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A Ferroptosis-Related Gene Signature for Predicting the Prognosis and Radiosensitivity in Nasopharyngeal Carcinoma

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Abbreviations and Acronyms

Abbreviation	Full Form
AUC	Area Under the Curve
BCA	Bicinchoninic Acid Assay
BPs	Biological Processes
CCs	Cellular Components
CCK-8	Cell Counting Kit-8
CI	Confidence Interval
Cox	Cox Proportional-Hazards Model
DCA	Decision Curve Analysis
DEGs	Differentially Expressed Genes
DMNC	Density of Maximum Neighborhood Component
DSS	Disease Specific Survival;
EPC	Edge Percolated Component
FINs	Ferroptosis Inducers
FPKM	Fragments Per Kilobase of transcript per Million mapped reads
FRDEGs	Ferroptosis-Related Differentially Expressed Genes
FRGs	Ferroptosis-Related Genes
GEO	Gene Expression Omnibus
GLUT3	Glucose Transporter Type 3
GO	Gene Ontology
GSEA	Gene Set Enrichment Analysis
GSVA	Gene Set Variation Analysis
HNSC	Head and Neck Squamous Cell Carcinoma
HNSCC	Head and Neck Squamous Cell Carcinoma
HPA	The Human Protein Atlas
HR	Hazard Ratio

Abbreviations and Acronyms

Abbreviation	Full Form
KEGG	Kyoto Encyclopedia of Genes and Genomes
KM	Kaplan-Meier
LASSO	Least Absolute Shrinkage and Selection Operator
MDA	Malondialdehyde
MCC	Matthews Correlation Coefficient
MDSCs	Myeloid-Derived Suppressor Cells
MFs	Molecular Functions
MNC	Maximum Neighborhood Component
MF	Molecular function
NPC	Nasopharyngeal Carcinoma
OS	Overall Survival
PFI	Progression-free interval
PPI	Protein-protein interaction
qPCR	Quantitative Real-Time PCR
RNA	Ribonucleic Acid
ROC	Receiver Operating Characteristic
ROS	Reactive Oxygen Species
SOD	Superoxide Dismutase
STRING	Search Tool for the Retrieval of Interacting Genes/Proteins
TCGA	The Cancer Genome Atlas
TF	Transcription Factor
TAZ	Transcriptional Co-activator with PDZ-binding Motif
UCSC	University of California, Santa Cruz
WB	Western Blot

Background: Radioresistance limits the efficacy of radiotherapy in nasopharyngeal carcinoma (NPC). We explored ferroptosis-related genes as candidate biomarkers and radiosensitivity regulators to counter this challenge.

Methods and Results: We screened ferroptosis-related differentially expressed genes (DEGs) using transcriptomic data from Gene Expression Omnibus (GEO, GSE48503), The Cancer Genome Atlas (TCGA), and ferroptosis gene sets from FerrDb and GeneCards. Functional enrichment and protein-protein interaction (PPI) network analyses were performed. A prognostic model was constructed and internally validated via Least Absolute Shrinkage and Selection Operator (LASSO) Cox regression. We knocked down and overexpressed candidate genes in C666-1 cells, then assessed clonogenic survival, apoptosis, and oxidative stress via malondialdehyde (MDA), superoxide dismutase (SOD), reactive oxygen species (ROS), and protein blotting. We identified 28 FRDEGs enriched in hypoxia and oxidative stress pathways. A robust prognostic signature based on *SLC2A3* and *TRIB3* was established, demonstrating significant predictive power for patient survival (AUCs: 0.700-0.836). Silencing *SLC2A3* or *TRIB3* significantly enhanced radiosensitivity by reducing clonogenic survival, while overexpression promoted radioresistance. Radiation-induced apoptosis and oxidative stress were markedly increased following knockdown of either individual gene, as indicated by elevated ROS/MDA and decreased SOD. Molecular analyses validated the computational prediction that *SLC2A3* and *TRIB3* regulate radiation response through apoptosis and oxidative stress.

Conclusions: The *SLC2A3/TRIB3* gene signature serves as a novel prognostic biomarker and predictor of radiosensitivity in NPC. Our integrated analysis confirms their functional roles in regulating apoptosis and oxidative stress, suggesting their potential as therapeutic targets for overcoming radioresistance.

Keywords: nasopharyngeal carcinoma, ferroptosis, radiotherapy, prognosis, biomarker

1. Introduction

According to global estimates from 2020, nasopharyngeal carcinoma (NPC) is a rare but highly aggressive tumor, responsible for approximately 133,354 incident cases and 80,008 deaths.¹ However, it exhibits a high incidence in southern China, with a rate of 30/100,000.¹ Due to the nasopharynx's proximity to critical neurovascular structures, treatment options of NPC are mainly

61 include radiotherapy, chemotherapy, or the concurrent use of the two, while surgical resection is
62 typically reserved for recurrent cases.² Radiotherapy could often achieve favorable initial response
63 for many patients, but because of radiation resistance, local recurrence or distant metastasis occurs
64 in 10% to 20% of patients after initial treatment. Thus, elucidating the molecular basis of
65 radioresistance and identifying robust biomarkers are critical for developing improved treatment
66 strategies and enhancing patient prognosis.

67 In the past few decades, biomarkers for poor prognosis of NPC after radiotherapy have been
68 sought by gene chip, microarray, and quantitative proteomic analysis. Although several biomarkers
69 are known to be associated with NPC radiosensitivity, including DNA damage repair proteins, cell
70 cycle redistribution related genes,³ our understanding of NPC radiosensitivity at the molecular level
71 remains limited. Characterized by iron-dependent lipid peroxidation, ferroptosis is a regulated cell
72 death process that plays a significant role in tumorigenesis and influences responses to cancer
73 therapy in multiple cancers.⁴ Recent studies have revealed links between ferroptosis and NPC: EBV
74 infection lowers NPC cell susceptibility to ferroptosis via overexpression of *SLC7A11/GPX4* and
75 activation of the p62-Keap1-NRF2 signaling pathway,⁵ while Cucurbitacin B downregulates *GPX4*
76 expression and triggers extensive lipid peroxidation to activate ferroptotic pathways in NPC cells.⁶
77 Additionally, activated *ACSL4* enhances the buildup of lipid peroxidation products, which results in
78 ferroptosis, and catalyzes polyunsaturated fatty acid-containing lipid biosynthesis.⁷ Given that
79 radiation triggers ROS-mediated lipid peroxidation, a core mechanism of ferroptosis, ferroptosis
80 may directly influence the radiosensitivity of NPC. Despite emerging links between ferroptosis and
81 cancer therapy resistance,^{8,9} it remains unclear whether FRGs could predict radiotherapy sensitivity
82 in NPC. Therefore, this study aims to systematically investigate the role of FRGs in predicting
83 prognosis and modulating radiosensitivity in NPC, with the goal of identifying potential therapeutic
84 targets and novel biomarkers to overcome treatment resistance.

85 We hypothesized that FRGs correlate with radioresistance and may serve as prognostic
86 biomarkers. To explore this, we systematically mined transcriptomic data and performed molecular
87 experiments to pinpoint ferroptosis-related differentially expressed genes (FRDEGs), aiming to
88 construct a predictive framework for radiosensitivity and clinical outcomes in NPC.

89

2. Materials and methods

2.1 Data Acquisition and Preprocessing

We retrieved gene expression profiles of NPC cell lines from the GEO dataset GSE48503 using the GEOquery R package (version 2.76.0). This dataset included miRNA data from three radioresistant and three radiosensitive NPC cell lines (GPL15019 platform), and mRNA data from two radioresistant and two radiosensitive cell lines (GPL570 platform). Two samples from each group were selected for analysis.

For validation, the Head and Neck Squamous Cell Carcinoma (HNSCC) dataset was retrieved from TCGA via TCGAbiolinks, comprising 502 tumor and 44 normal samples. Raw counts were converted to Fragments Per Kilobase of Transcript per Million mapped reads (FPKM) values, with clinical data sourced from University of California, Santa Cruz (UCSC) Xena.¹⁰

FRGs were compiled from GeneCards (search term: "ferroptosis") and the FerrDb database. After merging and removing duplicates, 684 unique genes were identified for analysis (Fig. 1; Table S1).

2.2 Bioinformatics Analysis Pipeline

2.2.1 DEGs Identification and Screening Criteria

The GSE48503 and TCGA-HNSC datasets were normalized via the limma package (version 3.64.1). We identified differentially expressed genes (DEGs) using the following criteria: $|\log_2FC| > 1$ and $P < 0.05$. FRDEGs were identified by intersecting NPC DEGs (radiosensitive vs. radioresistant) with the ferroptosis gene list. Results were displayed as Venn diagrams, volcano plots (ggplot2), and heatmaps (pheatmap).

2.2.2 Functional Enrichment Strategies

To identify the biological functions and pathways associated with the FRDEGs, we performed functional enrichment analyses using the clusterProfiler package.¹¹ Both Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO) term over-representation tests were applied, with statistical significance defined by an adjusted P-value (FDR) below 0.05 and a nominal P-value of less than 0.05.

2.2.3 Gene Set Enrichment Analysis

Gene Set Enrichment Analysis (GSEA) was conducted on the GSE48503 dataset to evaluate

the distribution of predefined gene sets across ranked gene lists. The reference gene set employed in this analysis was the C2.cp.v7.2. symbols collection, which was downloaded from the MSigDB resource. This gene set was divided into high- and low-correlation subsets for the analysis. Gene Set Variation Analysis (GSVA) was implemented on FRDEGs using "h.all.v7.4.symbols.gmt" gene set to compare functional enrichment between radioresistant and radiosensitive samples.

2.2.4 Tumor Microenvironment

We quantified the abundance of immune cells through single-sample GSEA (ssGSEA) implemented with the GSVA package. Differences were visualized with ggplot2.

2.3 Network Biology

2.3.1 Protein-Protein Interaction (PPI) Network Analysis

To investigate protein-level functional associations, a protein-protein interaction (PPI) network was reconstructed based on the STRING database. The resulting interaction network was subsequently imported into and visualized using the Cytoscape platform. Key nodes were identified using the Matthews Correlation Coefficient (MCC) algorithm.

2.3.2 Regulatory Network Prediction

miRNA-mRNA interactions were explored using the StarBase database. Drug-gene interactions and transcription factor (TF)-target relationships were predicted via DGIdb and TRRUST, respectively. Integrated networks were visualized in Cytoscape.

2.4 Clinical and Prognostic Modeling

2.4.1 ROC analysis

We plotted receiver operating characteristic (ROC) curves for prognostic FRDEGs as well as clinical variables in TCGA-HNSC cohort utilizing the survival ROC package. The area under the curve (AUC) quantified diagnostic efficacy.

2.4.2 Prognostic Model Building

Prognostic FRDEGs were selected via univariate Cox regression ($P < 0.1$) and incorporated into a multivariate Cox model. A Least Absolute Shrinkage and Selection Operator (LASSO) Cox regression model was applied to 23 FRDEGs to prevent overfitting. The optimal lambda (λ) was determined, and results were visualized via variable trajectory and risk factor graphs. According to

the individual risk score, patients were assigned to either a high-risk or a low-risk subgroup. The Kaplan-Meier (KM) estimator was then applied to compare the overall survival probabilities across the two risk groups, and the area under the time-dependent ROC curve was calculated as a measure of the model's prognostic power.

2.4.3 Clinical Correlation

A nomogram integrating significant prognostic factors was constructed to estimate 1-, 3-, and 5-year survival. We evaluated the model's predictive accuracy and clinical utility using calibration curves and decision curve analysis (DCA).

2.5 Molecular Characterization

2.5.1 Mutation Analysis

Genomic alterations of prognostic FRDEGs in HNSCC were analyzed using cBioPortal. The analysis focuses on mutation frequency and copy number variations.

2.5.2 Immunohistochemistry Validation

To compare tumor and normal tissues, we evaluated protein expression of FRDEGs using IHC datasets from the HPA database.

