gdc.cancer.gov/projects/TCGA-HNSC) and 63 cases retrieved from the Gene Expression Omnibus (GEO) repository. The GEO datasets included GSE12452 (31 nasopharyngeal carcinoma cases; https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE12452) and GSE37991 (32 oral squamous cell carcinoma cases; https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE37991). Transcriptome profiles and matched clinicopathological data were downloaded from the TCGA HNSCC cohort. Proteomic data were acquired from the Clinical Proteomic Tumor Analysis Consortium (CPTAC) Study S055 (https://proteomics.cancer.gov/programs/cptac). GSE12452 and GSE37991 were used for external validation. Follow-up survival data were extracted from the TCGA HNSCC cohort and further analyzed using the Gene Expression Profiling Interactive Analysis (GEPIA) 1.0 platform (http://gepia.cancer-pku.cn/).

Analysis of PDIA6 expression levels

PDIA6 mRNA expression in HNSCC tumor tissues and paired adjacent normal tissues was analyzed using TCGA. The Mann-Whitney U test was used to compare expression differences. PDIA6 protein

expression was verified using CPTAC with the Student's test. GEO2R was used to analyze expression differences in the GEO datasets.

Correlation between PDIA6 expression and clinicopathological characteristics

Patients were stratified into high and low *PDIA6* expression groups based on the median value. The correlation between *PDIA6* expression and clinicopathological characteristics was analyzed using the χ^2 test or Fisher's exact test, as appropriate. $P < 0.05$ was considered to indicate a statistically significant difference.

Survival analysis

Kaplan-Meier survival analysis was performed using GEPIA. Overall survival (OS) and disease-free survival (DFS) curves were plotted, and the log-rank test was used to compare survival differences. Univariate and multivariate Cox proportional hazard regression models were constructed to assess whether *PDIA6* expression was an independent prognostic factor.

Gene and protein interaction networks

The GeneMANIA database was used to predict *PDIA6*-interacting genes. The STRING database was used for protein-protein interaction (PPI) analysis with a confidence score > 0.7. The ClusterProfiler package in R software (v4.3.1) was used for Gene Ontology (GO) functional enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis.

Immune infiltration and chemokine correlation analysis

The Tumor Immune Estimation Resource (TIMER) databasewas used to analyze the correlation between *PDIA6* expression and immune cell infiltration. Spearman's rank correlation coefficient was calculated. The TISIDB database was used to analyze the correlation between *PDIA6* and chemokines/receptors.

Co-expressed genes and functional enrichment analysis

The LinkedOmics database was used to screen *PDIA6* co-expressed genes in the TCGA-HNSCC cohort. Gene set enrichment analysis (GSEA) was performed for GO and KEGG enrichment.

Statistical analysis

All statistical analyses were performed using R software (v4.3.1) and online tools. Mann-Whitney U test, Kruskal-Wallis test, χ^2 test, Fisher's exact test, Kaplan-Meier analysis, log-rank test, Cox regression and

Spearman's correlation were used. P < 0.05 was considered to indicate a statistically significant difference.

Results

PDIA6 expression in HNSCC and external validation

TCGA analysis demonstrated that PDIA6 mRNA expression was significantly higher in HNSCC tumor tissues compared with adjacent normal tissues (P < 0.05) (Fig. 1A). CPTAC proteomic analysis confirmed that PDIA6 protein expression was also significantly upregulated in HNSCC tissues (P < 0.05). (Fig. 1B)

Correlation between PDIA6 expression and HNSCC clinicopathological characteristics

At the protein level, TRIM56 is also frequently overexpressed in various tumor tissues. The expression level of PDIA6 in HNSCC was also upregulated (Fig. 1C). Validation using the GEO datasets GSE12452 (nasopharyngeal carcinoma) and GSE37991 (oral cancer) confirmed that PDIA6 is highly expressed in tumor tissues, with statistically significant differences (Fig. 1D).

TCGA database analysis revealed no significant correlation between PDIA6 expression and age or gender in HNSCC patients (Table 1). However, PDIA6 expression was significantly associated with T stage, lymph node metastasis, and clinical stage, with higher expression in advanced-stage disease (all P<0.05)((Table 1)).

