

Single-cell transcriptomics identifies PRDX1 and GPX4 as potential ferroptosis regulators and tumor microenvironment-related molecules in hepatocellular carcinoma

ZHOU ZHANG^{1*}, HUI CHEN^{2*}, CHEN QIAN^{3*}, NINGNING YANG⁴, ZIJING LIU³ and RUIRUI YANG⁵

¹Department of Clinical Laboratory, Affiliated Huishan Hospital of Xinglin College, Nantong University, Wuxi Huishan District People's Hospital, Wuxi, Jiangsu 214000, P.R. China; ²Center of Medical Laboratory, Changzhou Third People's Hospital, Changzhou, Jiangsu 213000, P.R. China; ³Department of Clinical Laboratory, Xishan People's Hospital of Wuxi City, Xishan Clinical Medical School, Kangda College of Nanjing Medical University, Wuxi, Jiangsu 214105, P.R. China; ⁴Department of Pathology, Xishan People's Hospital of Wuxi City, Xishan Clinical Medical School, Kangda College of Nanjing Medical University, Wuxi, Jiangsu 214105, P.R. China; ⁵Department of Science and Education, Affiliated Huishan Hospital of Xinglin College, Nantong University, Wuxi Huishan District People's Hospital, Wuxi, Jiangsu 214000, P.R. China

Received March 17, 2026; Accepted July 16, 2026

DOI: 10.3892/ol.2026.15886

Abstract. The present study aimed to investigate the expression and roles of peroxiredoxin 1 (PRDX1) and glutathione (GSH) peroxidase 4 (GPX4) in ferroptosis regulation and the tumor microenvironment in hepatocellular carcinoma (HCC). The expression levels of PRDX1 and GPX4 were assessed in HCC through analysis of datasets from The Cancer Genome Atlas (TCGA), GSE14520 from the Gene Expression Omnibus database and the Human Protein Atlas, along with HCC cell lines. Diagnostic and prognostic values were evaluated using receiver operating characteristic curves and Kaplan-Meier survival analyses. The single-cell dataset GSE149614 was employed to investigate the cellular localization and characteristics of specific cell subpopulations expressing PRDX1 or GPX4 within the HCC tumor microenvironment. The relationships between PRDX1, GPX4 and ferroptosis-related pathways were explored using the FerrDb database and Gene Set Enrichment Analysis. Additionally, their interactions with the immune microenvironment were examined utilizing the CIBERSORT algorithm. To validate the results of the bioinformatics analysis, the present study utilized small interfering (si)RNA technology to construct single gene knockdown and

dual gene co-knockdown models targeting PRDX1 and GPX4 in Hep3B cells. Subsequently, the proliferative activity of each group of cells was evaluated using the Cell Counting Kit-8 method and the levels of the intracellular lipid peroxidation product malondialdehyde (MDA) were detected using the MDA detection kit. The results of the present study demonstrated that the expression levels of PRDX1 and GPX4 were upregulated in HCC tissues and cell lines. The area under the curve for the combined diagnostic model of PRDX1 and GPX4 in HCC was 0.83. Furthermore, elevated expression levels of PRDX1 or GPX4 predicted reduced overall survival (OS), and their expression was weakly positively correlated in HCC. Single-cell transcriptomics demonstrated predominant expression of both genes in malignant cells. GPX4 was primarily detected in early-differentiation subgroups enriched in metabolic and ferroptosis pathways, whereas PRDX1 was predominantly expressed in intermediate-differentiation subgroups associated with antioxidative stress pathways. The dual-high PRDX1/GPX4 expression group exhibited increased reactive oxygen species, GSH and iron levels, whereas the dual-low group had prolonged OS. Immune infiltration analysis indicated a trend of higher M0 macrophage levels and lower CD4⁺ T-cell counts in the dual-high expression group. Furthermore, PRDX1 expression was positively correlated with multiple immune checkpoint genes, whereas GPX4 expression was markedly negatively correlated only with CD274 and TIGIT. The siRNA-PRDX1/siRNA-GPX4-1 group exhibited significantly inhibited HCC cell proliferation and promoted MDA production; however, proliferative activity was partially increased compared with that in the siRNA-PRDX1 group, whereas the levels of MDA accumulation were significantly reduced compared with in the siRNA-GPX4-1 group. In conclusion, the current study revealed the concerted co-expression of PRDX1 and GPX4 in malignant HCC, which was associated with ferroptosis evasion and immune suppression. Furthermore, the present findings suggested that PRDX1 may shift from an antioxidant to a pro-oxidant mediator under

Correspondence to: Professor Ruirui Yang, Department of Science and Education, Affiliated Huishan Hospital of Xinglin College, Nantong University, Wuxi Huishan District People's Hospital, 2 Zhanqian North Road, Luoshe, Huishan, Wuxi, Jiangsu 214000, P.R. China
E-mail: 13357925468@163.com

*Contributed equally

Key words: single-cell transcriptomics, hepatocellular carcinoma, peroxiredoxin 1, glutathione peroxidase 4, ferroptosis

GPX4 deficiency, thereby alleviating lipid peroxidation. These findings support targeting this axis as a future therapeutic breakthrough.

Introduction

Globally, hepatocellular carcinoma (HCC) represents the sixth most common type of cancer and the third leading cause of cancer-related deaths (1). As the primary histological subtype, HCC accounts for 85-90% of all liver malignancies (2,3). Current standard therapeutic interventions for HCC include surgical resection, liver transplantation, radiofrequency ablation, transarterial chemoembolization, hepatic arterial infusion chemotherapy and targeted molecular therapies (such as sorafenib, lenvatinib, regorafenib, and ramucirumab) (4). However, as most patients are diagnosed at intermediate to advanced stages, surgical options become impractical (5). Therefore, identification of biomarkers for early diagnosis and therapeutic targets remains urgently required.

Ferroptosis, first identified by Dixon in 2012 (6), is an emerging form of programmed cell death distinct from apoptosis, necrosis and autophagy. Ferroptosis is characterized by the iron-dependent accumulation of reactive oxygen species (ROS) and lipid peroxides, disrupting cellular redox homeostasis, and resulting in membrane condensation and eventual rupture (7). This cell death pathway is induced by metabolic intermediates and is strictly regulated by antioxidant mechanisms such as glutathione (GSH) peroxidase 4 (GPX4) and cystine/glutamate antiporter (8,9). Previous evidence has indicated that ferroptosis participates in numerous pathological processes, including cancer progression and ischemia-reperfusion injury (8,10). Moreover, it has been reported that ferroptosis inducers, alone or combined with conventional anticancer therapies, can effectively kill cancer cells (11-13), highlighting ferroptosis as a potential therapeutic target.

Intratumoral heterogeneity and cellular diversity within the tumor microenvironment (TME) currently present notable obstacles to early cancer diagnosis and precision medicine (14). This heterogeneity arises from variations in genetic makeup, pathology, differentiation and mutation profiles, reflecting tumor complexity and diversity throughout disease progression (15). Advancements in single-cell transcriptomics have provided deeper insights into tumor development, enabling precise identification of tumor subtypes and analysis of the TME (16,17). This approach helps elucidate intratumoral heterogeneity, cellular interactions within the microenvironment, predicts drug resistance, improves diagnostic accuracy and informs personalized treatments (18).

Peroxiredoxin 1 (PRDX1), a notable member of the PRDX family, functions as an antioxidant by eliminating intracellular ROS, protecting cells from oxidative damage. Moreover, PRDX1 and GPX4 maintain a close regulatory interaction in cancer, serving as crucial antioxidant proteins to inhibit ferroptosis and facilitate tumor development. For example, knockdown of PRDX1 in colorectal cancer cells leads to decreased GPX4 expression, elevated ROS accumulation and enhanced lipid peroxidation, thus triggering ferroptotic cell death (19). In breast cancer, inducing PRDX1 liquid-liquid phase separation reduces its peroxidase activity, disrupts ROS homeostasis, and promotes ferroptosis via lipid

peroxide accumulation mediated by the SLC7A11-GPX4 pathway (20). These observations imply that targeting PRDX1 alongside GPX4 inhibition could represent a promising anticancer strategy.

The present study aimed to utilize single-cell transcriptomics analysis to explore the expression profiles of PRDX1 and GPX4 in HCC, and to investigate their potential roles in ferroptosis regulation and the TME, thus providing theoretical support for strategies targeting ferroptosis resistance in HCC treatment.

Materials and methods

Bioinformatics online tools. The GEPIA2 database (<http://gepia2.cancer-pku.cn>) was employed to assess differential expression levels of PRDX1 and GPX4 across several cancer types. Specifically, under the 'Expression Analysis' section, the 'Expression DIY' feature and 'Multiple Gene Comparison' option were selected. Target gene names (PRDX1 or GPX4) were entered individually, with 'Match TCGA normal data' checked to include normal tissue references. A list of 23 TCGA cancer abbreviations (including LIHC and other solid tumors) were specified, and expression profiles were generated by clicking the 'Plot' button. The prognostic values of PRDX1 and GPX4 in HCC were analyzed using the 2022 version of the UALCAN database (<https://ualcan.path.uab.edu/>) (21,22). Within 'TCGA' section, 'TCGA Gene' was selected, gene names entered, and 'Liver hepatocellular carcinoma' chosen from the dataset menu. Survival analysis for PRDX1 and GPX4 in LIHC was performed using the UALCAN database. Kaplan-Meier survival curves were generated to evaluate the prognostic value, and the statistical significance of the difference between groups was determined using the Log-rank test.

Analysis of PRDX1 and GPX4 expression from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) databases. Transcriptomics RNA sequencing (RNA-seq) data of tumor and corresponding normal liver tissues from patients with HCC were obtained from TCGA-LIHC (Liver Hepatocellular Carcinoma) cohort (<https://portal.gdc.cancer.gov/projects/TCGA-LIHC>), with 50 paired tumor-normal samples included in the differential expression analysis. Microarray-based gene expression profiles were obtained from the GSE14520 dataset (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE14520>) (23), and 212 matched tumor-adjacent pairs (424 samples in total) were retained after strict quality control to exclude samples without paired counterparts or incomplete clinical annotations. R software (version 4.5.0; The R Foundation) was employed to filter the data. For TCGA-LIHC cohort, all primary HCC cases with available RNA-seq gene expression data and paired adjacent non-tumor liver tissues were included. Samples with non-HCC histology, those lacking expression data or those with incomplete pathological annotations were excluded. Genes with an expression level of <1 in more than half of the samples were filtered out. For the analysis of PRDX1 and GPX4 expression, the data from 50 matched tumor-adjacent pairs were extracted and differential expression was assessed using a paired Student's t-test.

For the GSE14520 dataset, all primary HCC samples and paired adjacent non-tumor samples with complete gene

expression profiles and clinical annotations were included; samples with missing expression values or ambiguous labeling were excluded. Differential expression levels of PRDX1 and GPX4 between tumor and adjacent non-tumor tissues were assessed using the Wilcoxon signed-rank test.