2.6 Experimental Validation

2.6.1 Cell Culture and Transfection

The C666-1 NPC cell line was propagated in RPMI-1640 medium (GIBCO, Cat. C11875500BT). Medium supplementation included 10% FBS (GIBCO, Cat. 10099-141C) and 1% penicillin-streptomycin (100×, Solarbio, Cat. P1400). Culture conditions were maintained at 37°C with 5% CO₂ in a humidified incubator (ESCO, Model CCL-170B-8). We plated 2×10⁵ cells per well in 6-well plates. At 20-30% confluency, transfection was performed using Lipo6000™ reagent with siRNA or overexpression plasmids targeting *SLC2A3* or *TRIB3*. After 6 hours, the medium was exchanged for fresh complete medium, followed by a 24-hour incubation. Transfection efficiency was assessed via quantitative real-time PCR (qPCR).

2.6.2 Irradiation and Experimental Groups

At 24 hours post-transfection, cells were subjected to irradiation at doses of 0, 4, 6, or 8 Gy, groups included siRNA-negative control (si-NC), siRNA-target (si-*SLC2A3*/si-*TRIB3*), overexpression-negative control (OE-NC), and overexpression (OE-*SLC2A3*/OE-*TRIB3*).

2.6.3 Colony Formation Assay

Cells were trypsinized, counted, and plated in 6-cm dishes with 1,000 cells per dish. The resulting colonies were fixed with 4% paraformaldehyde (Solarbio, Cat. BL539A), stained with 0.1% crystal violet (Solarbio, Cat. G1063), and manually counted. A colony was defined as a cluster of > 50 cells. The colony formation efficiency was computed as (colony count / initial number of seeded cells) $\times$ 100%.

2.6.4 RNA Extraction and Quantitative Real-Time PCR (qPCR)

We isolated total RNA from the cells using TriQuick Reagent (Solarbio, Cat. R1100). The concentration and purity of the extracted RNA were quantified via an ultra-micro nucleic acid detector (Suizhen, Model FC-1100). First-strand cDNA was synthesized from 1 μ g of total RNA using the Hifair® III 1st Strand cDNA Synthesis Kit (Yeasten, Cat. 11139ES60), which includes a gDNA digestion step. qPCR was then performed on an AriaMx Real-Time PCR System (Agilent) with Hieff® qPCR SYBR Green Master Mix (Yeasten, Cat. 11202ES03). The quantitative real-time PCR program was set with an initial denaturation step of 95°C for 60 s, followed by 35 amplification cycles (95°C for 5 s, 60°C for 30 s, and 72°C for 30 s). Target gene expression was normalized to the internal control and calculated using the comparative Ct ($2^{-\Delta\Delta Ct}$) method. All primer pairs utilized in this study are listed in Supplementary Table S2.

2.6.5 Western Blot Analysis (WB)

Cells were lysed using RIPA buffer (Solarbio, Cat. R0010) supplemented with PMSF (Solarbio, Cat. P0100) and a phosphatase inhibitor cocktail (Solarbio, Cat. P1260) at a ratio of 100:1:1. Concentrations of protein were verified via a bicinchoninic acid assay (BCA) protein assay kit (Biosharp, Cat. BL521A). Samples with equal amounts (adjusted to 2.0 mg/mL) were resolved via SDS-PAGE using 10%-15% gels, subsequently transfer onto PVDF membranes (Millipore, Cat. ISEQ00010). The PVDF membranes were pre-blocked with 8% non-fat dried milk (OXOID, Cat. YZ-LP0033B) for 1 h at 25°C. They were then incubated at 4°C overnight with target-specific primary antibodies (1:1000 dilution) under gentle shaking. After three rinses in TBST, the blots were treated with HRP-linked secondary antibodies (Abcam, ab6789 and ab6721; diluted 1:5000) and kept at 37°C for 1 hour with continuous rocking. The immunoreactive products were detected using an ECL kit (Biosharp, BL523A), and the resulting band densities were analyzed with the ImageJ program. Antibody details and dilutions are provided in Supplementary Table S3.

2.6.6 Flow Cytometry

Apoptosis was detected using Annexin V-APC and 7-AAD apoptosis detection kit (Lianke Bio, Cat. AP105-100-kit). Reactive Oxygen Species (ROS) levels were measured with DCFH-DA (10 μ mol/L; Beyotime, Cat. S0033S) in serum-free medium at 37°C for 20 min. After washing, both signals were analyzed on a CytoFLEX flow cytometer (Beckman Coulter), collecting 10,000 events per sample. Data analysis was done using FlowJo V10 software.

2.6.7 SOD Activity and MDA Content

Oxidative stress was evaluated by measuring superoxide dismutase (SOD) as well as the malondialdehyde (MDA) content. The corresponding kits were obtained from Nanjing Jiancheng Bioengineering Institute (Cat. A001-3 for SOD; A003-1 for MDA). SOD activity and MDA content were standardized to the total protein concentration of the cell lysates.

2.6.8 Cell Viability Assay

The Cell Counting Kit-8 (CCK-8; Biosharp, Cat. BS350B) was used to assess cell viability in triplicate to ensure data reliability. Briefly, transfected and/or irradiated C666-1 cells were plated into 96-well plates with 3,000 cells per well. After 24 h of post-treatment culture, the medium in each well was supplemented with 10 μ L of CCK-8 reagent. The plates were then returned to 37°C for a 1-hour incubation period. Final readings were obtained by measuring the absorbance at 450 nm with a microplate reader. Cell viability was calculated as follows:

*Note: A_{blank} , absorbance of wells with medium and CCK-8 only; A_{ctrl} , absorbance of control cells with CCK-8; A_{exp} , absorbance of treated cells with CCK-8.

2.7 Statistical analysis

Analyses used R software (v4.1.2) and GraphPad Prism 9.0. Data are mean $\pm$ SD. Group comparisons used Wilcoxon rank-sum or Kruskal-Wallis tests. Survival analysis used log-rank tests and Kaplan-Meier curves. Experimental data (≥ 3 replicates) were analyzed by two-way ANOVA. $P < 0.05$ was considered significant.

3. Results

3.1 NPC-Associated Differentially Expressed Genes and Their Functional Enrichment

The limma package was applied to standardize the data on radioresistant and radiosensitive NPC cell samples in the GSE48503 dataset (Fig. 2A). Subsequently, it was used to analyze the gene expression differences between these two groups. Analysis of GSE48503 identified 20,843 DEGs,

of which 265 genes met the threshold of $|\log_2FC| > 1$ and $P < 0.05$. Within this set, 234 transcripts displayed a positive $\log_2FC$, while 31 exhibited a negative $\log_2FC$ (Fig. 2B). In addition, the overlap between FRGs and DEGs from GSE48503 was analyzed, revealing 28 FRDEGs related to NPC metabolism (Figs. 2C-D). Fig. 2D shows that most of the 28 FRDEGs are more highly expressed in the radioresistant NPC cell samples (Group: resistant).

Functional enrichment analysis of the 28 FRDEGs was performed using GO and KEGG databases. For GO terms (Figs. 3A-B, Table S4), significant enrichment was observed in biological processes related to stress and stimulus responses, including the cellular and general response to ROS, cellular response to external stimulus, and response to metal ions. Regulation of stress-responsive transcription via the RNA Pol II promoter was also enriched. Within cellular components, only the perikaryon was significantly enriched. In the molecular function category, enrichment was identified for hydrolase and transporter activities, particularly carbon-oxygen lyase activity, glucose transporter activity, and hexose transmembrane transporter activity.

KEGG pathway enrichment analysis of the FRDEGs identified significant enrichment in several key pathways, as shown in Table S5. The top five enriched pathways included the HIF-1 signaling pathway, rheumatoid arthritis, mitophagy-animal, renal cell carcinoma, and the IL-17 signaling pathway (Fig. 3C). A network diagram visualizing the gene constituents within these top pathways is presented in Fig. 3D. To integrate the direction of gene expression changes with functional enrichment, a combined analysis was performed using the $\log_2FC$ values of the FRDEGs. This $\log_2FC$ -weighted enrichment analysis highlighted the most activated and suppressed functional categories (GO terms, Fig. 3E) and pathways (KEGG, Fig. 3F).

3.2 Pathway and Network Analysis of FRDEGs

According to the findings of the GSEA, the differential genes in GSE48503 exhibited a significant increase in four main biological characteristics (Fig. 4A): the HIF1 TF pathway (Fig. 4B), adipogenesis (Fig. 4C), the HIF2 pathway (Fig. 4D), the ATF2 pathway (Fig. 4E), as well as other pathways (Table S6).

To analyze the differential expression in hallmark gene sets between radioresistant NPC cell sample tissue (Group: resistant) and radiosensitive NPC cell sample tissue (Group: sensitive), GSVA

analysis was performed on the FRDEGs in the GSE48503 dataset (Fig. 4F). The results showed that there are 12 differently expressed hallmark gene sets between the resistant group and the sensitive group, in which 9 hallmark gene sets enriched in the resistance group include hypoxia, angiogenesis, Wnt/beta-catenin signaling, coagulation, late estrogen response, epithelial-mesenchymal transition, myogenesis, hedgehog signaling, and complement. Three hallmark gene sets were reduced in the resistance group, including MYC targets V2, DNA repair, and UV response.

In addition, immune infiltration disparities between resistant and sensitive groups were evaluated via ssGSEA-based analysis of 28 immune cell types in both datasets. Comparative analysis of the two cohorts revealed markedly distinct immune profiles. A total of eleven immune cell subsets showed differential abundance between the groups, encompassing various dendritic cell populations (activated and immature), CD4⁺ and CD8⁺ T-cell memory subsets (effector memory CD4⁺, central memory CD4⁺ and CD8⁺, and type 1 helper), as well as natural killer cells, eosinophils, monocytes, immature B cells, and myeloid-derived suppressor cells (MDSCs) (Figs. 4G-H).

We systematically characterized functional relationships among FRGs through PPI analyses using the STRING database (<https://cn.string-db.org/>). Genes were considered functionally connected if they established at least one interaction in the PPI network. Our analysis revealed three key findings: The global ferroptosis interactome, comprising 634 of 684 candidate genes (Fig. 5A), exhibited high connectivity with 618 genes (97.5%) forming interaction networks. Analysis of 265 DEGs identified 247 network components (Fig. 5B), including a core cluster of 176 tightly interconnected genes (71.3% connectivity). Most notably, 27 of 28 FRDEGs formed an interaction network (Fig. 5C), with 22 genes (81.5%) demonstrating particularly robust connectivity patterns (e.g., *IL6*, *VEGFA*, *SLC2A1*; full list in Table S7).

To prioritize key FRDEGs, we calculated MCC scores and visualized interactions via Cytoscape (Fig. 5D). *IL6* and *VEGFA* exhibited the highest MCC scores (indicating central network roles), while *ULK1*, *TUBA1A*, and others scored lowest (yellow-to-red gradient reflects score increase; see Table S7 for details).

The PPI network was analyzed for hub gene identification using the cytoHubba plugin integrated in Cytoscape. Eight topological measures were employed to rank the nodes, namely maximum neighborhood component (MNC), density of maximum neighborhood component (DMNC), MCC, Degree, EcCentricity, Closeness, EPC, and BottleNeck. Each method yielded a list of 15 top-ranked candidate genes. After comparing the results, we observed that MCC and Degree algorithms yielded identical results, while DMNC, MNC, edge percolated component (EPC), and Closeness algorithms showed consistent outcomes. Therefore, five representative algorithms (MCC, MNC, Degree, BottleNeck, and EcCentricity) were selected for the final analysis. The overlap of the top 15 genes from these five algorithms identified 10 hub genes: *BNIP3*, *CA9*, *DUSP1*, *FABP4*, *HNRNPD*, *IL6*, *JUN*, *LCN2*, *MUC1*, and *VEGFA*.

Using the StarBase database, we identified mRNA-miRNA interactions for 13 of the 22 FRDEGs, forming a network with 50 miRNAs and 95 interaction pairs (Fig. 6A; Table S8). Analysis via the TRRUST database revealed mRNA-TF interactions for 16 FRDEGs involving 83 TFs. Notably, *VEGFA* showed the highest connectivity with 48 TF partners, and JUN was found to function dually as both a target mRNA and a regulating TF (Fig. 6B; Table S9). DGIdb analysis predicted drug interactions for 12 FRDEGs, totaling 111 compounds. Among these, fenofibrate and insulin were each predicted to target three genes, while *VEGFA* and *TUBA1A* emerged as the most druggable nodes, being targeted by 24 and 22 drugs, respectively (Fig. 6C; Table S10).

