

KLF2 as a prognostic and potential therapeutic target associated with ferroptosis in serous ovarian cancer: An integrative bioinformatics and clinical validation study

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Abstract. Serous ovarian cancer (SOC), a prevalent histological subtype of ovarian cancer, poses a notable global threat to women's health. Ferroptosis has emerged as a pivotal focus in cancer research, presenting a promising novel therapeutic target. The present study aimed to identify key ferroptosis-related genes in SOC and evaluate their clinical implications. Gene expression profiles for SOC were obtained from the Gene Expression Omnibus database. Differential expression analysis and weighted gene co-expression network analysis, in conjunction with ferroptosis-related datasets, identified 22 differentially expressed genes associated with both SOC and ferroptosis. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analyses indicated their involvement in critical biological processes and pathways. Protein-protein interaction (PPI) network analysis identified four central hub genes: Kruppel-like factor 2 (KLF2), fatty acid binding protein 4 (FABP4), caveolin 1 (CAV1) and α -synuclein (SNCA). Validation using The Cancer Genome Atlas (TCGA) database confirmed significant downregulation of these genes in SOC ($P < 0.05$), with an assessment of their potential diagnostic value. Peripheral blood samples were collected from 20 patients with SOC (experimental group) and 20 healthy volunteers (control group). Reverse transcription-quantitative PCR demonstrated that KLF2 was significantly downregulated in patients with SOC ($P < 0.05$), while FABP4, CAV1 and SNCA did not show significant differences. Consequently,

subsequent experiments concentrated on KLF2. The down-regulation of KLF2 was further validated using the Human Protein Atlas database. Kaplan-Meier survival analysis revealed that low KLF2 expression was associated with a poor prognosis in patients with SOC. A nomogram suggested that KLF2 could serve as a prognostic biomarker for SOC. Immune infiltration analysis was performed based on TCGA data. Finally, interaction networks for microRNA-KLF2, transcription factor-KLF2 and chemical-KLF2 were constructed to predict potential molecular mechanisms and therapeutic drugs targeting KLF2 in SOC. These findings indicate that KLF2 may serve as a prognostic and potential therapeutic target related to ferroptosis in serous ovarian cancer, warranting further investigation.

Introduction

Ovarian cancer ranks as the eighth most common and fifth deadliest cancer among women globally. In 2022, an estimated 324,398 new cases were identified, with 206,839 associated mortalities, at incidence and mortality rates of 6.6 and 4.2 per 100,000 women each year, respectively (1). Serous ovarian cancer (SOC) constitutes 70 to 80% of all ovarian cancer cases, characterized by aggressive progression, late-stage diagnosis and a poor prognosis (2). Most patients present with peritoneal and pelvic metastases at diagnosis, and despite interventions such as debulking surgery and platinum-based chemotherapy, recurrence and drug resistance remain prevalent (3). Early diagnosis markedly improves patient outcomes, yet clinically applicable molecular markers remain scarce. While markers, including cyclin E1, minichromosome maintenance protein 10 and NIMA related kinase 2, have been reported (4-6), few studies have focused on ferroptosis-related genes for early detection or targeted therapies in SOC (7,8).

Ferroptosis is a novel form of cell death that differs from apoptosis, necrosis and pyroptosis (9). The core mechanism of ferroptosis involves iron-dependent accumulation of lipid reactive oxygen species (ROS); under the influence of ferrous iron or lipoxygenases, polyunsaturated fatty acids in cell membranes undergo peroxidation, resulting in membrane

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rupture and cell death (10). Crucial to this process is the downregulation of glutathione peroxidase 4 (GPX4), the key enzyme in the glutathione-based antioxidant system responsible for reducing lipid hydroperoxides (11). Morphologically, ferroptosis does not exhibit typical apoptotic features such as cell shrinkage, chromatin condensation, apoptotic body formation or cytoskeletal disintegration (12). Instead, electron microscopy shows shrunken mitochondria with increased membrane density (13). Biochemically, ferroptosis is resistant to inhibitors of apoptosis, pyroptosis or autophagy but can be suppressed by iron chelators and antioxidants (14). Thus, ferroptosis is primarily characterized by iron dependence and lipid peroxidation (15).