Prognostic value of PDIA6 in HNSCC

Kaplan-Meier survival analysis revealed that patients with high PDIA6 expression had significantly shorter OS and DFS compared with those with low expression (P < 0.05). Univariate Cox regression indicated that high PDIA6 expression was a risk factor for poor OS [hazard ratio (HR) = 1.35, 95% confidence interval (CI): 1.02–1.79, P = 0.036] and DFS (HR = 1.32, 95% CI: 1.01–1.72, P = 0.041). Multivariate Cox regression confirmed that high PDIA6 expression was an independent prognostic factor for poor OS (HR = 1.38, 95% CI: 1.03–1.85, P = 0.032) and DFS (HR = 1.34, 95% CI: 1.01–1.78, P = 0.043) (Fig. 1E).

Gene and protein interaction network analysis

GeneMANIA identified multiple genes closely interacting with PDIA6, including TXNDC5, XBP1 and P4HB (Fig. 2A). STRING analysis constructed a core PPI network of PDIA6. GO enrichment revealed that interacting molecules were primarily enriched in protein folding, ER protein processing and ER-associated degradation (ERAD) (Fig. 2B). KEGG analysis showed enrichment in ER protein processing, protein export and the PI3K-Akt signaling pathway (Fig. 2C).

Correlation between PDIA6 expression and immune infiltration

The correlation between PDIA6 mRNA expression and immune infiltration levels was analyzed using the TIMER database (Fig. 3A) with the Spearman's rank correlation method. The results demonstrated that PDIA6 expression was significantly correlated with the infiltration levels of CD4 + T cells ($r = 0.214$, $P < 0.001$), macrophages ($r = 0.164$, $P < 0.001$), and dendritic cells ($r = 0.134$, $P < 0.001$) in HNSCC. In addition, PDIA6 copy number variation was associated with the infiltration levels of B cells, CD8 + T cells, CD4 + T cells, neutrophils, and dendritic cells in HNSCC (Fig. 3B).

Similarly, the correlations between PDIA6 and immune cell markers were analyzed in TIMER (Table 2). PDIA6 levels were closely associated with TNFα, STAT5B, TIM-3, TAM-related markers, BDCA-4, among others. Therefore, PDIA6 may influence immunity in HNSCC through distinct mechanisms.

Correlation between PDIA6 and chemokines/receptors

TISIDB analysis showed that PDIA6 expression was significantly negatively correlated with anti-tumor chemokines and their receptors and positively correlated with pro-tumor chemokines CXCL5 and CXCL8 ($P < 0.05$) (Fig. 3C).

Co-expressed genes and functional enrichment analysis

A total of 8,877 PDIA6 co-expressed genes were identified. GO (Gene Ontology) enrichment was mainly observed in protein folding, ER stress response, extracellular matrix organization and immune response. PDIA6 expression was positively correlated with 5401 genes (dark red dots) and negatively correlated with 3476 genes (dark green dots) (Fig. 4A). Furthermore, a heatmap of the top 50 genes positively and negatively associated with PDIA6 expression (Fig. 4B).

Subsequently, gene set enrichment analysis (GSEA) was performed to annotate the gene ontology (GO) terms of PDIA6-coexpressed genes. GO annotation demonstrated that these coexpressed genes were primarily involved in protein folding, endoplasmic reticulum stress and several protein-binding processes. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis revealed that these proteins were enriched in protein processing in the endoplasmic reticulum, ECM-receptor interaction, primary immunodeficiency, as well as Th1 and Th2 cell differentiation (Fig. 4C).

Discussion

HNSCC is a highly heterogeneous malignancy with diverse etiological backgrounds and clinical outcomes, highlighting the urgent need to identify robust prognostic biomarkers and elucidate subtype-specific molecular mechanisms (1,8,9). The present study demonstrated that *PDIA6* was significantly upregulated in HNSCC and closely correlated with malignant progression and poor prognosis. Mechanistically, PDIA6 was involved in the regulation of ER homeostasis, oncogenic signaling pathways and the remodeling of an immunosuppressive TME. Notably, the present study provided novel

mechanistic insights into the potential roles of PDIA6 in viral carcinogenesis, immune evasion and therapeutic resistance in EBV-associated NPC.