3.3 Prognostic Modeling and Validation

As shown by the previous results, the expression of 28 FRDEGs and the occurrence of NPC are closely linked. To explore the expression differences in these 28 FRDEGs, we used the TCGA-HNSCC dataset as a validation set. In addition, the corresponding clinical data were analyzed. Statistical comparison via the Wilcoxon signed-rank test demonstrated significant differential expression for 23 FRDEGs between matched tumor and normal tissue samples (Figs. 7A–W). However, the expression differences of 5 FRDEGs, including *AKR1C3*, *IL6*, *JUN*, *TSC22D3*, and *TUBA1A*, were not statistically significant, and therefore they were excluded from further analysis.

Patient characteristics are summarized in Table S11.

Based on the median expression level of the 23 FRDEGs, the tumor samples were divided into two categories: those with high expression and those with low expression. Survival curves were then constructed and compared using the survival and survminer R packages (v4.1.3), and the prognostic significance of these genes was evaluated in NPC. The Kaplan-Meier curves of three FRDEGs were significant: *ENO2* ($P = 0.034$, Fig. 8A), *SLC2A3* ($P = 0.006$, Fig. 8B), and *TRIB3* ($P < 0.001$, Fig. 8C). ROC curves were drawn for these three genes based on the TCGA-HNSCC dataset (Figs. 8D–F), and their corresponding AUC values were 0.930 (*ENO2*), 0.700 (*SLC2A3*), and 0.836 (*TRIB3*).

We further constructed a LASSO Cox regression model using the 23 FRDEGs, which showed robust prognostic performance (Fig. 8G). Through LASSO regression analysis, 9 genes were retained in the prognostic model (*CBS*, *GABPB1-AS1*, *FABP4*, *HNRNPD*, *DUSP1*, *BNIP3*, *TRIB3*, *ARRDC3*, and *SLC2A3*), as shown in the variable trajectory plot (Fig. 8H). The risk factor plot (Fig. 8I) illustrated the risk grouping (based on median risk score), survival outcomes (survival time and status of samples), and expression patterns of the 9 genes in the model. Combining the OS KM curves of the 23 FRDEGs and the results of the LASSO regression model, we finally identified two FRDEGs (*SLC2A3* and *TRIB3*) with significant prognostic value.

The prognostic significance of FRDEGs was validated by systematic survival analyses. Univariate and multivariate Cox regression confirmed that M tumor stage (HR = 4.745, 95% CI:1.748-12.883, $P = 0.002$), radiation therapy (HR = 0.613, 95% CI:0.452-0.831, $P = 0.002$), *TRIB3* expression (HR = 1.674, 95% CI:1.276-2.196, $P < 0.001$) and *SLC2A3* expression (HR = 1.462, 95% CI:1.116-1.914, $P < 0.006$) were independent prognostic indicator (Table 1; Fig. 9A). Subsequent nomogram construction excluded the non-significant T stage variable, ultimately integrating three clinically actionable predictors (Fig. 9B), the final model demonstrated robust predictive accuracy. Calibration curves (Fig. 9C) for 1-, 3-, and 5-year survival showed high concordance between Cox model-predicted probabilities and actual observations, indicating robust prognostic performance.

DCA at 1-, 3-, and 5-year intervals (Figs. 9D-F) showed our model provided higher net benefit than alternative approaches across a range of threshold probabilities. We evaluated expression differences of the two prognostic FRDEGs, *SLC2A3* and *TRIB3*, between tumor and adjacent normal tissues, stratified by key clinical features (e.g., stage, treatment response). Differential expression analysis of prognostic FRDEGs (*SLC2A3* and *TRIB3*) revealed: *SLC2A3* expression was markedly elevated in tumor versus normal tissues ($P < 0.001$, Fig. 9G); *TRIB3* expression showed strong association with advanced tumor stage ($P < 0.001$, Fig. 9H).

3.4 Multi-dimensional Characterization of Key Genes (*SLC2A3* and *TRIB3*)

The prognostic relevance of *SLC2A3* and *TRIB3* in TCGA-HNSCC was investigated by assessing their associations with clinicopathological features. Survival analysis revealed significant correlations between gene expression and patient outcomes (Figs. 10A-F): high *SLC2A3* expression significantly correlated with poorer OS ($P < 0.001$) and DSS ($P = 0.012$) but not PFI ($P = 0.205$), while elevated *TRIB3* expression showed significant associations with worse OS ($P = 0.006$), DSS ($P < 0.001$) and PFI ($P = 0.003$). Pan-cancer analysis further demonstrated aberrant expression of both genes across multiple malignancies compared to normal tissues (Figs. 10G-H), suggesting broader oncogenic roles.

Somatic alterations in *SLC2A3* and *TRIB3* were analyzed using cBioPortal (v4.2) based on TCGA-HNSCC data (Fig. 11). Four major genetic alterations were identified: missense mutations (variant of uncertain significance, VUS), truncating mutations (VUS), amplifications, and deep deletions (Fig. 11A). *SLC2A3* was altered in 1.4% of cases, mostly through missense mutations (VUS: R228W, Q237K) and amplifications. These changes mapped to exon 6, which encodes the cytoplasmic domain. Computational analysis predicted that these amino acid substitutions would impair protein function (Fig. 11B). Post-translational modifications (PTMs) included phosphorylation and ubiquitination sites. *TRIB3* mutations were less frequent (0.4%), primarily consisting of truncating mutations (VUS: P116Rfs*94) in exon 3 and deep deletions (Fig. 11C). Phosphorylation was the predominant PTM observed for *TRIB3*.

Additionally, the transcript levels of *SLC2A3* and *TRIB3* in normal and HNSC tissues in the HPA database was examined, as was the immunohistochemical analysis of the RNA and protein distribution of *SLC2A3* and *TRIB3* in humans (Figs. 12A-B). High *SLC2A3* mRNA levels were found in bone marrow and lymphoid tissues. At the protein level, *SLC2A3* was predominantly detected in brain, respiratory system, male tissues, bone marrow, and lymphoid tissues. With regard to *TRIB3*, abundant transcripts were seen in endocrine tissues, liver, and gallbladder, while its protein was enriched in brain, endocrine tissues, and male tissues. The immunohistochemical staining patterns were additionally compared between HNSCC tumor tissues and their corresponding normal tissues. The results showed that tumor tissue was more darkly stained than normal tissue (Figs. 12C-D).

3.5 Functional Validation of *SLC2A3* and *TRIB3* in Radiosensitivity

Our computational models identified *SLC2A3* and *TRIB3* as top predictors of radiation response. To validate these predictions functionally, we first established a radiation model in C666-1 cells. Flow cytometric analysis of apoptosis and a CCK-8 assay confirmed that gradient irradiation induced a considerable increase in apoptosis and a concomitant decrease in cell viability. (Fig. 13A). We then successfully constructed *SLC2A3* and *TRIB3* knockdown and overexpression models, as verified by qPCR ($P < 0.01$; Fig. 13B). Crucially, clonogenic assays demonstrated that silencing *SLC2A3* or *TRIB3* significantly reduced clonogenic survival after radiation treatment ($P < 0.01$), while their overexpression increased it ($P < 0.01$) (Figs. 13C-D). These results directly validate *SLC2A3* and *TRIB3* as key determinants of clonogenic survival after radiation.

3.6 *SLC2A3* and *TRIB3* Modulate Radiation Response through Apoptosis and Oxidative Stress

Consistent with our pathway analysis predictions, functional studies revealed *SLC2A3* and *TRIB3* significantly modulated apoptosis and oxidative stress - key pathways identified by our computational models. Flow cytometry showed *SLC2A3* and *TRIB3* knockdown both increased radiation-induced apoptosis ($P < 0.001$), while their overexpression reduced apoptosis ($P < 0.05$) (Figs. 14A-B). In parallel, oxidative stress measurements confirmed model predictions: *SLC2A3* and *TRIB3* depletion both elevated ROS and MDA while reducing SOD activity (all $P < 0.01$), with the overexpression showing opposite effects (Figs. 14C-E). Collectively, these results experimentally confirm that apoptosis and oxidative stress are critical mechanisms

through which *SLC2A3* and *TRIB3* regulate radiation response.

3.7 Molecular and Phenotypic Confirmation of Model Predictions

At the molecular level, WB analysis was conducted to determine pro-apoptotic signaling. Knockdown of *SLC2A3* or *TRIB3* resulted in increased cleavage of caspase-3/9 and a higher Bax/Bcl-2 ratio, whereas overexpression of either gene suppressed these markers (Figs. 15A-F). qPCR analysis confirmed these regulatory patterns at the mRNA level (Figs. 15G-H), aligning with the predictions from our in-silico pathway analyses. Furthermore, in CCK-8 cell viability assays, the silencing of *SLC2A3* or *TRIB3* synergistically with radiation inhibited cell growth ($P < 0.01$). Conversely, overexpression promoted post-irradiation recovery ($P < 0.01$) (Fig. 15I).

4. Discussion

NPC is a malignancy in which radioresistance often leads to poor outcomes, creating an urgent need for predictive biomarkers.¹² Driven by lipid peroxidation, ferroptosis (an iron-dependent form of regulated cell death) has risen as a prospective therapeutic target in oncology.¹³ As the cornerstone of radiotherapy, ionizing radiation could trigger ferroptosis by increasing reactive oxygen species and modulating key regulators like *ACSL4*, *SLC7A11*, and *GPX4*, highlighting its potential role in radiosensitization.¹⁴ Research has indicated that ionizing radiation could induce ferroptosis and suppress tumor growth, suggesting that ferroptosis might serve as a crucial mediator in determining the response of NPC to radiotherapy.⁴ In this study, *SLC2A3* and *TRIB3* emerged as potential predictors of radiosensitivity and prognosis in NPC through our integrative analysis of GEO and TCGA datasets. These ferroptosis-related genes may be useful for tailoring radiotherapy.

Silencing *SLC2A3* or *TRIB3* in NPC cells significantly enhanced radiosensitivity in clonogenic assays, indicating that both genes are critical for radiation response. Upon manipulating *SLC2A3* or *TRIB3*, we observed altered caspase-3/9 cleavage, Bax/Bcl-2 ratio, and ROS/MDA/SOD levels, suggesting that these genes suppress apoptosis and alleviate oxidative stress to promote radioresistance. These phenotypes link to ferroptosis-associated networks, implying that *SLC2A3* and *TRIB3* modulate redox balance and apoptosis upon radiation. Together, these results identify *SLC2A3* and *TRIB3* as mediators of NPC radioresistance and implicate them in ferroptosis-related pathways. This dual role in apoptosis and oxidative stress offers a framework for targeting *SLC2A3*

and *TRIB3* to overcome radioresistance in NPC.

Our findings suggest that the pro-radioresistance function for *SLC2A3* and *TRIB3* in NPC is consistent with their established roles in stress adaptation and survival under adverse microenvironments. *SLC2A3* encodes glucose transporter 3 (GLUT3), which promotes glucose uptake and nucleotide synthesis to meet energy demands and maintain redox balance under radiation-induced stress.¹⁵ *TRIB3* is a comprehensive stress regulator. Its ability to sustain tumor cell stemness may also contribute to radioresistance in NPC.^{16,17} Thus, *SLC2A3* and *TRIB3* may affect radiotherapy responses via distinct but complementary routes.

Although bioinformatics analyses had linked *SLC2A3* and *TRIB3* to poor prognosis in head and neck cancers (a category that includes NPC),^{18,19} their direct functional role in mediating NPC radiosensitivity remained unexplored. The role of *TRIB3* in radioresistance appears context-dependent: it promotes resistance in esophageal squamous cell carcinoma through TAZ stabilization,²⁰ but its overexpression sensitizes triple-negative breast cancer to radiation via Notch1 suppression. In contrast, our findings demonstrate that both *SLC2A3* and *TRIB3* consistently promote radioresistance in NPC cells, highlighting the tumor-specific nature of their regulatory functions.