In both datasets, 'upregulation' was defined as a significantly higher expression in tumor samples compared with normal samples (two-sided $P < 0.05$). As the analysis was restricted to two pre-specified genes, no additional log2 fold-change cutoff or correction for multiple testing was applied.

PRDX1 and GPX4 protein expression from the Human Protein Atlas. Protein expression levels of PRDX1 and GPX4 in HCC tissues were evaluated using the Human Protein Atlas database (v25.0; <https://www.proteinatlas.org>). The key words 'liver' and 'cancer' were employed for data retrieval. Under 'Tissue' or 'Pathology', 'Liver' was selected, and the condition 'cancer' was specified. Immunohistochemical images and staining intensity scores from representative samples were compared: GPX4 [GPX4_normal: Female; age, 63 years; Liver; GPX4_tumor: Female; age, 77 years; Liver] and PRDX1 [PRDX1_normal: Female; age, 29 years; Liver; PRDX1_tumor: Female; age, 58 years; Liver].

Cell culture conditions. Normal human THLE-2 hepatocytes [American Type Culture Collection (ATCC) cat. no. CRL-2706] and human HCC Hep3B cells (ATCC cat. no. HB-8064) were sourced from the ATCC. Human HCC MHCC97-H (cat. no. SCSP-5092) and Huh-7 (cat. no. SCSP-526) cells were sourced from the National Collection of Authenticated Cell Cultures, Chinese Academy of Sciences. THLE-2 cells were cultured in BEGM Bullet Kit medium (cat. no. CC-3170; Lonza Group, Ltd.), which is the specific medium officially recommended by the manufacturer for maintaining this normal liver epithelial cell line, supplemented with 5 ng/ml epidermal growth factor (cat. no. CC-4175; Lonza Group, Ltd.), 70 ng/ml phosphoethanolamine (cat. no. P0503; MilliporeSigma) and 10% FBS (cat. no. 10099141; Gibco; Thermo Fisher Scientific, Inc.). Hep3B cells were maintained in MEM (cat. no. 11095080; Gibco; Thermo Fisher Scientific, Inc.) containing 10% FBS and 1% penicillin-streptomycin. MHCC97-H and Huh-7 cells were maintained in DMEM (cat. no. 11995065; Gibco; Thermo Fisher Scientific, Inc.) containing 10% FBS and 1% penicillin-streptomycin. All cells were cultured at 37°C in a humidified incubator with 5% CO₂.

Cell transfection. Hep3B cells were seeded at a density of 3×10^5 cells per well in a 6-well plate, with 2 ml culture medium added to each well. PRDX1 knockdown and GPX4 knockdown groups were set up separately and the dual-knockdown group was generated by co-transfection of siRNA-PRDX1-1 and siRNA-GPX4-1. The PRDX1 knockdown group included a negative control (NC) group [small interfering (si)RNA-NC] and three siRNA experimental groups targeting PRDX1 (siRNA-PRDX1-1, siRNA-PRDX1-2 and siRNA-PRDX1-3). The GPX4 knockdown group also included an NC group (siRNA-NC) and three siRNA experimental groups targeting GPX4 (siRNA-GPX4-1, siRNA-GPX4-2 and siRNA-GPX4-3). The final concentration of target siRNAs was 30 nM for each single-gene knockdown group, and 60 nM in total for the

dual-knockdown group (30 nM each for siRNA-PRDX1-1 and siRNA-GPX4-1). To ensure the comparability of biological effects, for the single-gene knockdown groups, an equivalent amount of siRNA-NC was added to reach the same 60 nM total siRNA concentration as the dual-knockdown group. Preliminary experiments verified that 60 nM total siRNA did not induce significant cytotoxicity in Hep3B cells. Transfections were performed using Lipofectamine® 3000 Transfection Reagent (cat. no. L3000015; Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Cells were incubated with the transfection mixture at 37°C for 6 h, after which the medium was replaced with fresh complete culture medium. At 24 h post-transfection, the knockdown efficiency of PRDX1 or GPX4 was detected separately. All siRNA sequences are shown in Table I.

Western blotting. THLE-2, Hep3B, Huh-7 and MHCC97-H cells were cultured until they reached ~80% confluence in a 100-mm culture dish, rinsed with PBS and subsequently lysed in 600 μ l NP-40 buffer (50 mM Tris-HCl, pH 7.4; 150 mM NaCl; 1% NP-40). Lysates were vortexed vigorously for 15 sec, three times at 5-min intervals on ice, then centrifuged at 12,000 $\times$ g for 30 min at 4°C. Protein levels were quantified using a BCA protein assay kit (cat. no. BB-3401; BestBio; www.bestbio.com.cn). For each sample, 50 μ g protein was mixed with 5X SDS-PAGE loading buffer and denatured at 95°C for 5 min. The proteins were then separated by SDS-PAGE on 12% gels and transferred to methanol-activated PVDF membranes. The membranes were blocked with 5% BSA (cat. no. SW3015; Beijing Solarbio Science & Technology Co., Ltd.) for 1 h at ambient temperature, rinsed three times with TBS-0.1% Tween, and incubated with antibodies against PRDX1 (1:5,000; cat. no. 15816-1-AP; Proteintech Group, Inc.) or GPX4 (1:500; cat. no. sc-166570; Santa Cruz Biotechnology, Inc.) separately for 3 h at room temperature. After additional washing, the membranes were incubated with HRP-conjugated secondary antibodies (1:5,000; cat. nos. RGAR001 or RGAM001; Proteintech Group, Inc.) for 1 h at room temperature. Detection was performed using BeyoECL Star reagent (cat. no. P0018AS; Beyotime Biotechnology) on a ChemiDoc MP Imaging System (cat. no. 1708280; Bio-Rad Laboratories, Inc.), and protein expression was semi-quantified with ImageJ software (version 1.53; National Institutes of Health), normalized to β -actin (1:20,000; cat. no. 66009-1-Ig; Proteintech Group, Inc.). Data are presented as the mean $\pm$ standard deviation from three independent biological replicates.

Cell proliferation assay. After digestion, the transfected Hep3B cells in each group were resuspended in complete culture medium. Hep3B cells were seeded in a 96-well plate at a concentration of 4,000 cells/well in 100 μ l culture medium ($n=4$ wells/group). After the cells adhered to the wells, this time point was recorded as 0 h. At 0, 24 and 48 h, 10 μ l Cell Counting Kit-8 reagent (cat. no. C0037; Beyotime Biotechnology) was added to each well of Hep3B cells and incubated at 37°C in a 5% CO₂ incubator for 1 h. Subsequently, the absorbance value was measured at a wavelength of 450 nm using an enzyme-linked immunosorbent assay plate reader.

Table I. Sequences of siRNAs targeting PRDX1 and GPX4.

siRNA Name	Sense sequence, 5'-3'	Antisense sequence, 5'-3'
siRNA-PRDX1-1	GAUGAGACUUUGAGACUAGUUTT	AACUAGUCUCAAGUCUCAUCTT
siRNA-PRDX1-2	CCAGAUGGUCAGUUUAAAGAUTT	AUCUUUAAACUGACCAUCUGGTT
siRNA-PRDX1-3	GCUUUCAGUGAUAGGGCAGAATT	UUCUGCCCUAUCACUGAAAGCTT
siRNA-GPX4-1	GCACAUGGUUAACCUGGACAATT	UUGUCCAGGUUAACCAUGUGCTT
siRNA-GPX4-2	GUGGAUGAAGAUGCAACCCAATT	UUGGGUUGGAUCUUAUCCACTT
siRNA-GPX4-3	GUGAGGCAAGACCGAAGUAAATT	UUUACUUCGGUCUUGCCUCACTT
siRNA-NC	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT

GPX4, glutathione peroxidase 4; NC, negative control; ns, not significant; PRDX1, peroxiredoxin 1; siRNA, small interfering RNA.

Measurement of malondialdehyde (MDA). The transfected Hep3B cells were lysed with cell lysis buffer (cat. no. P0013; Beyotime Biotechnology) on ice, after which, they were centrifuged at 10,000 x g for 10 min to obtain the supernatant and the protein concentration was determined using a BCA protein assay kit (cat. no. BB-3401; BestBio). Subsequently, the MDA detection working solution was prepared by diluting 7.5 ml TBA diluent, 2.5 ml TBA storage solution and 150 μ l of butylated hydroxytoluene, an antioxidant provided in the MDA assay kit (cat. no. S0131M; Beyotime Biotechnology), and the standard was diluted to 1, 2, 5, 10, 20 and 50 μ M with distilled water. The corresponding blanks, standards, and samples (0.1 ml) were added into 1.5 ml microcentrifuge tubes, and then mixed with 0.2 ml of MDA detection working solution. Afterwards, they were heated at 100°C for 15 min, cooled to room temperature and centrifuged at 1,000 x g for 10 min at room temperature. Supernatants (200 μ l) were then collected and the absorbance was measured in a 96-well plate at a wavelength of 532 nm using a microplate reader. Finally, the MDA concentration of the samples were calculated based on the standard curve and were converted to MDA content/unit protein based on protein concentration.

Diagnostic evaluation of PRDX1 and GPX4 in HCC. Transcriptomics and clinical data from TCGA-LIHC cohort were downloaded. Receiver operating characteristic (ROC) curves for PRDX1 and GPX4 were generated using the pROC package (v1.19.0.1) (24), with 'sample type' (tumor, 1; normal, 0) as the state variable and gene expression level as the test variable. A combined predictive model (PRDX1 + GPX4) was developed using a support vector machine (SVM; implemented via the e1071 package (v1.7-14, <https://CRAN.R-project.org/package=e1071>) and caret package (v6.0-94, <https://CRAN.R-project.org/package=caret>) and ROC curves were plotted using the pROC package.

Single-cell transcriptomics analysis

Data source and quality control. The single-cell transcriptomics dataset GSE149614 (25), containing 25,479 genes from 71,915 cells across 10 primary HCC tissues, 2 portal vein tumor thrombus tissues, 1 metastatic lymph node and 8 normal liver samples, was obtained from the GEO database. Quality control analysis was performed using Seurat software version 5.1.0 (<https://satijalab.org/seurat/>), excluding cells with

mitochondrial content of >20%, <200 genes or >8,000 genes. Normalization was achieved using the SCTransform method implemented in the Seurat R package (v5.1.0; <https://satijalab.org/seurat/>).