Functional analyses highlighted that PDIA6 serves a critical role in ER protein folding and the ER stress response, which is particularly relevant to NPC pathogenesis (10,11,15). EBV-encoded oncoproteins, especially LMP1, trigger chronic ER stress and sustain the unfolded protein response to promote cancer cell survival, proliferation and malignant transformation in NPC (17,18). PDIA6 may act as a downstream effector of EBV-induced ER stress, protecting tumor cells from proteotoxic damage and facilitating adaptation to stressful microenvironmental conditions (11,19).

The results indicated that *PDIA6* expression was significantly associated with lymph node metastasis in HNSCC, which is a characteristic clinical feature of NPC. PDIA6 may promote tumor metastasis by modulating extracellular matrix remodeling and chemokine-mediated cell migration and invasion (16,20). ER stress signaling has been reported to enhance radiotherapy resistance by promoting DNA damage repair and inhibiting apoptosis (21,22). Therefore, PDIA6 overexpression may enhance radioresistance in NPC and represent a potential therapeutic target.

PDIA6 may participate in the immune paradox of NPC, defined by prominent immune infiltration alongside potent immunosuppression (5,23). The present results revealed that *PDIA6* expression was closely correlated with the infiltration of Tregs, tumor-associated macrophages and exhausted T cells, which are major components of the immunosuppressive TME in EBV-associated NPC (23,24). *PDIA6* was associated with multiple chemokine axes that recruit immunosuppressive cells and impede antitumor immune responses. Targeting PDIA6 may represent a promising strategy to reverse immunosuppression and enhance the efficacy of immunotherapy.

Several limitations of the present study should be acknowledged. First, this study was based on retrospective analysis of public datasets; thus, prospective validation in clinical NPC samples and multicenter cohorts is warranted. Second, the precise molecular mechanisms by which PDIA6 regulates ER stress and immune remodeling in NPC remain to be experimentally verified. Further in vitro and in vivo studies are required to explore the interaction between PDIA6 and EBV-encoded proteins and evaluate the therapeutic potential of PDIA6 inhibition in EBV-positive NPC.

Conclusion

In summary, *PDIA6* is significantly upregulated in HNSCC and serves as a robust independent prognostic biomarker. PDIA6 contributes to HNSCC progression by regulating ER homeostasis, oncogenic signaling and the formation of an immunosuppressive TME. Notably, PDIA6 may function as a key regulator linking EBV infection, ER stress activation, immune evasion and therapeutic resistance in NPC. This study enhances understanding of the molecular heterogeneity of HNSCC and provides novel potential targets for prognostic evaluation and precision therapy in HNSCC patients, particularly those with NPC.

Declarations

Acknowledgements

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Availability of data and materials

The datasets analyzed during the present study are available from TCGA (https://portal.gdc.cancer.gov/), CPTAC (https://proteomics.cancer.gov/programs/cptac) and GEO (https://www.ncbi.nlm.nih.gov/geo/).

Authors' contributions

XS and YC designed the study. XS and WK analyzed the data. XS drafted the manuscript. YC revised the manuscript critically. All authors read and approved the final version of the manuscript.

Ethical approval

All data analyzed in this study were obtained from publicly available open-access databases. No human samples, animal experiments or clinical trials were conducted in this work. Accordingly, ethical approval was not required.

Consent to participate

This study did not involve recruitment of human participants or collection of individual clinical specimens. Written or verbal informed consent for participation is not applicable.

Consent to publish

No individual patient data, identifiable personal information or private clinical records are included in this manuscript. All utilized datasets are public resources. Thus, consent for publication is not applicable.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71(3):209-249.
2. Leemans CR, Braakhuis BJ and Brakenhoff RH. The molecular biology of head and neck cancer. Nat Rev Cancer 2011;11(1):9-22.