Due to its unique mechanism, ferroptosis has emerged as a promising target for cancer diagnosis and therapy (16). For example, the ferroptosis inducer erastin suppresses GA binding protein transcription factor (TF) subunit $\beta 1$ expression in hepatocellular carcinoma cells, leading to ROS accumulation and subsequent cell death (17). Recent evidence suggests that dysregulation of iron homeostasis can effectively eliminate persistent high-grade SOC (18). Additionally, non-coding RNAs (ncRNAs), including microRNAs (miRNA), long ncRNAs and circular RNAs, regulate ferroptosis-related gene expression through post-transcriptional mechanisms; these ncRNA signatures may serve as novel prognostic biomarkers in the tumor microenvironment, immunotherapy response and drug sensitivity, as demonstrated in gastric adenocarcinoma (19). Despite these advancements, the role of ferroptosis-related genes as early diagnostic and prognostic biomarkers in SOC remains insufficiently explored.

The present study aimed to identify key ferroptosis-associated genes in SOC utilizing an integrative bioinformatics and clinical assessment approach. The methodology involved screening differentially expressed genes (DEGs) and employing weighted gene co-expression network analysis (WGCNA) based on the GSE54388 dataset, intersecting these findings with known ferroptosis-related genes, and conducting functional enrichment and protein-protein interaction (PPI) analyses. The expression of candidate hub genes was assessed using reverse transcription-quantification PCR (RT-qPCR) using clinical blood samples, followed by evaluations of their diagnostic and prognostic value, immune infiltration correlations and construction of regulatory networks (miRNA, TF and chemical-gene interactions). The present study focused on identifying key ferroptosis-related genes in SOC and evaluating their clinical implications, with the aim of providing a scientific basis for further mechanistic and translational investigations.

Materials and methods

Patient datasets. As previously described (20), the gene expression profile GSE54388, which includes 16 samples of the SOC tumor epithelial component and 6 samples of human ovarian surface epithelium, was downloaded from the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/>) for the analysis of SOC. Data on ferroptosis-related genes were sourced from the FerrDb database (<http://www.zhounan.org/ferrdb/legacy/operations/download.html>), which currently encompasses 564 ferroptosis-related genes. RNA-sequencing

(RNA-seq) data in transcripts per million (TPM) format from The Cancer Genome Atlas (TCGA; <http://cancergenome.nih.gov/>) and the Genotype-Tissue Expression (GTEx; <https://gtexportal.org/home/index.html>) protocol were uniformly processed using the Toil process via the UCSC XENA database (<https://xenabrowser.net/datapages/>) and utilized as an external verification set.

A total of 20 adult female patients (age range, 30 to 77 years) diagnosed with high-grade SOC at Pidu District People's Hospital (Chengdu, China) between July 2024 and October 2025 were enrolled as the experimental group. All cases were histologically confirmed as high-grade SOC; no low-grade cases were included. According to the International Federation of Gynecology and Obstetrics 2018 staging system (21,22), 4 patients (20%) were classified as stage I, 5 (25%) as stage II, 8 (40%) as stage III and 3 (15%) as stage IV. BRCA1/2 germline mutation testing was available for 12 patients, of whom 2 (16.7%) carried pathogenic mutations; homologous recombination deficiency status was not routinely evaluated at the center and thus remains unavailable. Concerning cytoreductive outcomes, 12 patients (60%) achieved a complete gross resection (R0), 6 (30%) had residual tumor ≤ 1 cm (R1) and 2 (10%) had residual tumor > 1 cm (R2).

Inclusion criteria included: i) Pathological diagnosis of confirmed SOC, including both low-grade and high-grade SOC; ii) age range of 18 to 80 years; iii) treatment status of no prior systemic therapy for ovarian cancer (such as chemotherapy or targeted therapy); iv) complete clinical data and availability of comprehensive clinical information (including medical history, pathology reports and imaging examinations) for thorough analysis; and v) voluntary participation of patients in the study, with informed consent provided. Exclusion criteria included: i) Presence of other malignant tumors; ii) severe medical conditions such as notable systemic diseases involving the heart, liver or kidneys that may influence study results or patient survival; iii) recent severe infections or inflammatory responses that could alter peripheral blood markers; iv) co-existing endocrine disorders; v) immune system diseases that might interfere with the detection of ferroptosis markers; and vi) unqualified specimens, including peripheral blood samples failing to meet testing criteria, such as insufficient volume or hemolysis. Additionally, 20 healthy adult female volunteers (age range, 32-80 years) were selected as the control group. Written informed consent was obtained from all participants prior to enrollment, and participants were informed that their blood samples would be used for scientific research purposes. At the time of blood collection, none of the patients had received systemic treatment for ovarian cancer, such as chemotherapy or targeted therapy. A total of 2 ml of venous whole blood was collected from each participant, with samples processed immediately and stored at -80°C . Sample collection was conducted between July 2024 and October 2025. The present study received approval from the Ethics Committee of Pidu District People's Hospital [approval no. LWSPZ (2023) no. 09; 2023-4-11].