Dimensionality reduction, clustering and cell annotation. Principal component analysis (PCA) was performed using the top 2,000 highly variable genes, followed by visualization via Uniform Manifold Approximation and Projection (UMAP), and cell clustering were all performed using the Seurat R package (v5.1.0). Cells were clustered based on RNA shared nearest neighbor (RNA_snn) distance with a resolution setting of 0.6. Cell-type annotation was performed based on the canonical cell lineage marker genes reported in the published literature (26). The identity of each cluster was comprehensively determined by integrating the expression distribution of marker genes in FeaturePlot, the expression level and proportion in DotPlot (both visualized using the Seurat package; v5.1.0), as well as the specific expression characteristics of representative canonical markers. Cells with consistent expression of multiple specific signature genes were assigned to the corresponding cell type. Identified cell populations included malignant cells (APOA2⁺, ALB⁺, APOA1⁺, AMBP⁺, APOH⁺ and TTR⁺), T cells (IL7R⁺, CD2⁺ and CD3D⁺), B cells (MS4A1⁺, BANK1⁺ and CD79A⁺), myeloid cells (LYZ⁺, AIF1⁺ and HLA-DRA⁺), endothelial cells (SPARC⁺, TM4SF1⁺ and INSR⁺), fibroblasts (COL1A2⁺, FAP⁺ and ACTA2⁺), plasma cells (MZB1⁺, IGLL5⁺ and SSR4⁺), mast cells (TPSAB1⁺, TPSB2⁺ and CPA3⁺), natural killer (NK) cells (GZMB⁺, KLRD1⁺ and KLRF1⁺), plasmacytoid dendritic cells (pDCs; JCHAIN⁺, TCF4⁺ and TCL1A⁺) and cycling cells (TOP2A⁺ and MKI67⁺).

Analysis of malignant cell subpopulations and pseudotime trajectory. To further explore the intratumoral heterogeneity and dynamic developmental characteristics of HCC malignant cells, malignant cell subpopulations were separately extracted from the integrated single-cell dataset for secondary dimensionality reduction and reclustering analysis using the Seurat R package (v5.1.0). Specifically, the top 2,000 highly variable genes were selected to perform PCA, and the top 15 principal components were adopted for subsequent UMAP dimensionality reduction. Based on the RNA_snn algorithm, consistent and stable reclustering of malignant cells was performed with a resolution parameter of 0.1. Differentially expressed genes ($|\log_2\text{foldchange}| > 1$; false discovery rate,

<0.05) were identified using the FindMarkers function implemented in the Seurat R package (v5.1.0). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was performed using the clusterProfiler package (v4.14.6, <https://bioconductor.org/packages/clusterProfiler/>). To explore the dynamic differentiation trajectory of malignant cells, the Monocle2 algorithm (v2.36.0, <https://bioconductor.org/packages/monocle/>) was applied to reconstruct cell pseudotime developmental trajectories based on the DDRTree dimensionality reduction algorithm. The variable genes obtained from malignant cell reclustering were defined as ordering genes to arrange cells in pseudo-temporal order according to their differentiation status. To define the pseudotime origin, the root state was set to the cell cluster exhibiting the highest expression of hepatic progenitor markers and the lowest expression of mature hepatocyte functional genes, thereby orienting the trajectory from an undifferentiated to a more differentiated state. Finally, the dynamic expression change patterns of key functional genes along the pseudotime axis were systematically analyzed. Cell-cell communication between PRDX1/GPX4-high expressing malignant cells and other cell types was analyzed using the CellChat R package (v2.2.0; <https://github.com/sqjin/CellChat>). Malignant cells were stratified into high- and low-expression subgroups by median PRDX1/GPX4 gene expression; communication probabilities were computed, filtered with $\text{min.cell}=10$, and visualized via bubble plots after merging subgroup CellChat objects.

Molecular association and concerted effect of PRDX1/GPX4 on the ferroptosis regulatory network. A comprehensive list of ferroptosis-related genes, including drivers (ACSL4, TP53, HMOX1, METTL3, NCOA4, ALOX15, BECN1, TFRC and ATF3) and suppressors (NFE2L2, SLC7A11, SIRT1, PRDX6, FTH1, FTO and DHODH) was sourced from the FerrDb database (v2.0; <http://www.zhounan.org/ferrdb/>). Spearman rank correlation analysis was performed to assess the expression correlations between PRDX1/GPX4 and these ferroptosis-related genes, and heatmaps depicting gene expression profiles and correlation coefficients were generated using the pheatmap R package (version 1.0.12; <https://cran.r-project.org/package=pheatmap>). Gene Set Enrichment Analysis (GSEA) of ferroptosis, oxidative stress, lipid peroxidation, and iron metabolism pathways was performed using gene sets derived from the Molecular Signatures Database (MSigDB, version 7.5.1; <https://www.gsea-msigdb.org/gsea/msigdb/>). Ferroptosis suppressors scores were calculated based on suppressor gene expression, and their associations with PRDX1/GPX4 expression levels and overall survival (OS) were assessed. HCC patient samples from TCGA-LIHC cohort were stratified into PRDX1-high/GPX4-high, PRDX1-low/GPX4-low, PRDX1-high/GPX4-low and PRDX1-low/GPX4-high groups according to median expression levels, comparing ROS, MDA, GSH, iron scores and OS among groups. Protein-protein interaction networks related to PRDX1/GPX4 regulation of ferroptosis were developed using STRING (<https://string-db.org/>) and Cytoscape (<https://cytoscape.org/>).

Relationship between PRDX1/GPX4 expression and the immune microenvironment. The CIBERSORT algorithm (v1.03, <https://cibersort.stanford.edu/>) was used to evaluate

the abundance of immune cell infiltration in HCC samples from TCGA-LIHC cohort. According to the median expression values of PRDX1 and GPX4, all samples were divided into four groups based on the median expression of PRDX1 (cut-off: 8.33) and GPX4 (cut-off: 8.81): Both_High, Both_Low, PRDX1_High_GPX4_Low and PRDX1_Low_GPX4_High. The Kruskal-Wallis test was applied to compare the differences in immune infiltration between different groups (without post hoc test). Tumor microenvironment stromal and immune scores were calculated using the ESTIMATE R package (v1.0.13; <https://bioinformatics.mdanderson.org/estimate/>). Immune score data of HCC from TCGA were downloaded from the TIMER3 website (<http://timer.cistrome.org/>), and Spearman rank correlation scatter plots with PRDX1/GPX4 expression were generated. Tumor mutation burden (TMB) and microsatellite instability (MSI) data from TCGA-LIHC cohort were retrieved from cBioPortal (<https://www.cbioportal.org/>) to evaluate associations with PRDX1/GPX4 expression using Spearman rank correlation analysis.

Statistical analysis. Data analysis was performed using R software (v4.5.0) for bioinformatics cohorts and GraphPad Prism (v8.0.2; Dotmatics) for cellular experimental statistics and visualization. All cell-based assays were performed in three independent biological replicates, and quantitative results are expressed as mean $\pm$ standard deviation. The Shapiro-Wilk test was first performed to assess the normality of all continuous variables. For paired comparisons between tumor tissues and matched adjacent normal tissues, the paired Student's t-test was applied for normally distributed data (TCGA-LIHC cohort), while the Wilcoxon signed-rank test was used for non-normally distributed data (GSE14520 dataset). For comparisons between two independent groups, the unpaired Student's t-test or the Wilcoxon rank-sum test was selected according to the normality test results. Spearman's correlation was used for correlation analysis. For comparisons between multiple experimental groups and a single control group, one-way analysis of variance (ANOVA) was applied followed by Dunnett's t-test. For comparisons of grouped repeated measurement data, two-way repeated measures ANOVA was performed, with the Bonferroni method used for post hoc multiple comparisons. For all other pairwise comparisons across multiple groups, one-way ANOVA was conducted followed by Tukey's test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Elevated expression of PRDX1 and GPX4 in HCC tissues and cells. Pan-cancer analysis revealed elevated expression levels of PRDX1 and GPX4 across multiple cancer types (Fig. 1A and B). Furthermore, analysis of TCGA cohort and GSE14520 dataset confirmed higher expression of PRDX1 and GPX4 in HCC tissues (Fig. 1C and D). Protein expression data from the Human Protein Atlas also indicated that both proteins were upregulated in HCC tissues (Fig. 1E). Western blot analysis further confirmed increased protein expression of PRDX1 and GPX4 in Hep3B, Huh-7 and MHCC97-H cells (Fig. 1F).

Clinical significance of PRDX1 and GPX4 in HCC. ROC curve analyses yielded area under the curve (AUC) values of

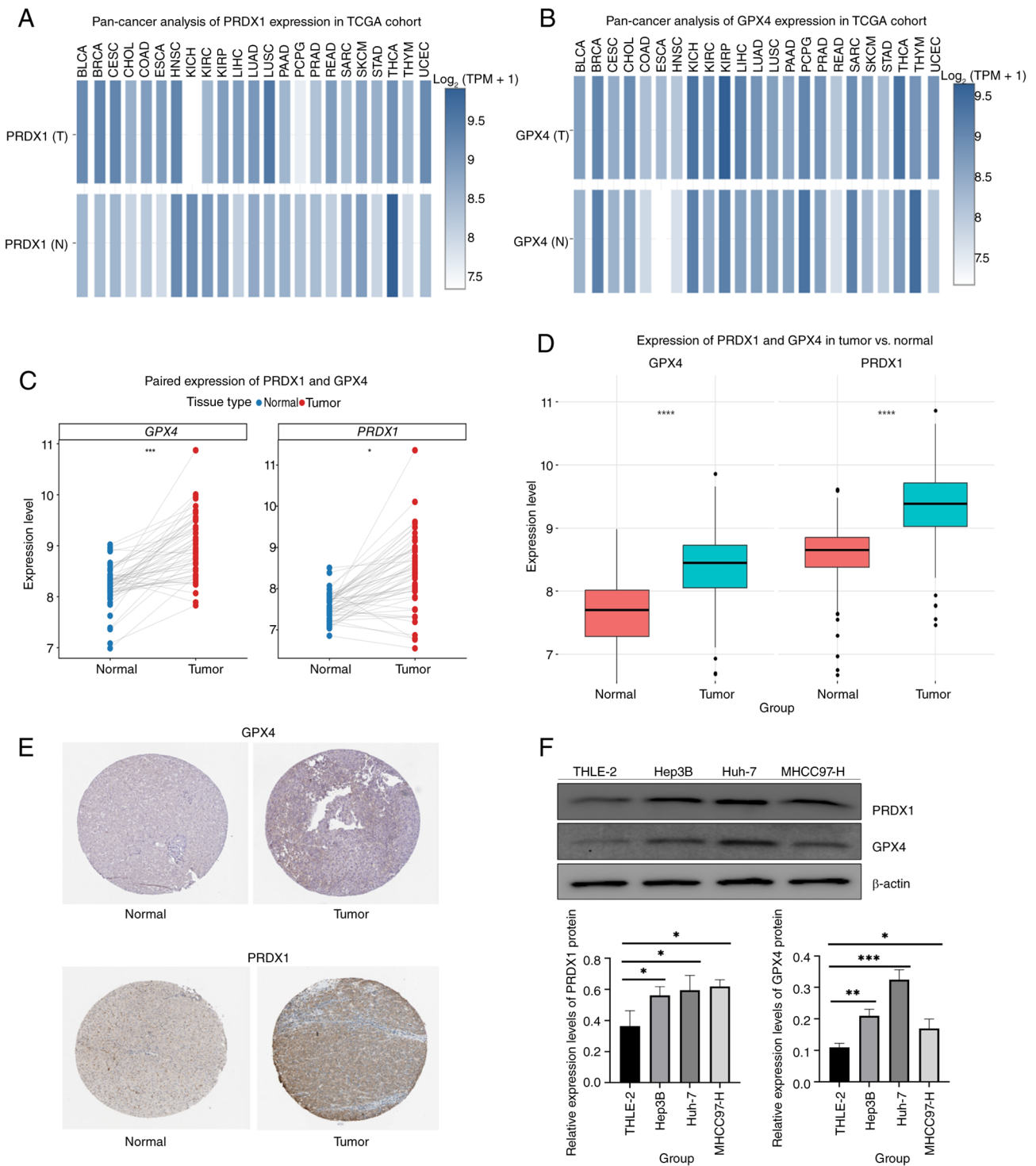


Figure 1. PRDX1 and GPX4 expression in HCC tissues and cells. Expression of (A) PRDX1 and (B) GPX4 across pan-cancer types, analyzed using GEPIA2 (based on TCGA cohort). The color scale represents $\log_2$ -transformed TPM values [$\log_2(\text{TPM}+1)$]. PRDX1 and GPX4 expression levels in HCC tissues from (C) TCGA cohort and (D) GSE14520 dataset. (E) Protein expression levels of PRDX1 and GPX4 in HCC tissues in the Human Protein Atlas database. (F) Comparison of PRDX1 and GPX4 protein expression in THLE-2, Hep3B, Huh7 and MHCC97-H cell lines. Data are presented as the mean $\pm$ SD from three independent biological replicates. Scale bar, 100 μm . * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. GPX4, glutathione peroxidase 4; HCC, hepatocellular carcinoma; PRDX1, peroxiredoxin 1; TCGA, The Cancer Genome Atlas; TPM, transcripts per million.