3. Vermorken JB, Mesia R, Rivera F, Remenar E, Kawecka J, Lorusso V, et al. Platinum-based chemotherapy plus cetuximab in head and neck cancer. N Engl J Med 2008;359(11):1116-1127.
4. Chen W, Luo M, Liu Y, Zhang H, Yang Z, Qin B, et al. Nasopharyngeal carcinoma: epidemiology, pathogenesis, and treatment advances. Signal Transduct Target Ther 2022;7(1):359.
5. Chan ATC, Teo PM and Johnson PJ. Nasopharyngeal carcinoma. Lancet 2019;394(10200):641-655.
6. Johnson DE, Grandis JR, Takata Y and Carlson DL. Head and neck squamous cell carcinoma. Nat Rev Dis Primers 2020;6(1):92.
7. Yip KL, Khoo AS and Wong TS. Biomarkers in nasopharyngeal carcinoma. Semin Cancer Biol 2021;73:188-198.
8. Giaccone G, Wang Y and Faivre S. Emerging biomarkers and targeted therapy for head and neck cancer. Nat Rev Clin Oncol 2015;12(1):33-46.
9. Reusch U, Hasseus B, Lewin F, Sandin F, Angström A, Marklund A, et al. Molecular subtypes in head and neck cancer: current understanding and clinical implications. EBioMedicine 2020;59:102938.
10. Bulleid NJ and Ellgaard L. Protein disulphide isomerases: a diversity of functions beyond disulphide formation. Nat Rev Mol Cell Biol 2011;12(1):48-60.
11. Ren J, Han L and Zhang Y. PDIA6 regulates endoplasmic reticulum stress and cell proliferation in gastric cancer. J Cell Physiol 2021;236(8):5623-5635.
12. Wang Y, Liu J and Zhang H. The PDI family: novel regulators of tumor progression and therapeutic targets. Oncogene 2022;41(28):2375-2388.
13. Li X, Jiang Y, Chen L, Chen X, Chen J, Chen Z, et al. PDIA6 mediates cisplatin resistance by regulating ER stress and autophagy in non-small cell lung cancer. J Cell Mol Med 2020;24(11):6249-6261.
14. Huang S, Zhang Y, Tang W, Chen J, Chen L and Chen X. Prognostic value and immune landscape of endoplasmic reticulum stress-related genes in head and neck squamous cell carcinoma. J Pers Med 2023;13(2):267.
15. Liu X, Chen S, Wu Y, Chen J, Chen L and Chen X. Multiomics profiling reveals endoplasmic reticulum stress as a key driver of metastasis and immune evasion in HNSCC. Mol Oncol 2024;18(2):345-362.
16. Zlotnik A and Yoshie O. Chemokines: multiple levels of regulation. Nat Rev Immunol 2012;12(5):326-338.
17. Liu Y, Li W and Chen X. LMP1-induced endoplasmic reticulum stress promotes nasopharyngeal carcinoma cell proliferation via the PERK-eIF2α-ATF4 pathway. Oncol Rep 2020;44(3):1063-1074.
18. Young LS, Dawson CM and Wang F. EBV and cancer. Nat Rev Cancer 2016;16(12):789-802.
19. Hetz C and Papa FR. The unfolded protein response: integrating stress signals through the ER membrane. Nat Cell Biol 2018;20(8):829-838.
20. Chen Y, Zhang L, Lin J, Chen J, Chen L and Chen X. Radiotherapy resistance in nasopharyngeal carcinoma: molecular mechanisms and therapeutic strategies. Cancer Lett 2023;562:216-248.
21. Tai W, Chen J, Li J, Chen L and Chen X. ER stress and autophagy in radioresistance of cancer. Semin Cancer Biol 2022;79:94-104.

22. Zhang Q, Wei P, Huang Y, Chen J, Chen L and Chen X. Targeting PDIA6 enhances chemotherapy-induced immunogenic cell death in pancreatic cancer. J Hematol Oncol 2023;16(1):142.
23. Wang H, Li J, Zhao X, Chen J, Chen L and Chen X. Immune suppression and therapeutic implications in the EBV-associated nasopharyngeal carcinoma microenvironment. Mol Cancer 2021;20(1):146.
24. Fridman WH, Pagès F, Sautès-Fridman C, Galon J and Tartour E. The immune contexture in cancer prognosis and treatment. Nat Rev Clin Oncol 2017;14(7):401-417.

Tables

Tables are available in the Supplementary Files section.