The therapeutic potential of targeting *SLC2A3* and *TRIB3* is considerable. Based on their pro-radioresistance mechanisms, we propose that targeting these genes, via *SLC2A3*/GLUT3 inhibitors or *TRIB3*-targeting modalities, may synergize with radiotherapy by inducing ferroptosis, thereby offering a promising strategy to overcome radioresistance in NPC. This approach is particularly rational when combined with existing PD-1/PD-L1 inhibitors.²¹ Ferroptotic cell death can enhance antitumor immunity through the release of DAMPs and neoantigens, which may help reverse the immunosuppressive tumor microenvironment of NPC.²² This immune-sensitizing effect is especially valuable. Most current NPC radiosensitivity biomarkers focus on DNA repair defects alone and do not translate into therapeutic options. Our model goes further, predicting resistance while revealing druggable metabolic-immune connections.

This mechanism is particularly relevant in NPC, given that its pathogenesis is closely associated with altered cellular metabolism and oxidative stress. Both EBV infection and hypoxic microenvironment contribute to these processes,^{23,24} rendering NPC cells potentially vulnerable to ferroptosis-inducing strategies. Therefore, targeting ferroptosis represents a mechanistically

grounded strategy to exploit inherent vulnerabilities of the tumor, suggesting that our findings may hold unique value for both basic research and clinical translation (Table S12).^{12,25-50}

This study has limitations. Our validation was performed in cell lines, and animal experiments are needed to confirm the relevance of these findings. The prognostic model, derived from public databases, should be further assessed in prospective cohorts. In addition, focusing only on FRGs may miss other important NPC prognosis-related factors. Future work combining multi-omics data and experimental models might provide a more complete picture of NPC biology and therapy response.

The *SLC2A3/TRIB3* ferroptosis signature was identified as a determinant of radiosensitivity and prognosis in NPC. Functional assays confirmed that these genes regulate apoptosis and oxidative stress, providing mechanistic support for their therapeutic targeting. Beyond serving as predictive markers, *SLC2A3/TRIB3* may be directly exploitable for intervention. Their combination with ferroptosis inducers or immunomodulators could enhance radiotherapy efficacy and improve patient outcomes in NPC.

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Conflict of Interest

The authors declare no conflicts of interest.

Author contributions

JHZ, FBW and LFL: conceptualization. MG and CYZ: investigation. ZPC and JQW: data curation. QQH, JHZ, ZWX and KZ: writing — original draft preparation, QQH, and LFL: experimental verification. ZC and MG: methodology. HLY: software. JHZ, FBW and LFL: funding acquisition. All authors contributed to article revision, read, and approved the submitted version.

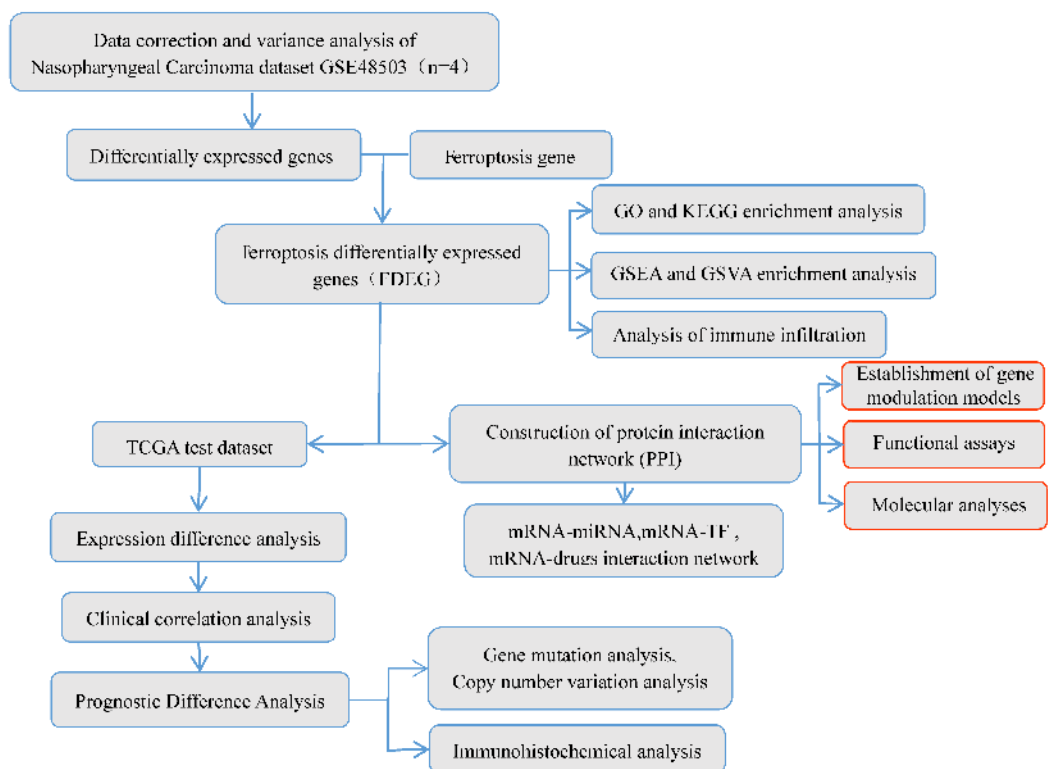
Ethics Statement

Not applicable.

Data availability statement

The datasets for this study could be found in the GEO (<https://www.ncbi.nlm.nih.gov/geo/>), TCGA (<https://gdc.xenahubs.net/>) databases.

525 **Figure legends:**



526

527 **Fig. 1** Bioinformatics analysis workflow. The overall pipeline encompassed data normalization,
528 analysis of differential expression, enrichment analysis of functions, construction of molecular
529 networks, and experimental validation, as detailed in Sections 2.2–2.7.

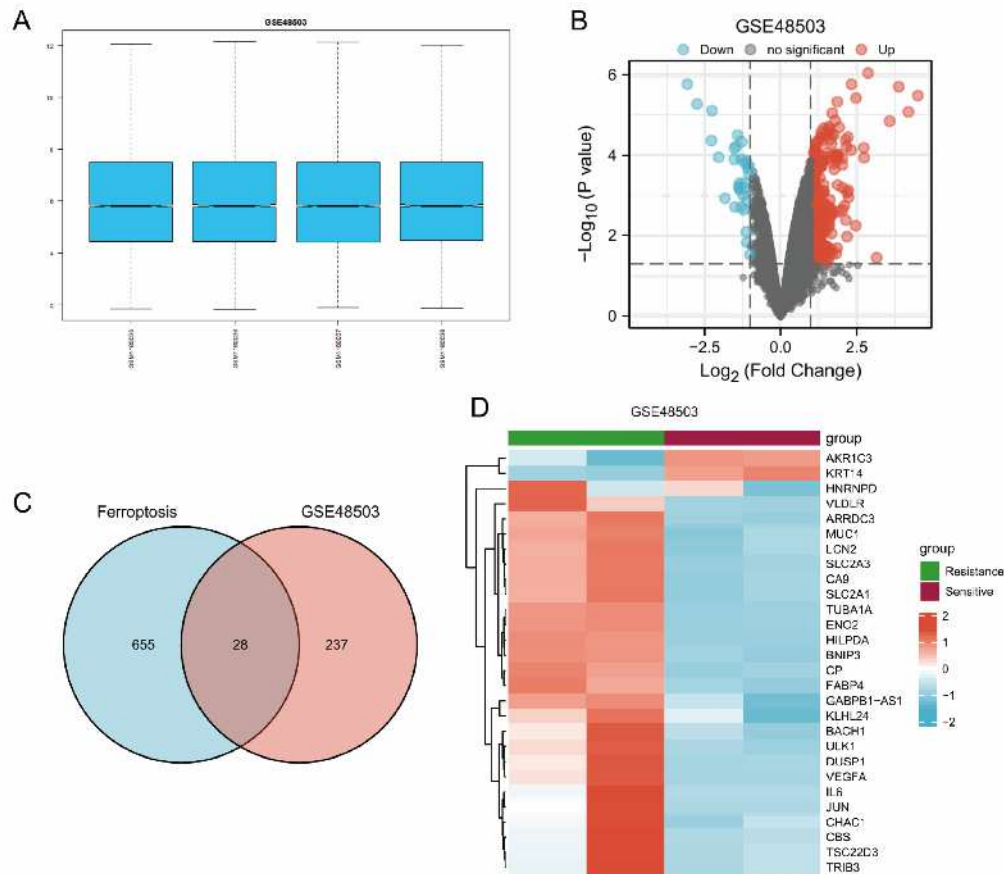
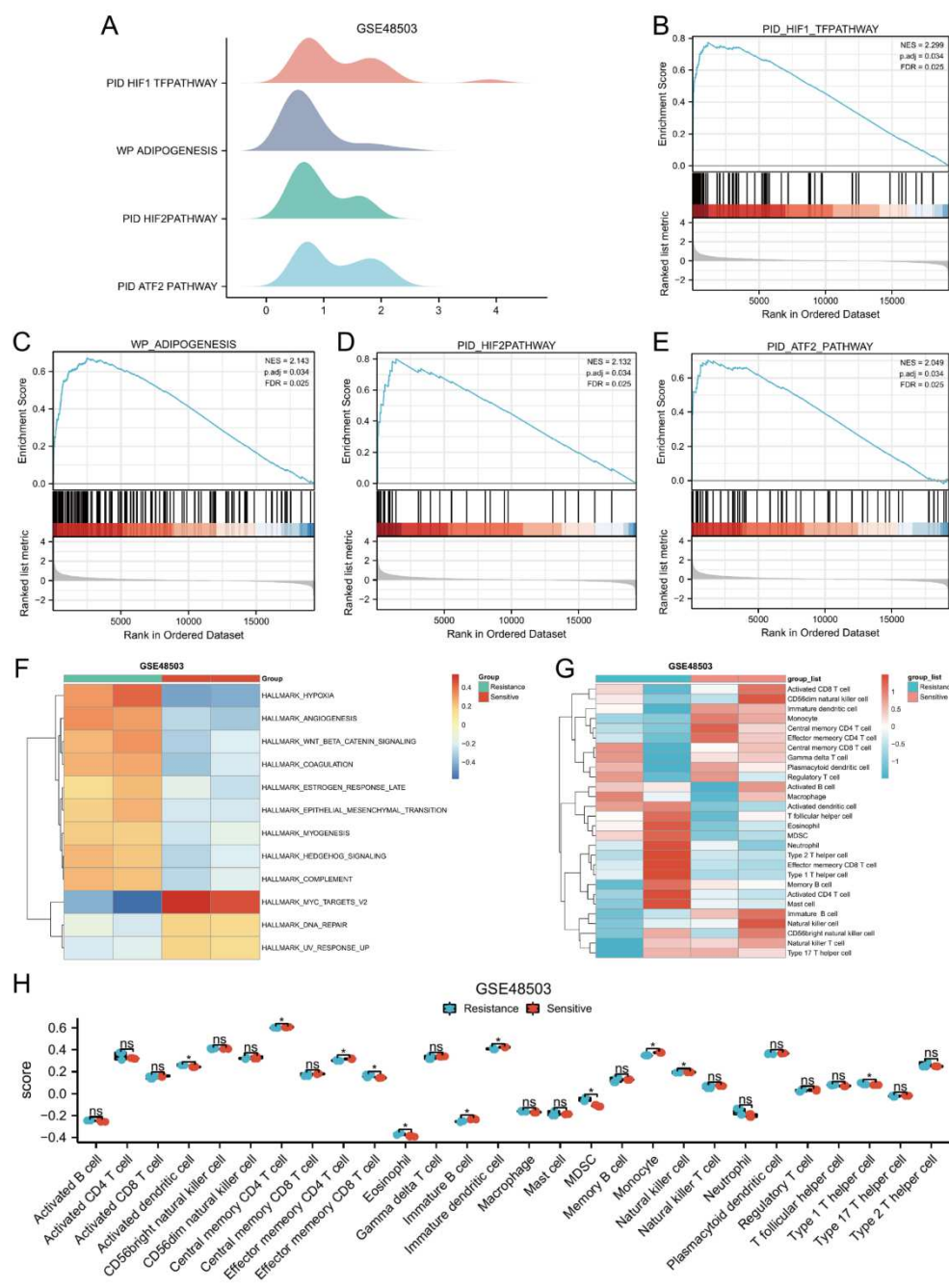


Fig. 2 NPC-related DEGs. **(A)** Data correction results for the four samples in dataset GSE48503. **(B)** Volcano plot of differentially expressed genes between radioresistant NPC cell samples (resistant) and radiosensitive NPC cell samples (sensitive) in the GSE48503 dataset. **(C)** Venn diagram of FRGs and DEGs in the GSE48503 dataset. **(D)** Heatmap of FRDEGs in the GSE48503 dataset. Abbreviations: NPC, nasopharyngeal carcinoma; FRGs, ferroptosis-related genes; DEGs, differentially expressed genes.