0.642 for PRDX1, 0.713 for GPX4, and 0.83 for the combined PRDX1-GPX4 model in diagnosing HCC (Fig. 2A). Survival analyses demonstrated shorter OS in patients with elevated PRDX1 or GPX4 expression (Fig. 2B and C). Additionally, a very weak positive correlation between PRDX1 and GPX4 expression ($\rho = 0.21$) was noted (Fig. 2D). PRDX1 expression

showed a very weak positive association with sex ($\rho = 0.23$), whereas GPX4 expression was very weakly correlated with age ($\rho = 0.12$).

Cellular localization of PRDX1 and GPX4 in the HCC microenvironment. A total of 11 distinct cellular subpopulations

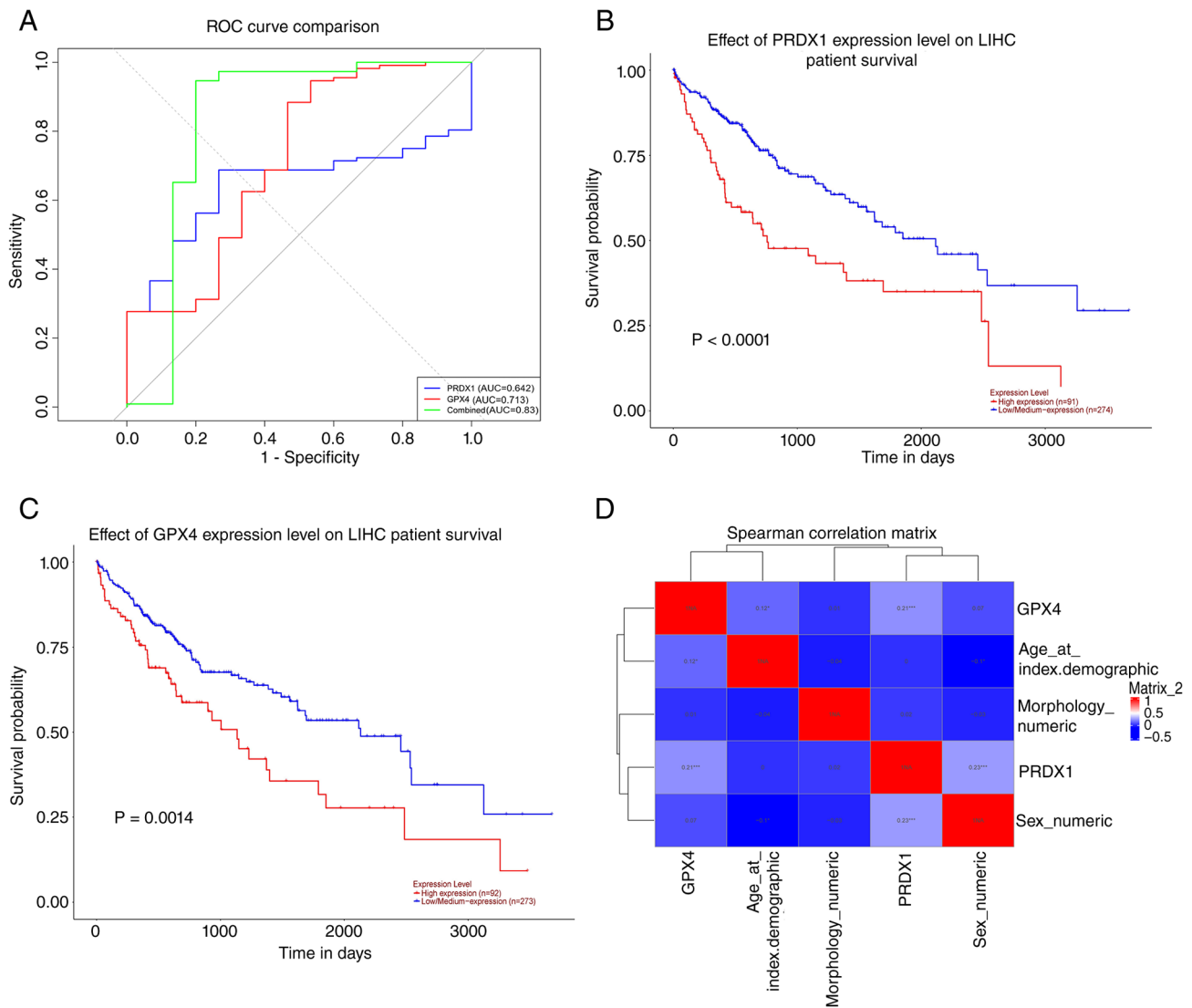


Figure 2. Clinical importance of PRDX1 and GPX4 in HCC based on data from TCGA-LIHC cohort. (A) Combined diagnostic efficacy of PRDX1 and GPX4 for HCC. Prognostic value of (B) PRDX1 and (C) GPX4 in HCC survival analysis. (D) Correlation between PRDX1/GPX4 expression and clinical characteristics of patients with HCC. * $P < 0.05$, *** $P < 0.001$. LIHC, liver hepatocellular carcinoma; AUC, area under the curve; GPX4, glutathione peroxidase 4; HCC, hepatocellular carcinoma; PRDX1, peroxiredoxin 1.

were identified within the single-cell dataset GSE149614, comprising malignant cells, T cells, B cells, myeloid cells, endothelial cells, cancer-associated fibroblasts, plasma cells, mast cells, NK cells, pDCs and cycling cells (Fig. 3A). PRDX1 and GPX4 were predominantly expressed in malignant and myeloid cells but scarcely expressed in lymphocytes (Fig. 3B). Significant heterogeneity in PRDX1/GPX4 expression and microenvironmental composition was observed among patient samples (Fig. 3C).

Dynamic expression and functional features of PRDX1 and GPX4 in malignant cell subpopulations. Through malignancy scoring and pseudotime trajectory analysis, reclustered malignant cells were ordered along a differentiation continuum and classified into three sequential states (State 1-State 3). As illustrated in Fig. 4A, State 1 represented early-differentiation stem-like cells with low malignancy, State 2 was the intermediate-differentiation transition subset, and State 3 corresponded to late-differentiation cells with high

malignancy. Dynamic expression profiles along the pseudotime axis demonstrated that GPX4 was predominantly enriched in State 1 stem-like cells, whereas PRDX1 was markedly upregulated in State 2 intermediate-differentiation subpopulations. KEGG pathway enrichment showed genes upregulated in the high GPX4 expression group were enriched in pathways related to metabolic reprogramming, ferroptosis and viral infection, including 'Biosynthesis of amino acids', 'Ferroptosis', 'Glycolysis/Gluconeogenesis' and 'Virion-Hepatitis viruses' (Fig. 4B). Furthermore, PRDX1-high expressing groups were enriched in pathways associated with oxidative stress and GSH metabolism, including 'Glutathione metabolism' and the 'Pentose phosphate pathway' (Fig. 4B). Cell-cell communication analyses indicated strong interactions between PRDX1/GPX4 high-expressing malignant cells and endothelial cells, T cells and fibroblasts via ligand-receptor pairs such as secreted phosphoprotein 1 (SPP1) and angiopoietin-like 4 (ANGPTL4) (Fig. 4C).