Figures

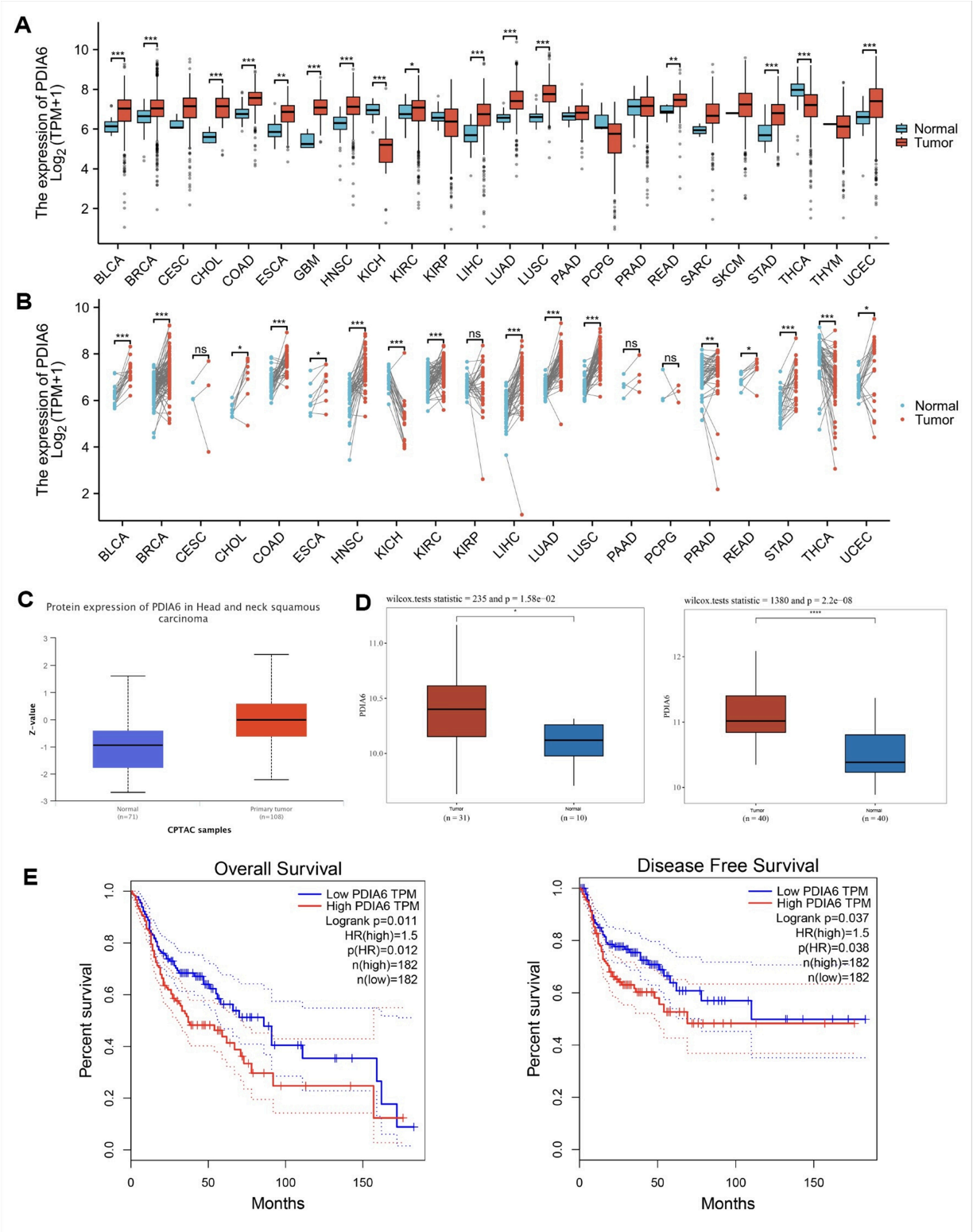


Figure 1

Expression and prognostic analysis of PDIA6 in HNSCC

(A) PDIA6 mRNA expression difference between HNSCC tumor tissues and adjacent normal tissues in the TCGA database; (B) PDIA6 protein expression comparison between HNSCC tissues and normal tissues in the CPTAC database; (C) PDIA6 expression difference between tumor and normal tissues in

the GSE12452 (left) and GSE37991 (right) datasets; (D) OS (left) and DFS (right) curves of HNSCC patients with high/low PDIA6 expression. *P*<0.05, **P**<0.01, ***P**<0.001.