546 $\log_2FC < 0$). Abbreviations: KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene
547 Ontology; $\log_2FC$, $\log_2$ fold change.



548

549 **Fig. 4** Functional enrichment analysis of GSEA and evaluation of the tumor microenvironment with

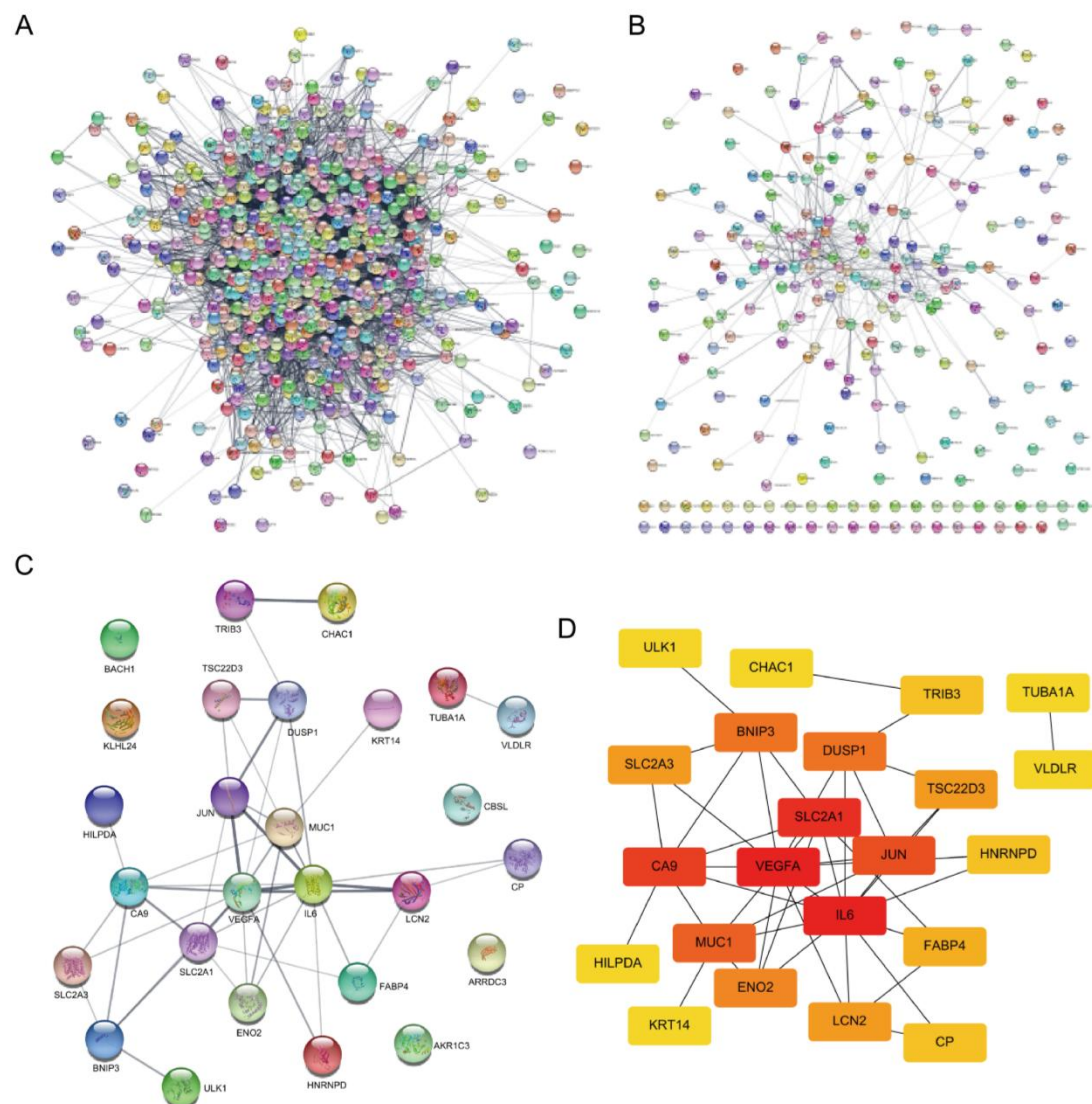
550 ssGSEA. **(A)** Four main biological features of the NPC dataset GSE48503 were surfaced via GSEA

551 analysis. **(B-E)** Key genes identified in the NPC dataset GSE48503 are enriched in Pid Hif1

552 Tfp pathway (B), Adipogenesis (C), Pid Hif2 Pathway (D), and Pid Atf2 Pathway (E). **(F)** GSVA

553 analysis of FRDEGs in the GSE48503 dataset. **(G-H)** Immune infiltration in a heatmap (G) and a

554 group comparison map (H). Abbreviations: GSVA, Gene Set Variation Analysis; GSEA, Gene Set



556
557 **Fig. 5** Screening of hub genes from the PPI network. **(A)** PPI network of FRGs. **(B)** PPI of the DEGs
558 in the GSE48503 dataset. **(C)** PPI network of FRDEGs. **(D)** PPI network of 22 FRDEGs with the
559 MCC algorithm. Abbreviations: PPI, Protein-protein interaction; DEGs, FRDEGs, ferroptosis-
560 related differentially expressed genes; MCC, Matthews correlation coefficient.

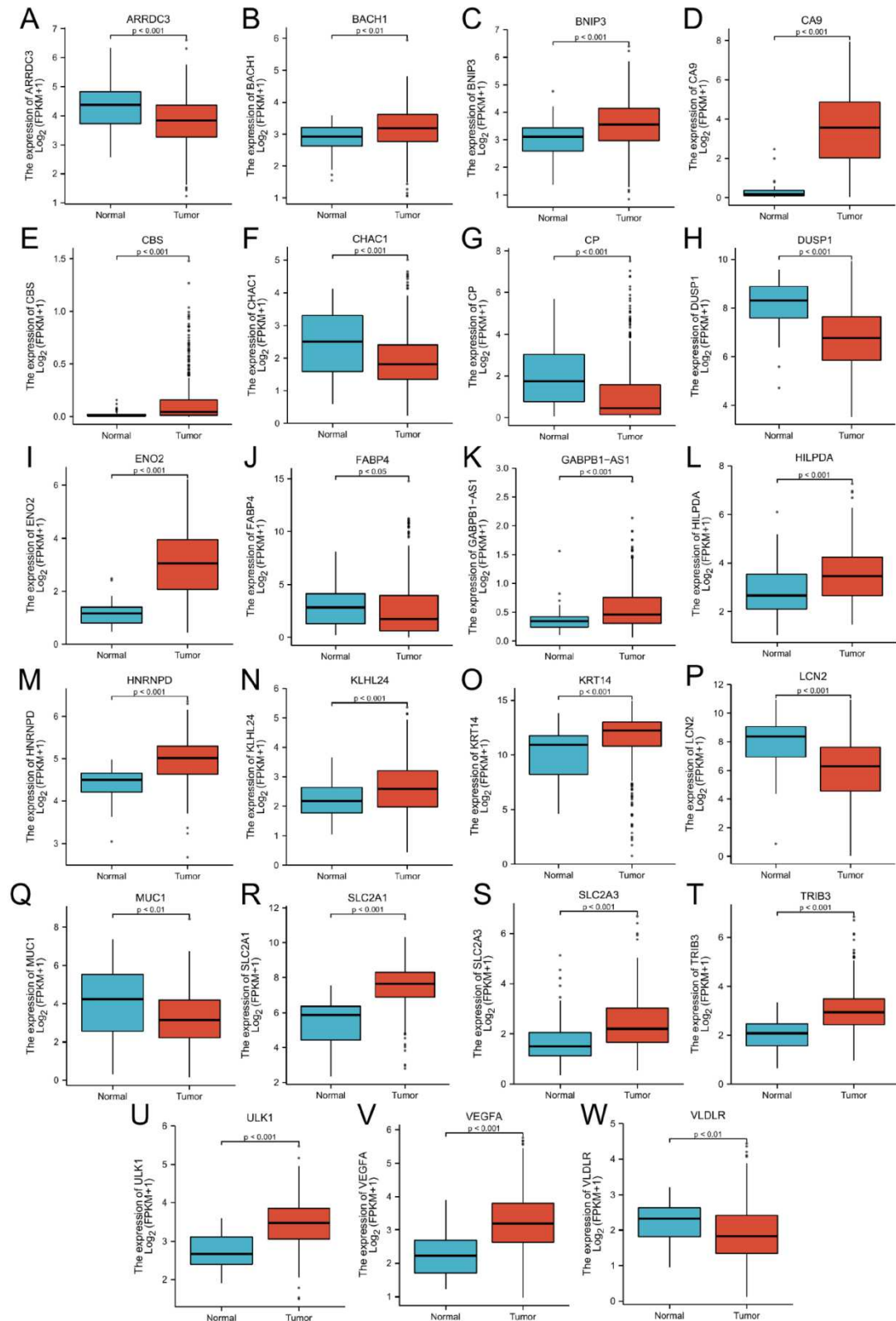
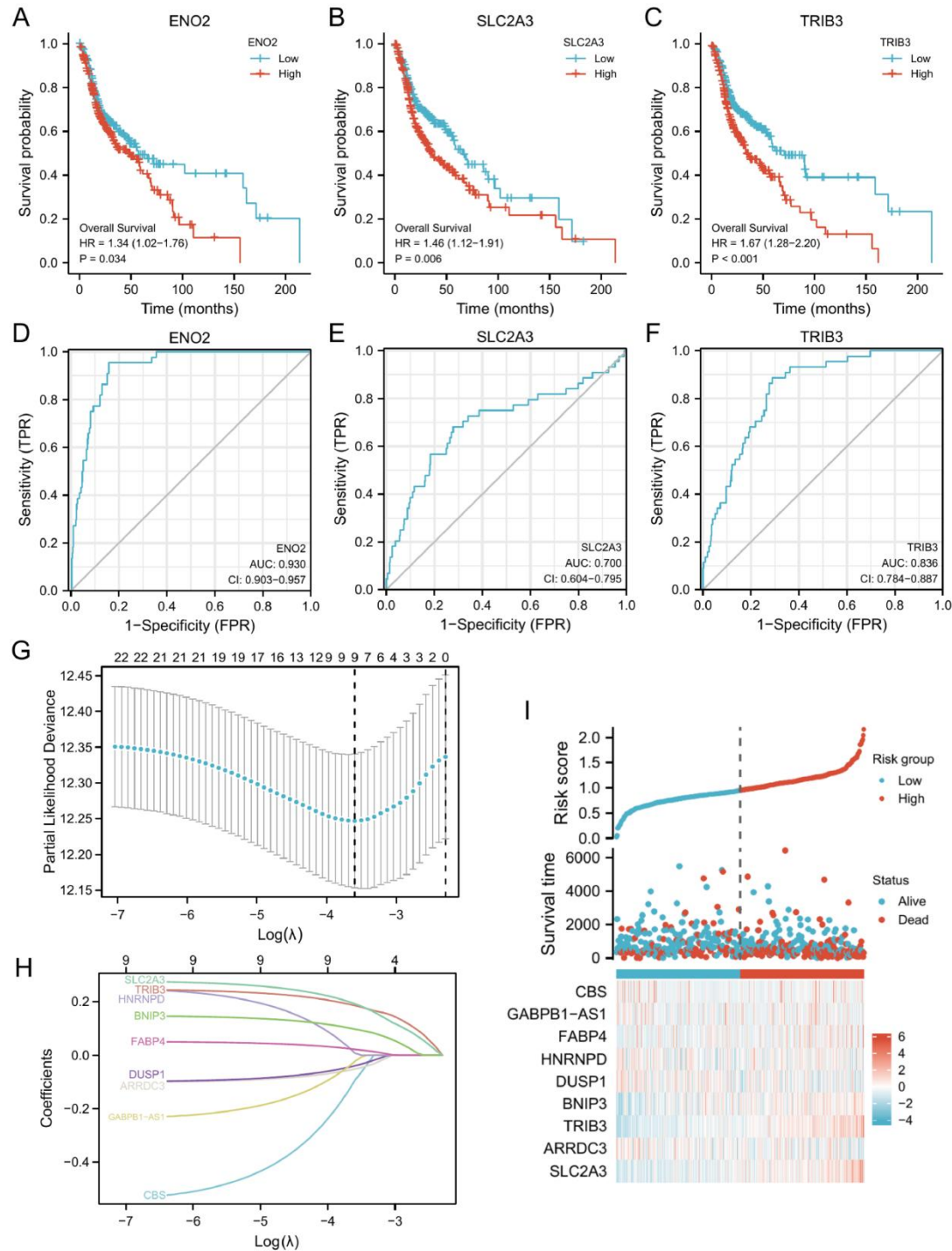