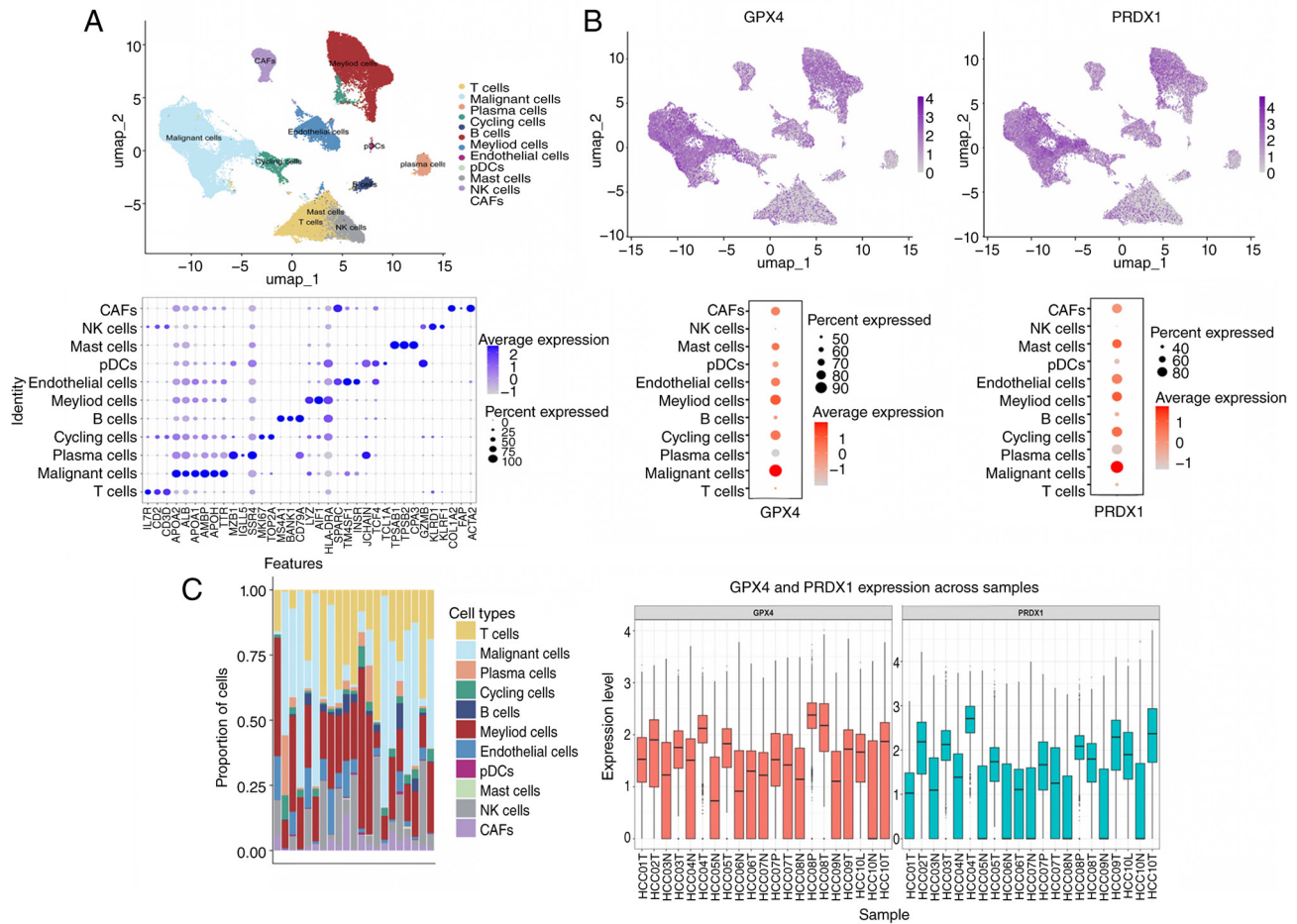


Figure 3. Cellular localization of PRDX1 and GPX4 in the hepatocellular carcinoma microenvironment based on GSE149614 dataset. (A) UMAP visualization of single-cell clusters and dot plot of marker gene expression. (B) PRDX1/GPX4 expression in different cell subpopulations. (C) Variation in cellular composition and PRDX1/GPX4 expression levels among individual patient samples. CAF, cancer-associated fibroblast; GPX4, glutathione peroxidase 4; NK cells, natural killer cells; pDCs, plasmacytoid dendritic cells; PRDX1, peroxiredoxin 1; UMAP, Uniform Manifold Approximation and Projection.

Association and concerted role of PRDX1 and GPX4 in ferroptosis regulation. Using the FerrDb database, 18 genes associated with ferroptosis were identified (Fig. 5A). PRDX1 was positively correlated with the ferroptosis suppressors GPX4, PRDX6, FTH1 and SLC7A11. By contrast, PRDX1 was negatively correlated with the classical ferroptosis driver TP53, as well as with SIRT1 (Fig. 5B), which is generally considered to suppress ferroptosis in certain contexts (27,28). Similarly, GPX4 was positively correlated with suppressors PRDX1, PRDX6, FTH1 and NFE2L2, and negatively correlated with drivers ALOX15, ACSL4 and TP53 (Fig. 5B). GSEA revealed that low GPX4 expression was enriched in iron metabolism and oxidative stress pathways, whereas high PRDX1 expression was associated with ferroptosis and oxidative stress pathways (Fig. 5C). The ferroptosis suppressors score was higher in the PRDX1-high group (Fig. 5D). Combined analyses revealed higher ROS, GSH and iron scores in the dual-high PRDX1/GPX4 group, whereas the dual-low group displayed longer OS (Fig. 5E). Protein interaction network analysis highlighted that PRDX1 and GPX4 suppress ferroptosis through three central functional nodes: Glutathione Metabolism, Lipid Peroxidation and Iron Metabolism. As shown in the topological PPI network in Fig. 5F, PRDX1 and GPX4 are closely associated with these three core hubs and occupy a central position in the ferroptosis regulatory network.

Relationship of PRDX1 and GPX4 expression with the HCC immune microenvironment. CIBERSORT analysis indicated increased M0 macrophages and reduced CD4⁺ T-cell infiltration in the dual-high PRDX1/GPX4 expression group (Fig. 6A). PRDX1 expression was positively correlated with immune checkpoints PDCD1, CD274, CTLA4, LAG3, HAVCR2 and TIGIT, whereas GPX4 expression negatively correlated with checkpoints CD274 and TIGIT (Fig. 6B). ESTIMATE analyses demonstrated very weak negative correlations between PRDX1/GPX4 expression and stromal score (Fig. 6C). Neither gene expression was correlated with TMB or MSI (Fig. 6D).

Effect of knocking down PRDX1 and GPX4 on cell proliferation and lipid peroxidation (MDA) levels. To verify the results of bioinformatics analysis, the current study designed and synthesized siRNAs targeting PRDX1 and GPX4. After transfection into Hep3B cells, the siRNAs with the best knockdown efficiency (siRNA-PRDX1-1 and siRNA-GPX4-1) were selected for subsequent experiments (Fig. 7A). HCC cell proliferation experiments showed that the siRNA-PRDX1, siRNA-GPX4-1 and siRNA-PRDX1/siRNA-GPX4-1 groups exhibited significantly inhibited proliferation; however, the cell proliferation of the siRNA-PRDX1/siRNA-GPX4-1 group showed a partial increase compared with the siRNA-PRDX1 group (Fig. 7B).

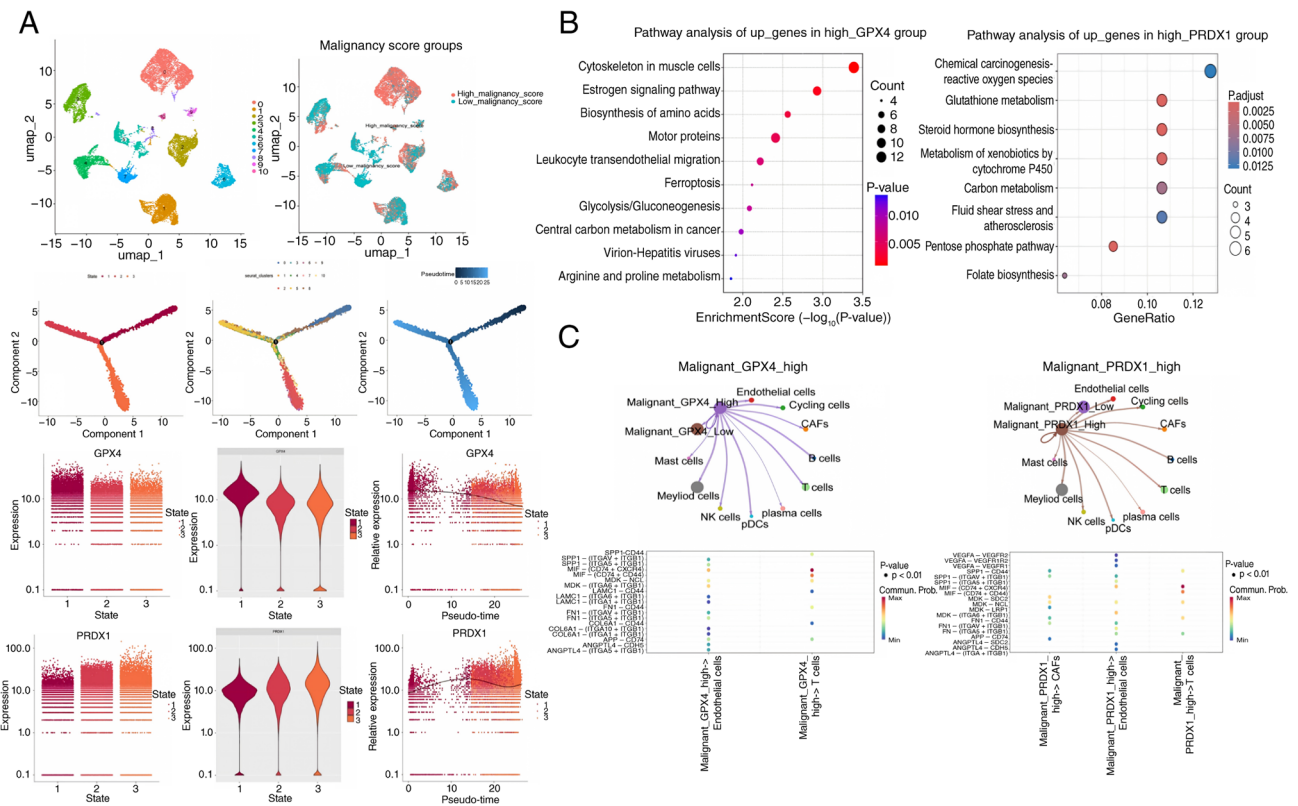


Figure 4. Dynamic expression and functional features of PRDX1 and GPX4 in malignant cell subpopulations based on GSE149614 dataset. (A) Dynamic changes in PRDX1 and GPX4 expression during tumor cell evolution, analyzed using malignancy scoring and pseudotime trajectory. (B) Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis of significantly upregulated genes in PRDX1 and GPX4 high-expression malignant cells. (C) Cell communication analysis showing interactions between PRDX1 and GPX4 high-expression malignant cells and other cell types within the microenvironment. CAF, cancer-associated fibroblast; GPX4, glutathione peroxidase 4; NK cells, natural killer cells; pDCs, plasmacytoid dendritic cells; PRDX1, peroxiredoxin 1; UMAP, Uniform Manifold Approximation and Projection.

The MDA detection results showed that all three silencing treatments significantly promoted the generation of intracellular MDA, but the cumulative level of intracellular MDA in the siRNA-PRDX1/siRNA-GPX4-1 group was significantly lower than that in the siRNA-GPX4-1 group (Fig. 7C).

Discussion

Ferroptosis has emerged as a distinct iron-dependent programmed cell death mechanism characterized by lipid peroxidation, which markedly impacts tumor initiation, progression and therapeutic resistance (29-32). The regulation of ferroptosis involves intricate mechanisms operating at multiple cellular levels. A critical aspect of ferroptosis is the disruption of equilibrium between the generation and elimination of intracellular lipid ROS, leading to excessive lipid peroxide accumulation and subsequent cell membrane rupture (7,8). Zheng *et al* (33) demonstrated that donafenib in combination with GSK-J4 synergistically elevated HMOX1 expression and intracellular ferrous ion concentration, consequently promoting ferroptosis in HCC cells. Another investigation reported that TRIM3 interacts directly with SLC7A11/xCT via its NHL domain, facilitating K11-linked ubiquitination at residue K37, thus triggering proteasomal degradation of SLC7A11; this mechanism promotes ferroptosis and inhibits tumor growth in non-small cell lung cancer (34). Yan *et al* (35) further reported that TIPE can suppress

ferroptosis by interacting with MGST1-ALOX5, thereby enhancing colorectal cancer cell proliferation.