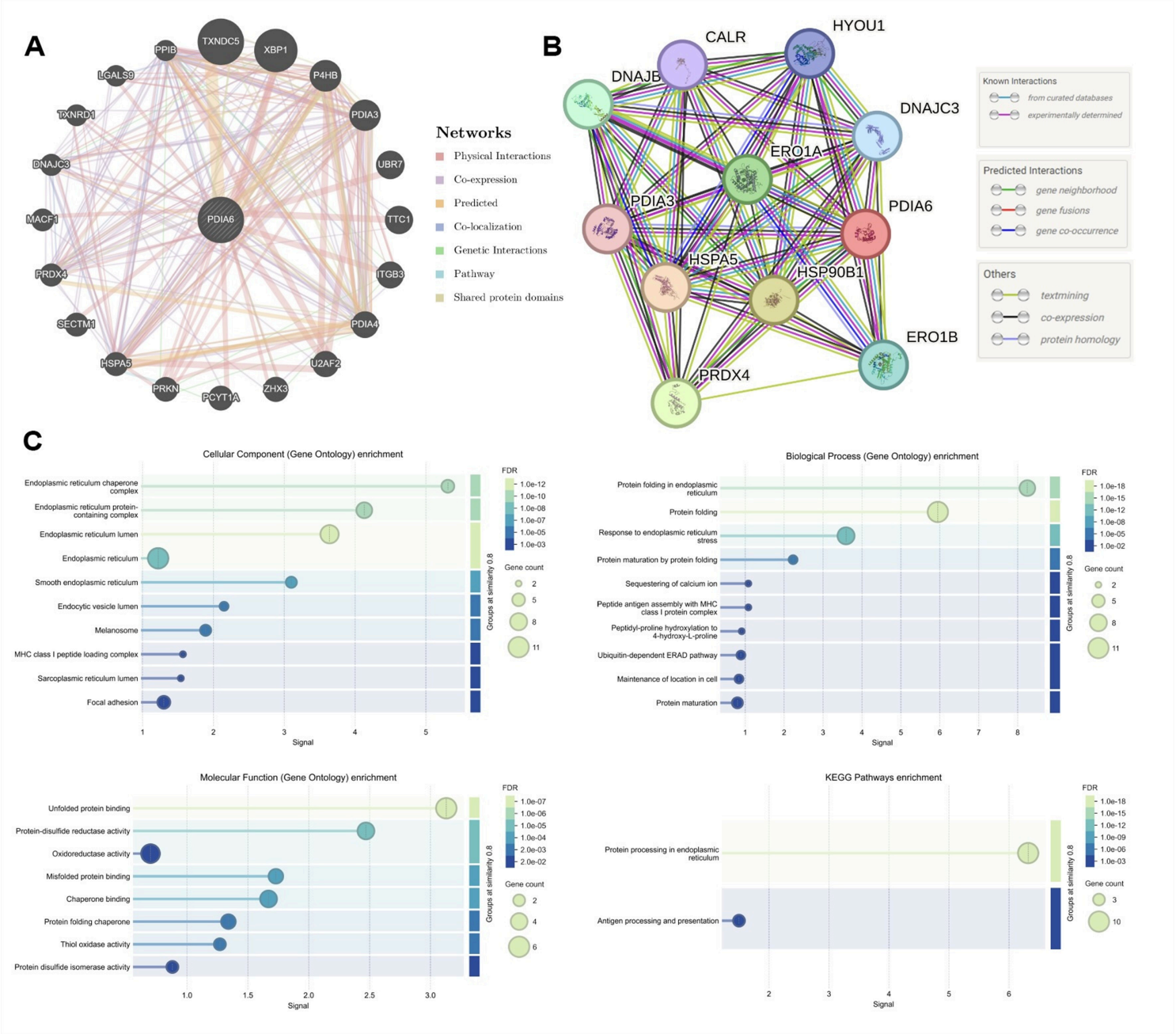


Figure 2

Gene-protein interaction network and functional enrichment analysis of PDIA6 in HNSCC

(A) Gene interaction network of PDIA6 constructed by GeneMANIA; (B) PPI network of PDIA6 constructed by STRING (confidence score >0.7); (C) GO functional enrichment (CC/BP/MF) and KEGG pathway enrichment analysis of PDIA6-interacting molecules.