Differential gene expression screening. DEGs within the GSE54388 dataset (23) were analyzed employing the 'limma' package (<https://bioconductor.org/packages/limma>) in R software (version 4.0; Posit Software, PBC). Gene

expression differences were evaluated based on P-values and the logarithm of the fold-change (logFC). DEGs meeting the thresholds of $P < 0.05$ and $|\log_2FC| > 1$ were deemed statistically significant and visualized using volcano plots. Principal component analysis (PCA) was performed using the 'Rtsne' package (<https://github.com/jkrijthe/Rtsne>), and results were presented as heatmaps generated using the 'ggplot2' package (<https://ggplot2.tidyverse.org>).

WGCNA. WGCNA was performed on the GSE54388 gene expression profile utilizing the cloud platform (<https://cloud.oebiotech.com/>) and executed using the 'WGCNA' (24) R package (version 1.72) to identify modules associated with SOC and derive SOC-related genes. Data visualization was conducted using R and Python (version 3.11.0; Python Software Foundation). Genes with low variability were removed based on a standard deviation value of ≤ 0.5 . A signed co-expression network was constructed, with the soft-thresholding power β determined by assessing values ranging from 1 to 30; the optimal β was selected as the lowest value achieving a scale-free topology model fit (signed R^2) ≥ 0.9 while maintaining relatively high mean connectivity. Using this power, a topological overlap matrix (TOM) was computed, and the dissimilarity (1-TOM) was employed for hierarchical clustering with average linkage. Modules were initially identified through dynamic tree cutting (deepSplit=2; minimum module size=30), resulting in modules that were subsequently merged at a cutHeight of 0.25 (module eigengene correlation ≥ 0.75), yielding final modules, including a grey module for unassigned genes. For each module, the module eigengene was correlated with the trait of interest (SOC) using Pearson correlation; modules with $|\text{correlation}| \geq 0.3$ and P-value < 0.05 were considered significantly associated.

Core gene screening and Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses. The differentially expressed SOC genes obtained from the GSE54388 dataset, potential genes identified through WGCNA analysis and ferroptosis-related genes were intersected. A Venn diagram illustrating the SOC ferroptosis genes was generated using the 'ggplot2' package. Subsequently, selected genes underwent GO (<https://geneontology.org/>) and KEGG (<https://www.kegg.jp/>) enrichment analyses. GO analysis encompassed biological process (BP), cellular component (CC) and molecular function (MF).

PPI network analysis. PPIs were analyzed utilizing the online STRING 11.0 database (<https://cn.string-db.org/>), enabling the construction of a PPI network of ferroptosis genes associated with SOC. The network regulatory modules of DEGs were established using the MCODE plugin within Cytoscape 3.9.1 software (<http://www.cytoscape.org>).

External data sets validate key genes for screening. Corresponding TCGA data for ovarian serous cystadenocarcinoma and normal tissue data from GTEx were extracted using UCSC XENA (<https://xenabrowser.net/datapages/>). This dataset included GTEx normal tissues (88 cases) and TCGA tumor tissues (427 cases). Receiver operating characteristic (ROC) curve analysis was employed to compare the predictive

accuracy of linear predictors derived from the combined model and clinicopathological prognostic factors, utilizing the 'pROC' (25) package for the analysis.