The present study explored the cooperative inhibitory roles of PRDX1 and GPX4 on ferroptosis in HCC progression using single-cell transcriptomics analysis. Initially, the expression levels of PRDX1 and GPX4 in HCC were confirmed utilizing data from TCGA, GSE14520 and Human Protein Atlas databases, and THLE-2, Hep3B, Huh-7 and MHCC97-H cell lines. The findings indicated substantial upregulation of both PRDX1 and GPX4 in HCC, aligning with previous observations (36-38). Subsequently, their diagnostic performance and prognostic significance were evaluated. The AUC values of PRDX1 and GPX4 for HCC diagnosis were 0.642 and 0.713, respectively, whereas the AUC of the combined PRDX1-GPX4 model increased to 0.83, indicating improved diagnostic performance. Survival analyses indicated significantly reduced OS among patients exhibiting high expression levels of PRDX1 or GPX4 compared with those with lower expression levels, signifying worse prognostic outcomes. Thus, PRDX1 and GPX4 may potentially serve as valuable biomarkers for diagnosing and predicting HCC outcomes; however, further validation through multicenter studies is necessary. Additionally, PRDX1 showed a weak positive association with patient sex, whereas GPX4 showed a weak positive association with age.

Subsequently, dimensionality reduction and clustering analyses were performed on the GSE149614 dataset, and 11 major cellular subpopulations were identified. Analysis of

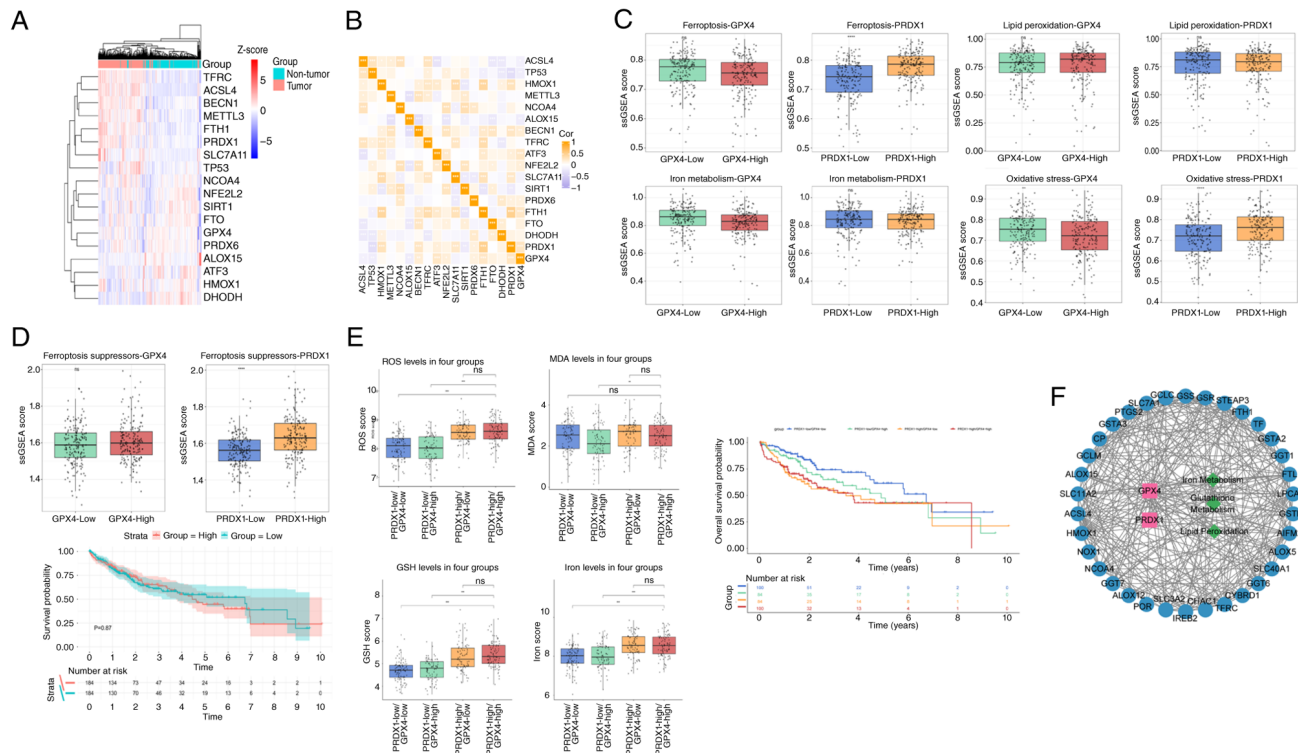


Figure 5. Association and concerted role of PRDX1 and GPX4 in ferroptosis regulation. (A) Heatmap illustrating expression of key ferroptosis-related genes based on GSE14520 dataset. The color scale represents row-scaled normalized gene expression values (Z-score). (B) Spearman correlations between PRDX1/GPX4 and key ferroptosis-associated genes based on GSE14520 dataset. (C) GSEA for PRDX1/GPX4 and related ferroptosis pathways based on data from TCGA-LIHC cohort. (D) Relationship between ferroptosis suppressor scores, PRDX1/GPX4 expression and patient prognosis based on data from TCGA-LIHC cohort. (E) Associations between PRDX1/GPX4 co-expression, metabolic markers and prognosis. Boxplots (upper) show ROS, MDA, GSH and iron levels across four PRDX1/GPX4 expression subgroups. Kaplan-Meier curves (lower) depict overall survival differences among the four groups based on data from TCGA-LIHC cohort (Log-rank $P=0.017$). (F) Integrated network depicting the regulatory role of PRDX1/GPX4 in ferroptosis. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$ GPX4, GSH peroxidase 4; GSEA, Gene Set Enrichment Analysis; GSH, glutathione; MDA, malondialdehyde; PRDX1, peroxiredoxin 1; ROS, reactive oxygen species; TPM, transcripts per million.

cellular distribution revealed that PRDX1 and GPX4 were predominantly expressed within malignant cells and myeloid populations, with minimal expression observed in lymphocyte subsets such as T, NK and B cells. Furthermore, marked variability in PRDX1/GPX4 expression and the immune microenvironment composition was evident among different individuals. These results imply that the ferroptosis-inhibiting functions of PRDX1 and GPX4 primarily operate within tumor cells rather than immune cells, aligning with prior studies that demonstrated targeting this pathway predominantly impacts cancer cells and modulates anti-tumor immunity in the microenvironment (39,40).

Re-clustering of malignant cells then identified 11 distinct clusters. Combined malignancy scoring and pseudotime analysis revealed that GPX4 was highly expressed during early differentiation stages, indicating that highly malignant, stem-like cells depend on GPX4 to resist ferroptosis. Conversely, PRDX1 was prominently expressed during intermediate differentiation stages, suggesting a protective role during metabolic transitions from stem-cell to differentiated-cell metabolism. KEGG analysis indicated that genes upregulated in GPX4-high expression cells were associated with pathways associated with metabolic reprogramming, ferroptosis and viral infection. Specifically, activation of metabolic pathways, including glycolysis/gluconeogenesis and amino acid biosynthesis, highlighted active metabolic processes supplying

energy and macromolecules necessary for rapid proliferation. Additionally, enrichment of estrogen and hepatitis virus pathways implied roles in hormonal regulation and virus-related hepatocarcinogenesis. Genes upregulated in PRDX1-high expression cells were primarily involved in redox balance and xenobiotic metabolism pathways, notably ROS chemical carcinogenesis and GSH metabolism. Additionally, enrichment analysis highlighted activation of the pentose phosphate pathway, contributing NADPH essential for GSH regeneration and bolstering the antioxidant defense mechanisms (41,42) in cells exhibiting high PRDX1 expression. These results align with the protective role of PRDX1 during cellular metabolic transitions. Cell communication analysis demonstrated robust interactions between PRDX1/GPX4-high expressing malignant cells and endothelial cells, T cells and fibroblasts via ligand-receptor pairs such as SPP1 and ANGPTL4.

Correlation analysis of 18 key ferroptosis-related genes from the FerrDb database revealed PRDX1 was positively correlated with the ferroptosis suppressors GPX4, PRDX6, FTH1 and SLC7A11 (43-45). By contrast, PRDX1 was negatively correlated with the classical ferroptosis driver TP53, as well as with SIRT1, which is generally considered to suppress ferroptosis in certain contexts (27,28). Similarly, GPX4 was positively correlated with suppressors PRDX1, PRDX6, FTH1 and NFE2L2, and negatively correlated with drivers ALOX15, ACSL4 and TP53 (46-48). PRDX1-high expressing

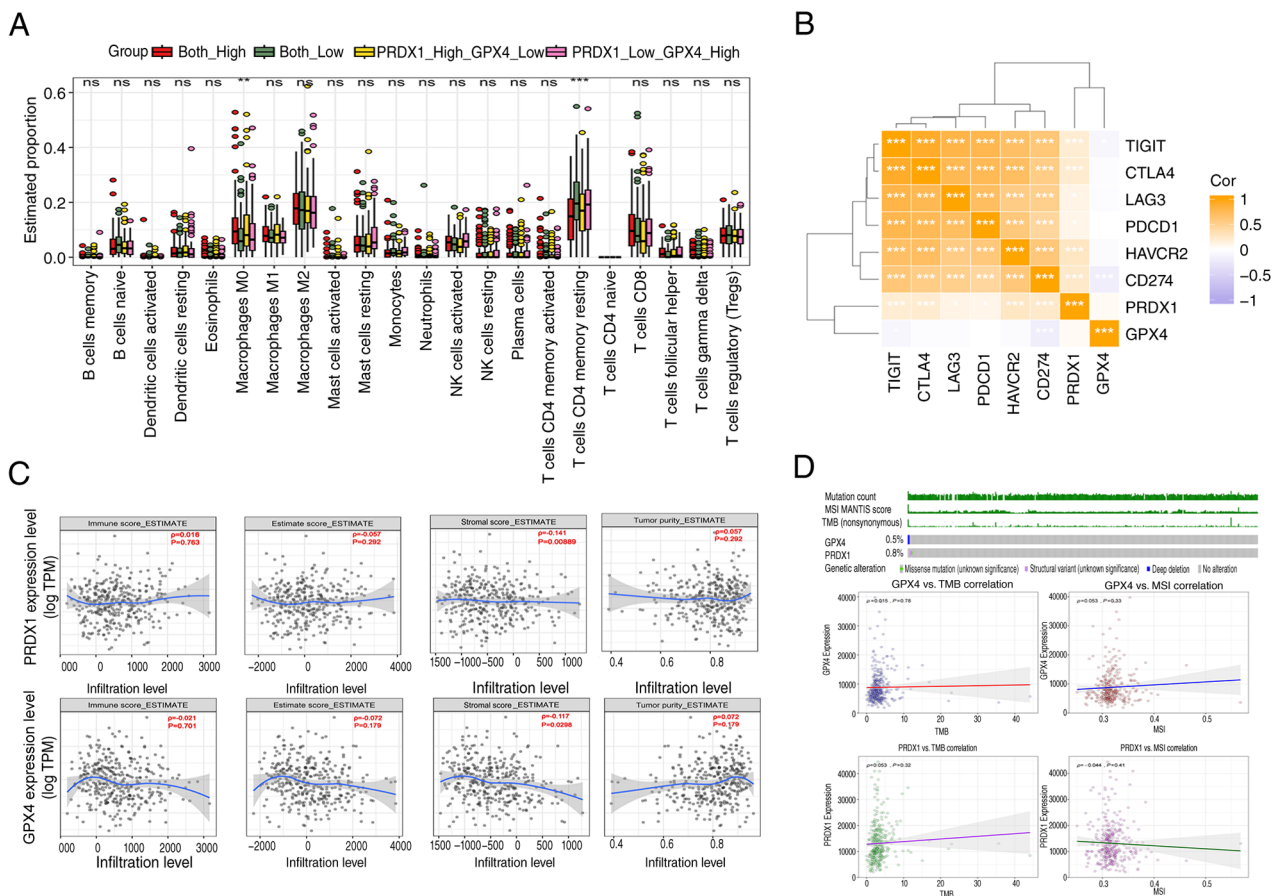


Figure 6. Relationship of PRDX1 and GPX4 expression with the HCC immune microenvironment based on TCGA-LIHC cohort. (A) Comparison of estimated immune cell proportions among different PRDX1/GPX4 expression subgroups.. (B) Correlation between PRDX1/GPX4 expression and immune checkpoint molecule expression. (C) Associations between PRDX1/GPX4 expression and immune-related scores. (D) Correlation analysis of PRDX1/GPX4 expression with TMB and MSI. *P<0.05, **P<0.01, ***P<0.001. GPX4, glutathione peroxidase 4; HCC, hepatocellular carcinoma; ICI, immune cell infiltration; MSI, microsatellite instability; PRDX1, peroxiredoxin 1; TMB, tumor mutation burden.