Fig. 7 The expression of 23 FRDEGs. (A-W) The difference in expression of 23 FRDEGs: *ARRDC3* (A), *BACH1* (B), *BNIP3* (C), *CA9* (D), *CBS* (E), *CHAC1* (F), *CP* (G), *DUSP1* (H), *ENO2* (I), *FABP4* (J), *GABPB1-AS1* (K), *HILPDA* (L), *HNRNPD* (M), *KLHL24* (N), *KRT14* (O), *LCN2* (P), *MUC1* (Q), *SLC2A1* (R), *SLC2A3* (S), *TRIB3* (T), *ULK1* (U), *VEGFA* (V), and *VLDLR* (W) between HNSC

573 (tumor) and adjacent tissues (normal). Abbreviations: HNSC, Head and Neck Squamous Cell
 574 Carcinoma.



575
 576 **Fig. 8** Prognosis analysis of FRDEGs and construction of a prognosis model. (A–C) KM curve of
 577 prognosis analysis of the FRDEGs *ENO2* (A), *SLC2A3* (B), and *TRIB3* (C). (D–F) ROC curve of
 578 the FRDEGs *ENO2* (D), *SLC2A3* (E), and *TRIB3* (F). (G) LASSO regression prognostic model of
 579 FRDEGs. (H–I) LASSO regression prognostic model variable locus diagram (H) and risk factor

diagram (I) of FRDEGs. Abbreviations: FRDEGs, ferroptosis-related differentially expressed genes; KM, Kaplan-Meier; ROC, receiver operating characteristic; LASSO, Least Absolute Shrinkage and Selection Operator.

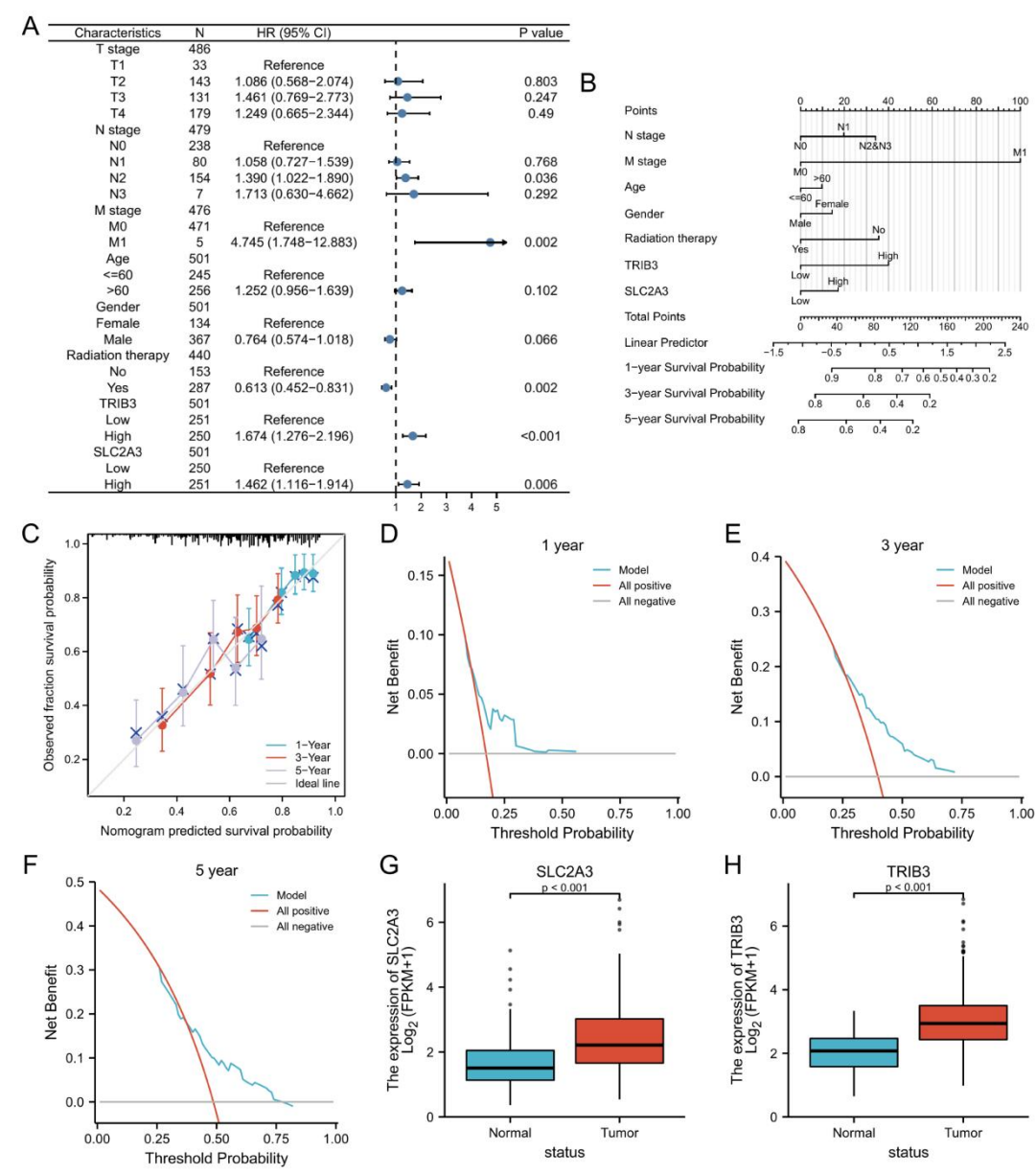


Fig. 9 Prognosis of two FRDEGs (*SLC2A3*, *TRIB3*). (A–C) Cox regression analysis (univariate and multivariate) for prognostic FRDEGs: Forest chart (A), nomogram (B), 1-, 3-, and 5-year calibration curves (C). (D–F) DCA diagram of 1-year (D), 3-years (E), and 5-years (F) of prognostic calibration analysis. LASSO Cox regression prognosis model. (G–H) Correlation analysis between *SLC2A3* (G) and *TRIB3* (H) and clinical tumor stage.

Note: The X-axis of the calibration curve is the survival probability predicted by the model, and the

Y-axis is the survival probability displayed by the actual data. The X-axis in the DCA diagram represents the probability threshold or threshold probability, and the Y-axis represents the net income. Abbreviations: DCA, decision curve analysis; FRDEGs, ferroptosis-related differentially expressed genes; LASSO, Least Absolute Shrinkage and Selection Operator.

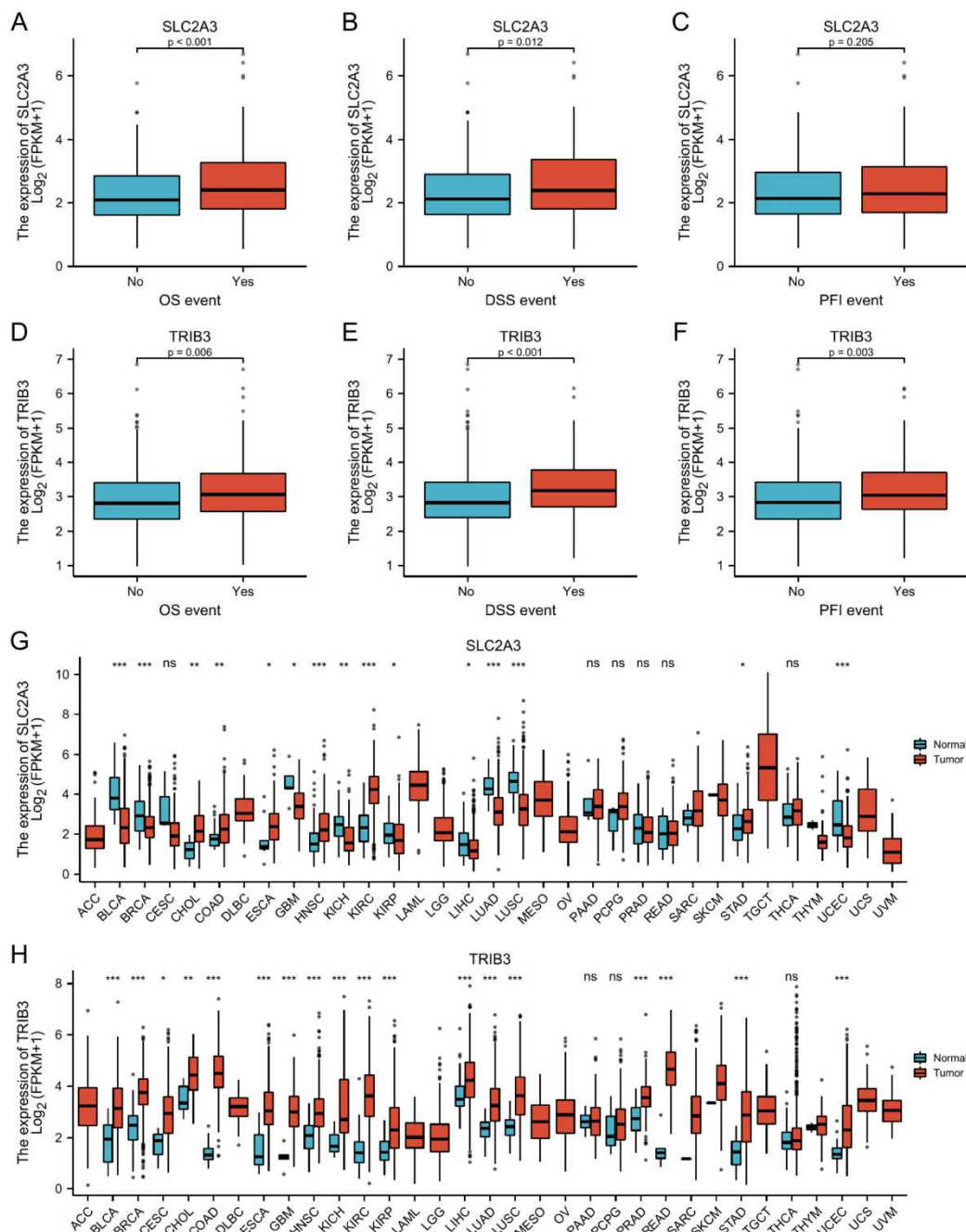
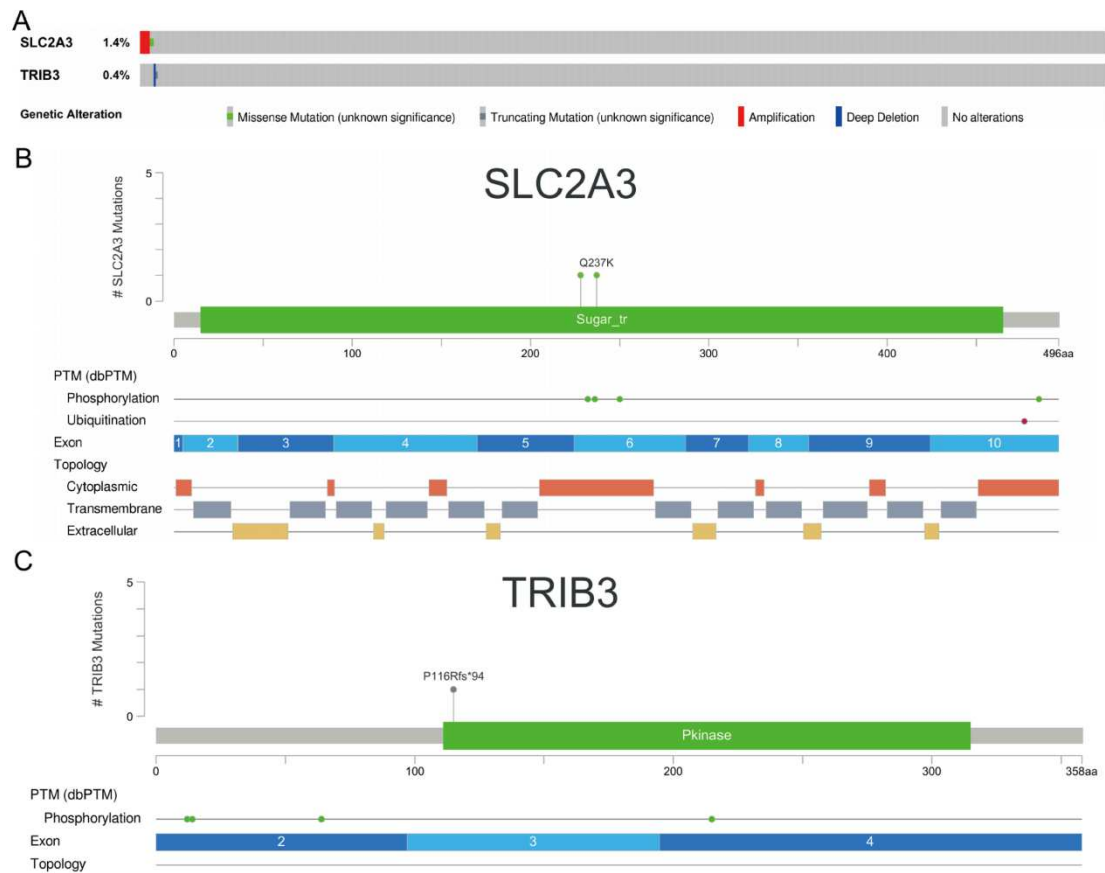