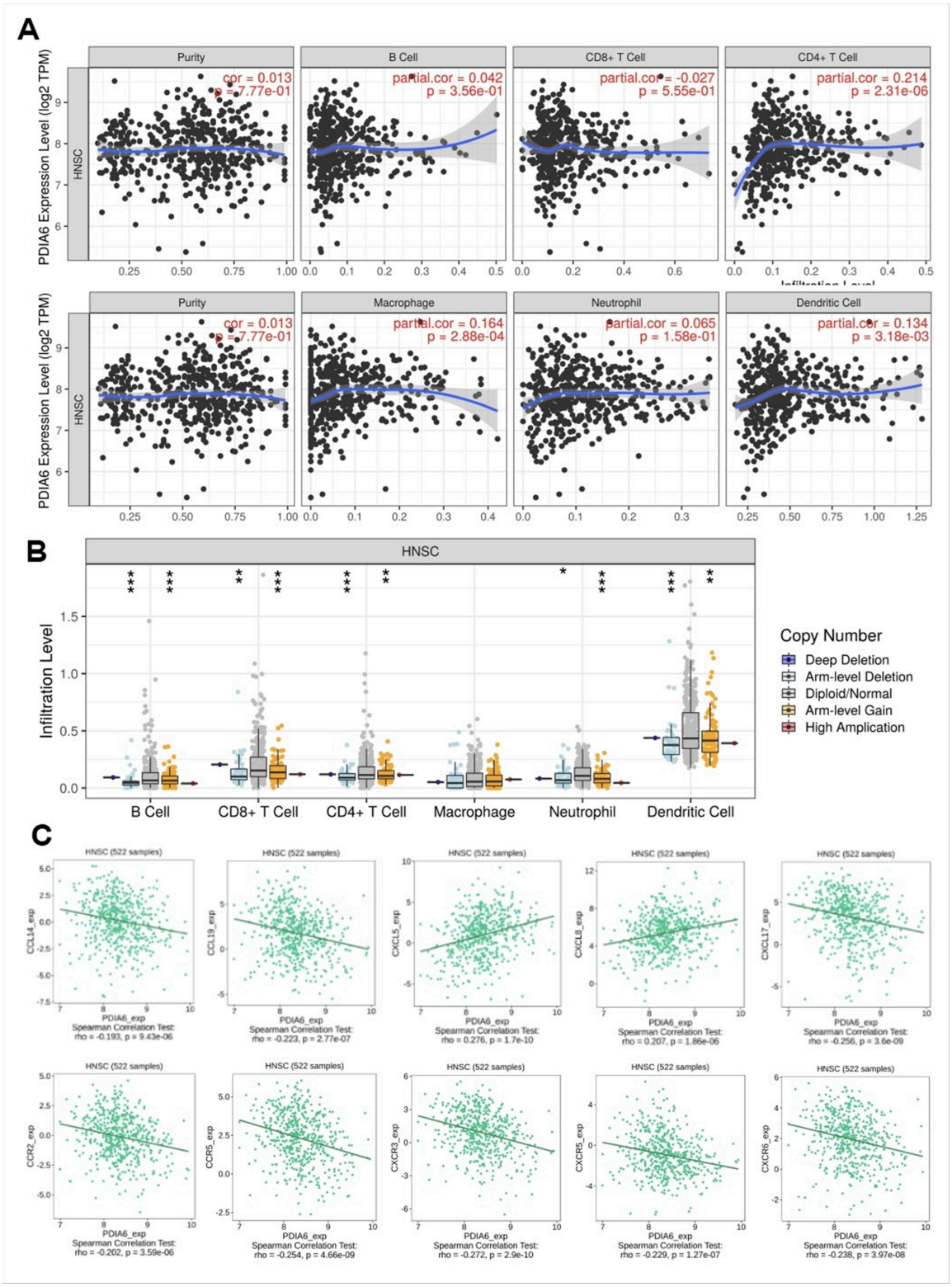


Figure 3

Correlation analysis of PDIA6 expression with immune infiltration and chemokines in HNSCC

(A) Spearman correlation analysis between PDIA6 expression and infiltration levels of six immune cell types in HNSCC; (B) The effect of PDIA6 copy number variation on immune cell infiltration in HNSCC

(*P*<0.05, **P**<0.01, ***P**<0.001); (C) Correlation analysis between PDIA6 expression and chemokines/receptors in HNSCC (Spearman correlation)