cells exhibited enrichment in ferroptosis and oxidative stress pathways, indicating adaptive resistance under ferroptosis pressure. Both genes jointly regulate a dynamic ferroptosis balance within the TME. Notably, the PRDX1-high group displayed increased ferroptosis resistance scores, suggesting PRDX1 confers ferroptosis resistance through a comprehensive defensive network, potentially contributing to poor HCC prognosis. The PRDX1/GPX4 dual-high expression group exhibited elevated ROS, GSH and iron scores, whereas the dual-low expression group exhibited prolonged OS, indicating that these genes maintain redox homeostasis by constructing a multi-layered ferroptosis defense, promoting HCC progression. Moreover, functional analyses suggested that PRDX1/GPX4 primarily inhibit ferroptosis through regulating GSH metabolism, lipid peroxidation and iron metabolism. Collectively, these multi-layered findings suggested that PRDX1 and GPX4 may act as critical modulators of ferroptosis resistance, potentially facilitating HCC progression through a comprehensive defensive network.

Subsequently, the association of PRDX1/GPX4 expression with immune microenvironment remodeling in HCC was explored. CIBERSORT analysis indicated an increased number of M0 macrophages and decreased CD4⁺ T cells in the PRDX1/GPX4-high expression group, highlighting their involvement in immune microenvironment reshaping. Immune

checkpoint analysis illustrated distinct immune regulatory roles for PRDX1 and GPX4. PRDX1 was positively correlated with multiple checkpoint molecules, including PDCD1, CD274, CTLA4, LAG3, HAVCR2 and TIGIT, suggesting involvement in immune evasion via multi-targeted T-cell exhaustion. GPX4 was negatively correlated with CD274 and TIGIT, implying a role in modulating checkpoint expression through oxidative homeostasis maintenance. Both PRDX1 and GPX4 had weak negative correlations with stromal scores and showed no relationship with TMB or MSI, indicating their mediation of ferroptosis resistance and immune evasion functions independently from genomic mutations and primarily through functional mechanisms.

To validate the bioinformatics predictions, siRNA-mediated knockdown of PRDX1 and GPX4 was employed in Hep3B cells and subsequent functional analyses were performed. As expected, single knockdown of either PRDX1 or GPX4 significantly suppressed Hep3B cell proliferation and elevated intracellular MDA levels, consistent with their established antioxidant and tumor-promoting roles, thereby confirming the initial bioinformatics findings. Notably, although the combined knockdown of PRDX1 and GPX4 markedly inhibited proliferation, its activity exhibited a partial recovery compared with the PRDX1 single knockdown group, yet remained lower than that of the GPX4 single knockdown group. To elucidate the

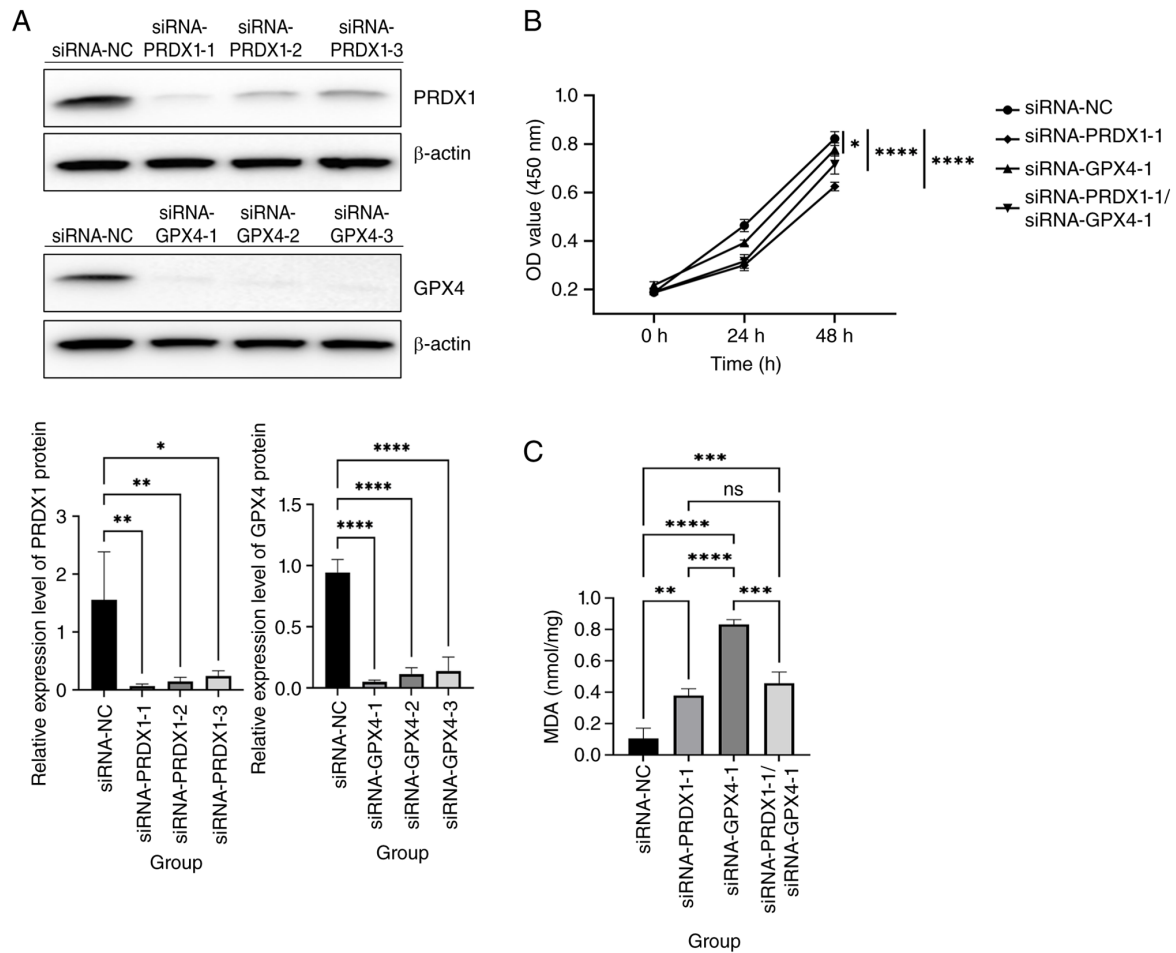


Figure 7. Effect of knocking down PRDX1 and GPX4 on cell proliferation and lipid peroxidation (MDA) levels using Hep3B cells. (A) Silencing efficiency validation of siRNAs targeting PRDX1 and GPX4. (B) Effects of PRDX1 and GPX4 on the proliferation of Hep3B cells. (C) Effects of PRDX1 and GPX4 on MDA generation in Hep3B cells. Data are presented as the mean $\pm$ SD from three independent biological replicates. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. GPX4, glutathione peroxidase 4; MDA, malondialdehyde; NC, negative control; ns, not significant; PRDX1, peroxiredoxin 1; siRNA, small interfering RNA.

underlying mechanism responsible for this non-additive effect, intracellular MDA levels were measured. Notably, the double knockdown group displayed significantly lower MDA accumulation than the GPX4 single knockdown group, indicating that PRDX1 silencing partially alleviates the severe lipid peroxidation induced by GPX4 deficiency. Based on these results, it may be hypothesized that under the intense lipid peroxidation stress triggered by GPX4 loss, PRDX1 switches from its canonical antioxidant role to a pro-oxidant mediator. This functional shift may partially explain why the proliferative activity of the double knockdown group was moderately restored relative to the PRDX1 single knockdown group. Nevertheless, this hypothesis is currently derived from phenotypic observations and warrants further validation through dynamic oxidative stress tracking, protein post-translational modification analyses and targeted rescue experiments.

In conclusion, the present comprehensive bioinformatics analysis, supported by *in vitro* functional validation, revealed a previously underestimated co-expression pattern of PRDX1 and GPX4 in malignant HCC cells, which is closely related to ferroptosis evasion and an immunosuppressive TME. Notably, the experimental results indicate that although GPX4 deficiency can severely induce lipid peroxidation, silencing PRDX1 at the same time can significantly alleviate this

oxidative damage. This suggests that under the stress of GPX4 deficiency, PRDX1 may transition from its typical antioxidant role to a pro-oxidant mediator. These findings broaden the understanding of the concerted interaction between PRDX1 and GPX4 in reshaping the redox pattern of HCC, and provide a theoretical basis for further exploration of this concerted axis as a potential therapeutic breakthrough.

Acknowledgements

Not applicable.

Funding

The present study was funded by the Scientific Research Program of Wuxi Health Commission (grant no. Q202415), the Clinical Medicine Special Research Fund Project of Nantong University (Key Project; grant no. 2024JZ049), the University Student Innovation and Entrepreneurship Program (grant no. S202010439049), the Soft Science Research Project of Wuxi Science and Technology Association (grant no. KX-25-C309) and the Scientific Research and Development Foundation of Kangda College of Nanjing Medical University (grant no. KD2025KYJJ209).