Fig. 10 Clinical analysis and pan-cancer analysis of FRDEGs in prognosis. (A–C) Association analysis between *SLC2A3* and clinical OS (A), DSS (B), and PFI (C). (D–F) Association analysis between *TRIB3* and clinical OS (D), DSS (E), and PFI (F). (G–H) Unpaired pan-cancer analysis of *SLC2A3* (G) and *TRIB3* (H). Abbreviations: PFI: progression-free interval; DSS: disease specific

600 survival; OS: overall survival.



601

602 **Fig. 11** Gene mutation analysis of FRDEGs for prognosis. **(A)** Mutation analysis results of *SLC2A3*

603 and *TRIB3* in TCGA-HNSC dataset. **(B-C)** Gene mutation analysis of the DEGs *SLC2A3* (B) and

604 *TRIB3* (C) related to cell death in the TCGA-HNSC dataset. All data are from the cBioPortal

605 database. Abbreviations: DEGs, Differentially Expressed Genes; HNSC, Head and Neck Squamous

606 Cell Carcinoma; TCGA, The Cancer Genome Atlas; FRDEGs, ferroptosis-related differentially

607 expressed genes.

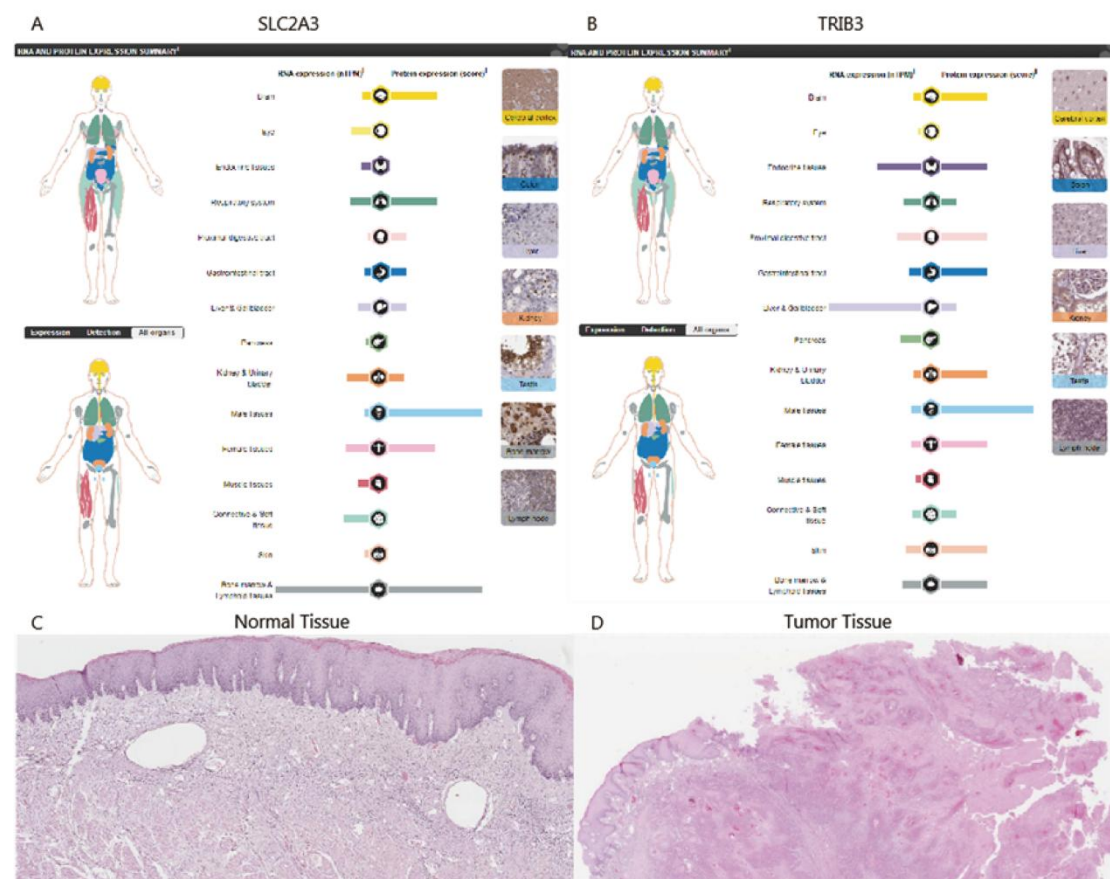


Fig. 12 Immunohistochemistry of FRDEGs in prognosis. **(A)** mRNA and protein expression levels of *SLC2A3* in normal human tissues. **(B)** mRNA and protein expression levels of *TRIB3* in normal human tissues. **(C-D)** The HPA database uses immunohistochemistry to evaluate normal tissue (C) and tumor tissue (D) from HNSC patients. All data are from the HPA database. Abbreviations: HPA, The Human Protein Atlas.

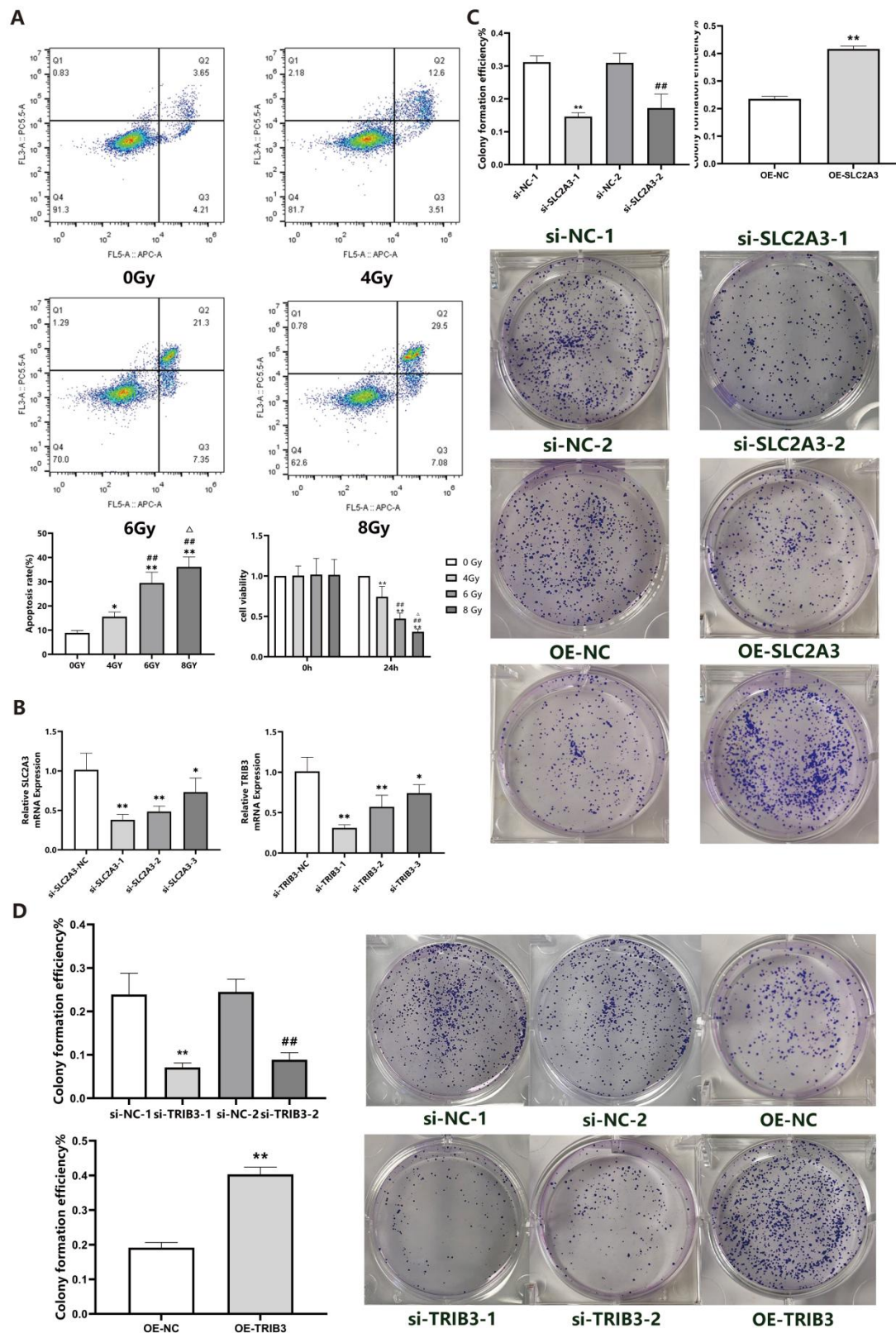


Fig. 13 Validation of *SLC2A3* and *TRIB3* as key determinants of radiation response in C666-1 cells.

(A) Radiation-induced phenotypes in C666-1 cells. Flow cytometric analysis of apoptosis and CCK-8 assay demonstrated that gradient irradiation markedly increased the apoptosis rate and decreased

618 cell viability at 24h. Data are expressed as mean $\pm$ SD (*P < 0.05, **P < 0.01 vs. 0 Gy group; ##P
619 < 0.01 vs. 4 Gy group; $\triangle$ P < 0.05, vs 6 Gy). (B) qPCR analysis confirming the successful
620 knockdown of *SLC2A3* and *TRIB3*. Data are presented as mean $\pm$ SD (*P < 0.05, **P < 0.01 vs. NC
621 group). (C, D) Clonogenic assay evaluating the impact of *SLC2A3* (C) and *TRIB3* (D) on
622 radiosensitivity. Silencing *SLC2A3* or *TRIB3* significantly reduced clonogenic survival after
623 irradiation, while their overexpression enhanced it. Data are presented as mean $\pm$ SD (*P < 0.05,
624 **P < 0.01 vs. respective si-NC group; ##P < 0.01 vs. respective OE-NC group).