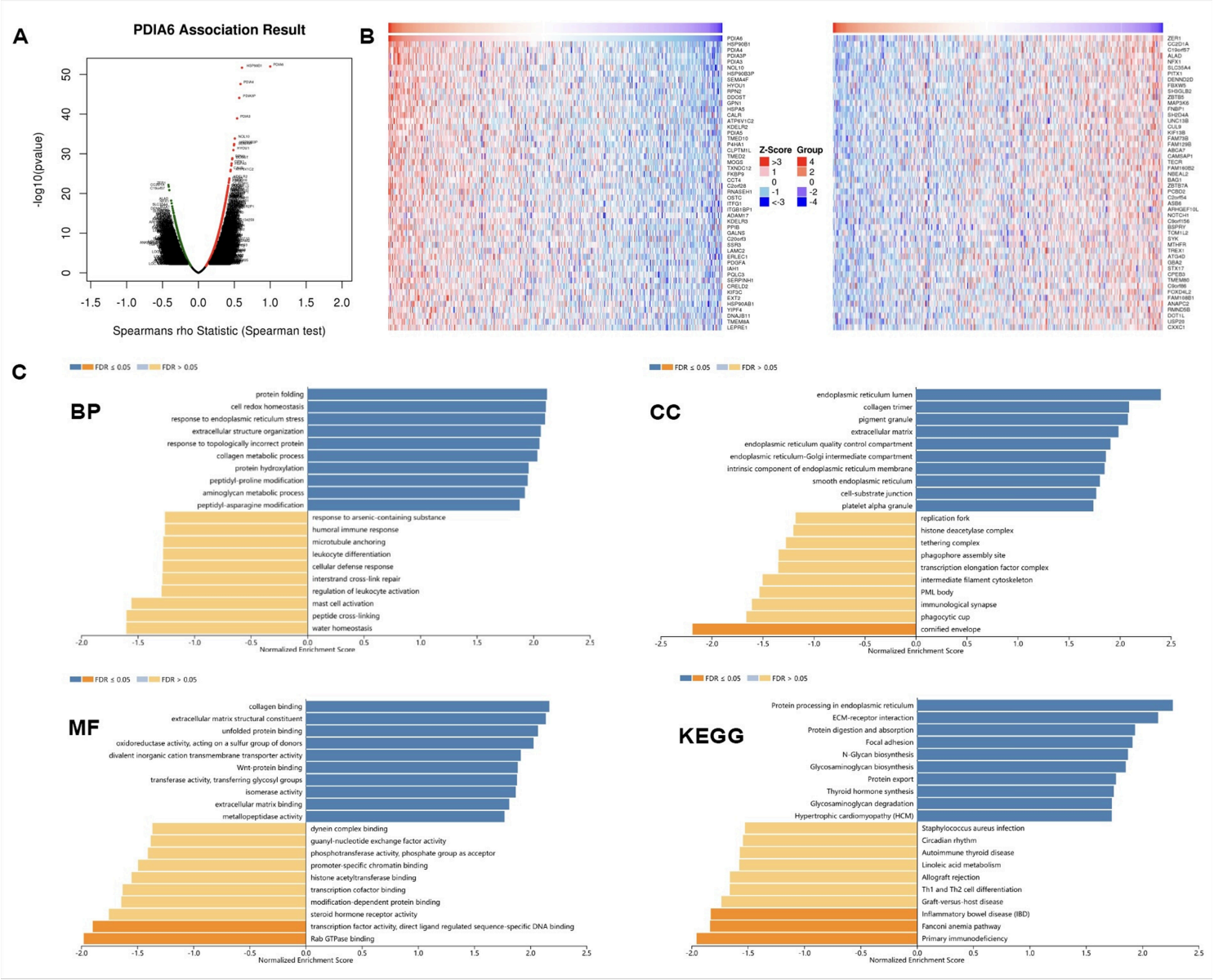


Figure 4

Co-expressed genes and functional enrichment analysis of PDIA6 in HNSCC

(A) Volcano plot of PDIA6 co-expressed genes in the TCGA-HNSCC cohort (red dots: positive correlation, green dots: negative correlation); (B) Heat map of the top 50 positively (left) and negatively (right) correlated genes with PDIA6; (C) GO functional enrichment (CC/BP/MF) and KEGG pathway enrichment analysis of PDIA6 co-expressed

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.docx
- Table2.docx