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

ZZ and RY were responsible for the conception and design of the study. ZZ, HC, CQ, NY and ZL performed the data analysis. ZZ, HC, CQ and RY drafted the manuscript. ZZ, HC and RY confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I and Jemal A: Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 74: 229-263, 2024.
- Feng J, Li J, Wu L, Yu Q, Ji J, Wu J, Dai W and Guo C: Emerging roles and the regulation of aerobic glycolysis in hepatocellular carcinoma. *J Exp Clin Cancer Res* 39: 126, 2020.
- Ringelhan M, Pfister D, O'Connor T, Pikarsky E and Heikenwalder M: The immunology of hepatocellular carcinoma. *Nat Immunol* 19: 222-232, 2018.
- Singal AG, Kanwal F and Llovet JM: Global trends in hepatocellular carcinoma epidemiology: Implications for screening, prevention and therapy. *Nat Rev Clin Oncol* 20: 864-884, 2023.
- Toh MR, Wong EYT, Wong SH, Ng AWT, Loo LH, Chow PKH and Ngeow J: Global epidemiology and genetics of hepatocellular carcinoma. *Gastroenterology* 164: 766-782, 2023.
- Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, Patel DN, Bauer AJ, Cantley AM, Yang WS, *et al*: Ferroptosis: An iron-dependent form of nonapoptotic cell death. *Cell* 149: 1060-1072, 2012.
- Dixon SJ and Olzmann JA: The cell biology of ferroptosis. *Nat Rev Mol Cell Biol* 25: 424-442, 2024.
- Liu J, Kang R and Tang D: Signaling pathways and defense mechanisms of ferroptosis. *FEBS J* 289: 7038-7050, 2022.
- Chen X, Yu C, Kang R, Kroemer G and Tang D: Cellular degradation systems in ferroptosis. *Cell Death Differ* 28: 1135-1148, 2021.
- Tang D, Kang R, Berghe TV, Vandenabeele P and Kroemer G: The molecular machinery of regulated cell death. *Cell Res* 29: 347-364, 2019.
- Yamaguchi Y, Kasukabe T and Kumakura S: Piperlongumine rapidly induces the death of human pancreatic cancer cells mainly through the induction of ferroptosis. *Int J Oncol* 52: 1011-1022, 2018.
- Hao S, Yu J, He W, Huang Q, Zhao Y, Liang B, Zhang S, Wen Z, Dong S, Rao J, *et al*: Cysteine dioxygenase 1 mediates erastin-induced ferroptosis in human gastric cancer cells. *Neoplasia* 19: 1022-1032, 2017.
- Alvarez SW, Sviderskiy VO, Terzi EM, Papagiannakopoulos T, Moreira AL, Adams S, Sabatini DM, Birsoy K and Possemato R: NFS1 undergoes positive selection in lung tumours and protects cells from ferroptosis. *Nature* 551: 639-643, 2017.
- Hanahan D and Coussens LM: Accessories to the crime: Functions of cells recruited to the tumor microenvironment. *Cancer Cell* 21: 309-322, 2012.
- Vitale I, Shema E, Loi S and Galluzzi L: Intratumoral heterogeneity in cancer progression and response to immunotherapy. *Nat Med* 27: 212-224, 2021.
- Stuart T and Satija R: Integrative single-cell analysis. *Nat Rev Genet* 20: 257-272, 2019.
- Shi J, Zhang Y, Xu L and Wang F: Single-cell transcriptomics reveals tumor microenvironment remodeling in hepatocellular carcinoma with varying tumor subclonal complexity. *Front Genet* 15: 1467682, 2024.
- Sun F, Li H, Sun D, Fu S, Gu L, Shao X, Wang Q, Dong X, Duan B, Xing F, *et al*: Single-cell omics: Experimental workflow, data analyses and applications. *Sci China Life Sci* 68: 5-102, 2025.
- Song Y, Wang X, Sun Y, Yu N, Tian Y, Han J, Qu X and Yu X: PRDX1 inhibits ferroptosis by binding to Cullin-3 as a molecular chaperone in colorectal cancer. *Int J Biol Sci* 20: 5070-5086, 2024.
- Fan X, Liu F, Wang X, Wang Y, Chen Y, Shi C, Su X, Tan M, Yan Q, Peng J, *et al*: LncFASA promotes cancer ferroptosis via modulating PRDX1 phase separation. *Sci China Life Sci* 67: 488-503, 2024.
- Chandrashekar DS, Karthikeyan SK, Korla PK, Patel H, Shovon ARS, Athar M, Netto GJ, Qin ZS, Kumar S, Manne U, *et al*: UALCAN: An update to the integrated cancer data analysis platform. *Neoplasia* 25: 18-27, 2022.
- Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi BVSK and Varambally S: UALCAN: A portal for facilitating tumor subgroup gene expression and survival analyses. *Neoplasia* 19: 649-658, 2017.
- Roessler S, Jia HL, Budhu A, Forgues M, Ye QH, Lee JS, Thorgerirsson SS, Sun Z, Tang ZY, Qin LX and Wang XW: A unique metastasis gene signature enables prediction of tumor relapse in early-stage hepatocellular carcinoma patients. *Cancer Res* 70: 10202-10212, 2010.
- Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez JC and Müller M: pROC: An open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinformatics* 12: 77, 2011.
- Lu Y, Yang A, Quan C, Pan Y, Zhang H, Li Y, Gao C, Lu H, Wang X, Cao P, *et al*: A single-cell atlas of the multicellular ecosystem of primary and metastatic hepatocellular carcinoma. *Nat Commun* 13: 4594, 2022.
- Sun Y, Wu L, Zhong Y, Zhou K, Hou Y, Wang Z, Zhang Z, Xie J, Wang C, Chen D, *et al*: Single-cell landscape of the ecosystem in early-relapse hepatocellular carcinoma. *Cell* 184: 404-421.e16, 2021.
- Zhang Y, Kong F, Li N, Tao L, Zhai J, Ma J and Zhang S: Potential role of SIRT1 in cell ferroptosis. *Front Cell Dev Biol* 13: 1525294, 2025.
- Zeng J, Guo J, Huang S, Cheng Y, Luo F, Xu X, Chen R, Ma G and Wang Y: The roles of sirtuins in ferroptosis. *Front Physiol* 14: 1131201, 2023.
- Li D, Zhang M, Liu J, Li Z and Ni B: Potential therapies for HCC involving targeting the ferroptosis pathway. *Am J Cancer Res* 14: 1446-1465, 2024.
- Zhou Q, Meng Y, Li D, Yao L, Le J, Liu Y, Sun Y, Zeng F, Chen X and Deng G: Ferroptosis in cancer: From molecular mechanisms to therapeutic strategies. *Signal Transduct Target Ther* 9: 55, 2024.
- Mou Y, Wang J, Wu J, He D, Zhang C, Duan C and Li B: Ferroptosis, a new form of cell death: Opportunities and challenges in cancer. *J Hematol Oncol* 12: 34, 2019.
- Zhang C, Liu X, Jin S, Chen Y and Guo R: Ferroptosis in cancer therapy: A novel approach to reversing drug resistance. *Mol Cancer* 21: 47, 2022.
- Zheng C, Zhang B, Li Y, Liu K, Wei W, Liang S, Guo H, Ma K, Liu Y, Wang J and Liu L: Donafenib and GSK-J4 synergistically induce ferroptosis in liver cancer by upregulating HMOX1 expression. *Adv Sci (Weinh)* 10: e2206798, 2023.
- Wang Z, Shen N, Wang Z, Yu L, Yang S, Wang Y, Liu Y, Han G and Zhang Q: TRIM3 facilitates ferroptosis in non-small cell lung cancer through promoting SLC7A11/xCT K11-linked ubiquitination and degradation. *Cell Death Differ* 31: 53-64, 2024.
- Yan C, Yu S, Zhang J, Li Z, Lin Z, Zhang S, Li H, Ye Z, Huang J, Ye Y and Zhuang G: TIPE inhibits ferroptosis in colorectal cancer cells by regulating MGST1/ALOX5. *Mol Cancer Res* 23: 143-154, 2025.

36. Zhang Z, Zhou P, Liu M and Pei B: Expression and prognostic role of PRDX1 In Gastrointestinal Cancers. *J Cancer* 14: 2895-2907, 2023.
37. Pan R, Zhao Z, Xu D, Li C and Xia Q: GPX4 transcriptionally promotes liver cancer metastasis via GRHL3/PTEN/PI3K/AKT axis. *Transl Res* 271: 79-92, 2024.
38. Yu W, Li Y, Gao C, Li D, Chen L, Dai B, Yang H, Han L, Deng Q and Bian X: MDH2 promotes hepatocellular carcinoma growth through ferroptosis evasion via stabilizing GPX4. *Int J Mol Sci* 25: 11604, 2024.
39. Wiernicki B, Maschalidi S, Pinney J, Adjemian S, Berghe TV, Ravichandran KS and Vandenabeele P: Cancer cells dying from ferroptosis impede dendritic cell-mediated anti-tumor immunity. *Nat Commun* 13: 3676, 2022.
40. Caforio M, Iacovelli S, Locatelli F and Folgiero V: Inducing ferroptosis to improve cancer therapy: A promising tool for enhancing immunotherapy. *J Exp Clin Cancer Res* 45: 10, 2026.
41. Circu ML and Aw TY: Glutathione and modulation of cell apoptosis. *Biochim Biophys Acta* 1823: 1767-1777, 2012.
42. Stincone A, Prigione A, Cramer T, Wamelink MMC, Campbell K, Cheung E, Olin-Sandoval V, Grüning NM, Krüger A, Alam MT, *et al*: The return of metabolism: Biochemistry and physiology of the pentose phosphate pathway. *Biol Rev Camb Philos Soc* 90: 927-963, 2015.
43. Ito J, Nakamura T, Toyama T, Chen D, Berndt C, Poschmann G, Mourão ASD, Doll S, Suzuki M, Zhang W, *et al*: PRDX6 dictates ferroptosis sensitivity by directing cellular selenium utilization. *Mol Cell* 84: 4629-4644 e9, 2024.
44. Cheng X, Ge X, Zhang C, Yang X, Yu Z, Zhang M, Cao W, Ni Q, Liu Y, He S and Yuan Y: Tryptophan suppresses FTH1-driven ferritinophagy, a key correlate of prognosis in hepatocellular carcinoma. *Cell Prolif* 59: e70074, 2026.
45. Koppula P, Zhuang L and Gan B: Cystine transporter SLC7A11/xCT in cancer: ferroptosis, nutrient dependency, and cancer therapy. *Protein Cell* 12: 599-620, 2021.
46. Zhang S, Guan M, Ren T, Li N, Ding Q, Sun D and Zhu H: Prexasertib exerts a synergistic effect on the antitumor activity of Lenvatinib through ALOX15-mediated ferroptosis in hepatocellular carcinoma. *Int Immunopharmacol* 150: 114278, 2025.
47. Jiang Y, Hui D, Pan Z, Yu Y, Liu L, Yu X, Wu C and Sun M: Curcumin promotes ferroptosis in hepatocellular carcinoma via upregulation of ACSL4. *J Cancer Res Clin Oncol* 150: 429, 2024.
48. Chen D, Chu B, Yang X, Liu Z, Jin Y, Kon N, Rabadan R, Jiang X, Stockwell BR and Gu W: iPLA2beta-mediated lipid detoxification controls p53-driven ferroptosis independent of GPX4. *Nat Commun* 12: 3644, 2021.



Copyright © 2026 Zhang et al. This work is licensed under a Creative Commons Attribution 4.0 International (CC BY-NC 4.0) License