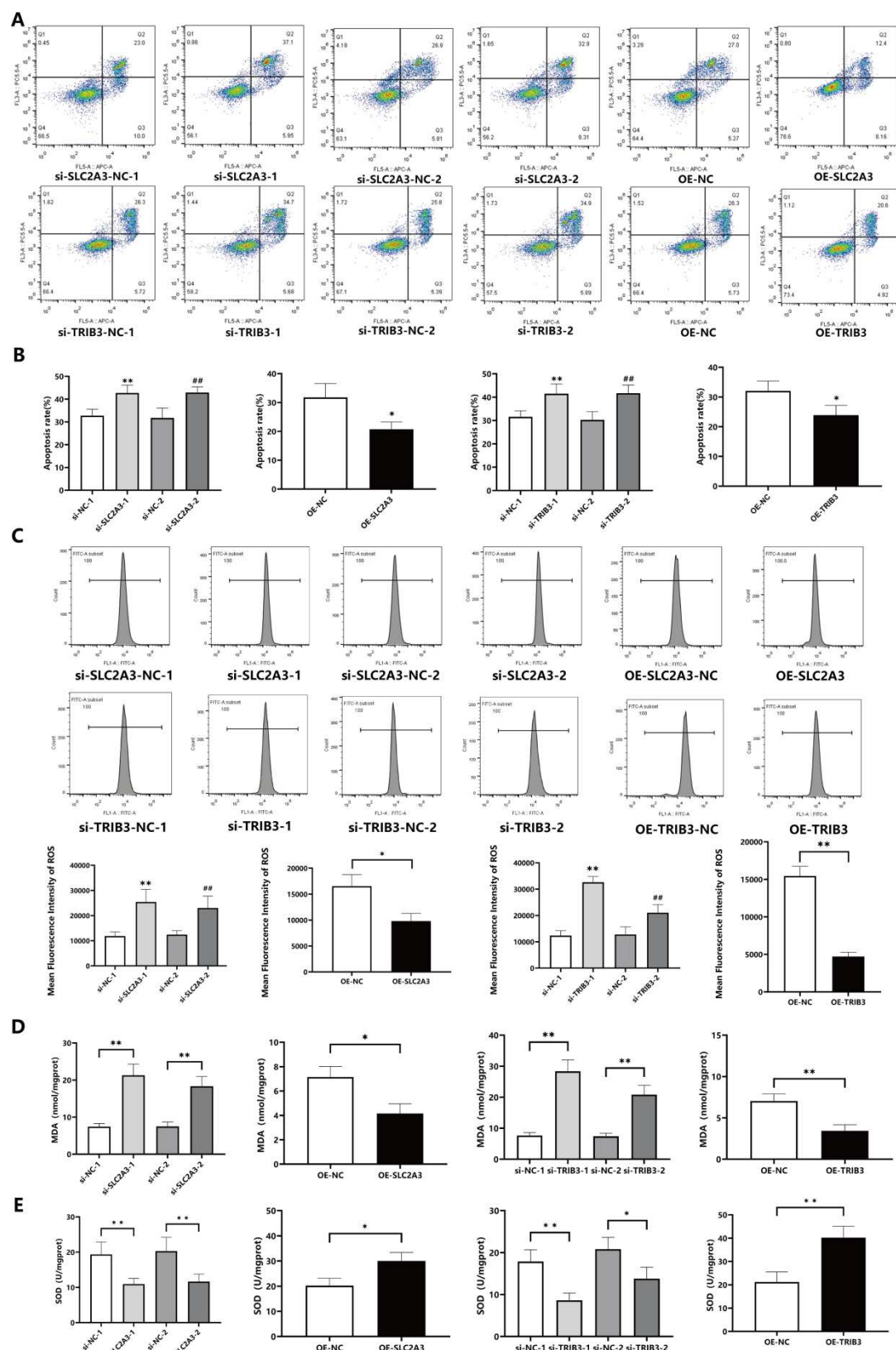


Fig. 14 Regulation of radiation-induced apoptosis and oxidative stress by *SLC2A3* and *TRIB3*. (A, B) Flow cytometry analysis of apoptosis. Knockdown of *SLC2A3* or *TRIB3* significantly increased radiation-induced apoptosis, while their overexpression exerted a protective effect. (C-E) Oxidative

stress detection. Depletion of *SLC2A3* or *TRIB3* significantly increased ROS and MDA levels, and decreased SOD activity, whereas overexpression exhibited opposite effects. These results experimentally confirm that apoptosis and oxidative stress are critical mechanisms by which *SLC2A3* and *TRIB3* regulate radiation response phenotypes.

Note: Data are expressed as mean $\pm$ SD. *P < 0.05, **P < 0.01 vs. counterpart control group; ##P < 0.01 vs. radiation-only group. Abbreviations: SOD, superoxide dismutase; MDA, malondialdehyde; ROS, reactive oxygen species.

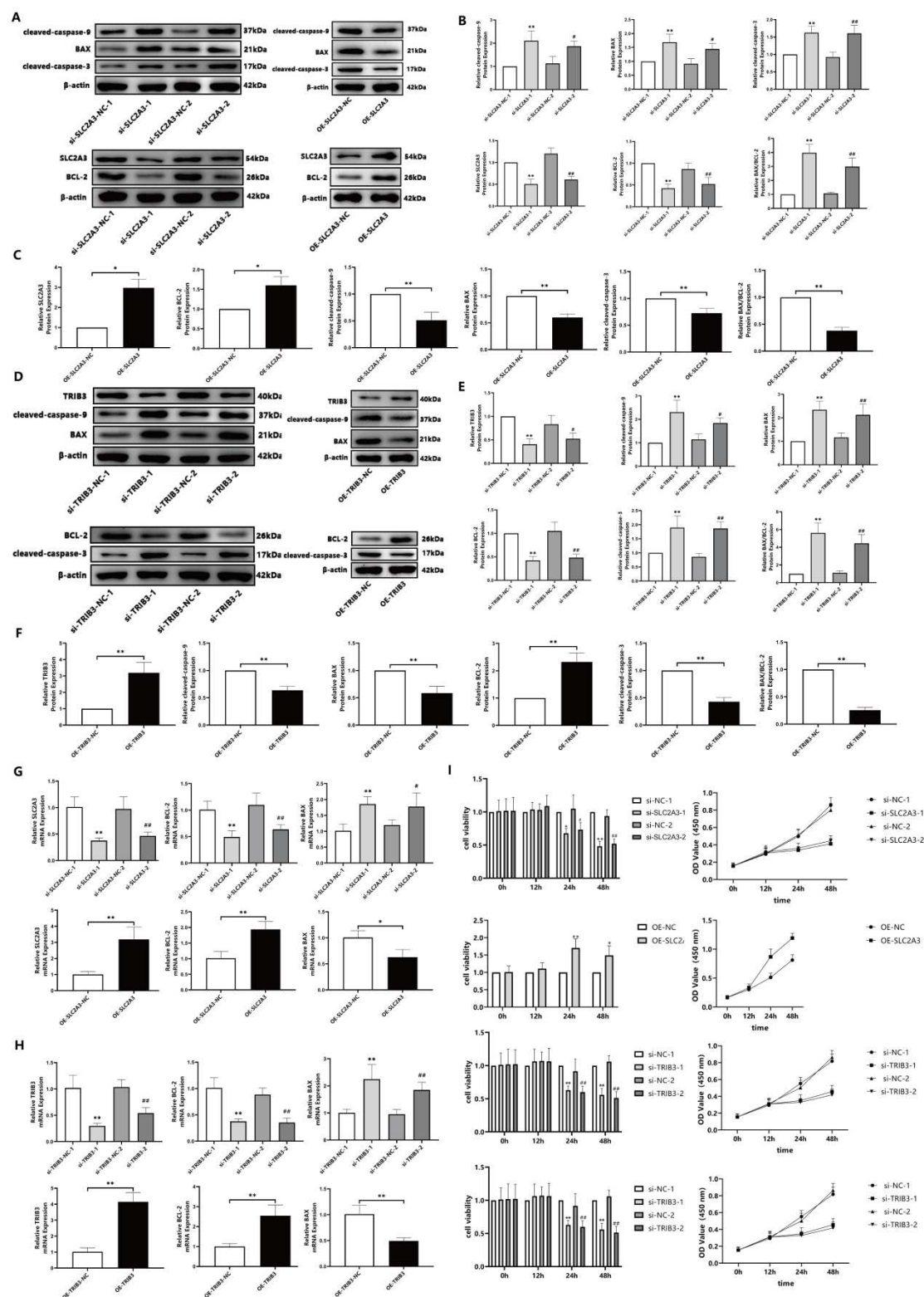


Fig. 15 Molecular and functional validation of pro-apoptotic signaling regulated by *SLC2A3* and *TRIB3*. (A-F) Western blot analysis of pro-apoptotic markers. (A-C) Knockdown of *SLC2A3* increased the cleavage of caspase-3 and caspase-9 and elevated the Bax/Bcl-2 ratio, whereas its overexpression suppressed these markers. (D-F) *TRIB3* knockdown similarly enhanced caspase-3/9

TRIB3. (A-F) Western blot analysis of pro-apoptotic markers. (A-C) Knockdown of *SLC2A3*

increased the cleavage of caspase-3 and caspase-9 and elevated the Bax/Bcl-2 ratio, whereas its

overexpression suppressed these markers. (D-F) *TRIB3* knockdown similarly enhanced caspase-3/9

cleavage and the Bax/Bcl-2 ratio, while its overexpression inhibited these pathways. (G, H) qPCR analysis of pro-apoptotic gene expression post-irradiation. The mRNA expression levels of key apoptotic genes were consistent with the protein-level changes observed by Western blot. (I) CCK-8 cell viability assay. Silencing of *SLC2A3* or *TRIB3*, in combination with radiation, synergistically inhibited cell growth over 48 hours. Conversely, overexpression of either gene promoted significant post-irradiation recovery.

Note: Data are expressed as mean $\pm$ SD. *, # $P < 0.05$ vs. respective control group; *, ## $P < 0.01$ vs. respective control group.

Table

Table 1. Identification of clinical features associated with OS via COX regression analysis

Characteristics	Total(N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
T stage	486				
T1	33	Reference			
T2	143	1.086 (0.568-2.074)	0.803		
T3	131	1.461 (0.769-2.773)	0.247		
T4	179	1.249 (0.665-2.344)	0.490		
N stage	479				
N0	238	Reference			
N1	80	1.058 (0.727-1.539)	0.768		
N2	154	1.390 (1.022-1.890)	0.036*		
N3	7	1.713 (0.630-4.662)	0.292		
M stage	476				
M0	471	Reference			
M1	5	4.745 (1.748-12.883)	0.002**	5.278 (1.635-17.038)	0.005
Age	501				
≤ 60	245	Reference			
> 60	256	1.252 (0.956-1.639)	0.102		

Table 1. Identification of clinical features associated with OS via COX regression analysis

Characteristics	Total(N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Gender	501				
Female	134	Reference			
Male	367	0.764 (0.574-1.018)	0.066	0.771 (0.552-1.076)	0.127
Radiation therapy	440				
No	153	Reference			
Yes	287	0.613 (0.452-0.831)	0.002**	0.606 (0.442-0.832)	0.002**
<i>TRIB3</i>	501				
Low	251	Reference			
High	250	1.674 (1.276-2.196)	< 0.001***	1.865 (1.362-2.555)	< 0.001***
<i>SLC2A3</i>	501				
Low	250	Reference			
High	251	1.462 (1.116-1.914)	0.006**	1.381 (1.009-1.892)	0.044*

Note: *P < 0.05, **P < 0.01, ***P < 0.001.

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