Assess candidate genes by RT-qPCR experiments. Total RNA was extracted from blood samples using a Blood RNA Extraction Kit (Biosharp Life Sciences), ensuring the removal of genomic DNA during the extraction process. Reverse transcription was conducted using the PrimeScript™ RT reagent Kit with gDNA Eraser (Takara Biotechnology Co., Ltd.) according to the manufacturer's instructions. qPCR was subsequently performed using TB Green™ Premix Ex Taq™ II (Takara Biotechnology Co., Ltd.) to quantify the expression levels of DEGs, with GAPDH serving as the endogenous reference. Primer sequences (Table I) were synthesized and purified via the ULTRAPAGE method by Shanghai Sheng Gong Biology Engineering Technology Service, Ltd. Each 20- μ l qPCR mixture underwent the following thermal cycling conditions: Initial denaturation at 95°C for 30 sec, followed by 45 cycles of denaturation at 95°C for 5 sec, annealing at 55°C for 30 sec and extension at 72°C for 30 sec. Threshold cycle (Cq) values were acquired using QuantStudio™ Design & Analysis SE Software (version 1.5.2; Thermo Fisher Scientific, Inc.). Relative mRNA expression levels were calculated employing the $2^{-\Delta\Delta Cq}$ method (26), where $\Delta Cq = Cq_{\text{target gene}} - Cq_{\text{internal reference}}$ and $\Delta\Delta Cq = Cq_{\text{experimental}} - \Delta Cq_{\text{control}}$. Gene expression differences are presented as FCs derived from $2^{-\Delta\Delta Cq}$.

Human Protein Atlas (HPA) database analysis. The HPA database (<http://www.proteinatlas.org/>) was utilized to validate Kruppel-like factor 2 (KLF2) expression in SOC. To access the data, 'KLF2' was entered in the homepage search, followed by selecting 'TISSUE' and then 'CERVIX' for normal tissue images; additionally, 'PATHOLOGY' and 'CANCER-OVARIAN CANCER' were selected to obtain corresponding cancer images. The images used in this study are available from the HPA (v24.proteinatlas.org) at <https://v24.proteinatlas.org/ENSG00000127528-KLF2/tissue>. Image credit: HPA (27). The HPA is licensed under the Creative Commons Attribution-ShareAlike 4.0 International License (28).

Immune infiltration analysis. Assessment of immune cell infiltration is essential for disease research and prognosis prediction. To quantify immune cell infiltration in the relevant dataset, single-sample Gene Set Enrichment Analysis (ssGSEA) was employed using the R package 'GSVA' (v1.46.0) (29). For each sample, ssGSEA computes an enrichment score by ranking all genes based on their expression levels and assessing the empirical cumulative distribution function of genes within a specific gene set relative to those outside of it; the resulting score reflects the relative abundance of a specific immune cell type within that sample. The gene signatures utilized comprised 24 immune cell type-specific markers derived from the study by Bindea *et al* (30), which are well-validated and encompass a broad range of innate and adaptive immune cell populations, with detailed information provided in the supplementary materials of that publication (30). For the ferroptosis-related gene in SOC, its association with each immune cell infiltration score was evaluated using Spearman's

Table I. Primer sequences.

Primer name	Forward primer (5'-3')	Reverse primer (5'-3')
GAPDH	TGACTTCAACAGCGACACCCA	CACCCTGTTGCTGTAGCCAAA
CAV1	GGAGATCGACCTGGTCAACC	GCCGTCAAACTGTGTGTCC
SNCA	GTGGCTGCTGCTGAGAAAAC	CACCACTGCTCCTCCAACAT
KLF2	GAAGCCCTACCACTGCAACT	CATGTGCCGTTTCATGTGCA
FABP4	GCTTTGCCACCAGGAAAGTG	TCCTGGCCCAGTATGAAGGA

CAV1, caveolin 1; SNCA, α -synuclein; KLF2, Kruppel-like factor 2; FABP4, fatty acid binding protein 4.

rank correlation coefficient (ρ), implemented via the `cor.test` function in R (stats package; <https://www.R-project.org/>). A correlation was deemed statistically significant at a two-sided P-value <0.05 . All visualizations were generated using the 'ggplot2' (v3.4.4) package. Notably, ssGSEA-based immune infiltration provides computational estimates dependent on the quality and specificity of the selected gene signatures, which are not direct measurements of cellular abundance; thus, these results should be interpreted as hypothesis-generating and ideally validated through orthogonal methods such as immunohistochemistry or flow cytometry in future studies.

miRNA, TF and drug prediction. To construct miRNA-gene interactions, TF-gene interactions and protein-chemical interactions for the identified key genes, the online software NetworkAnalyst (version 3.0; <https://www.networkanalyst.ca/>) was utilized.

Statistical analysis. All statistical analyses were conducted using R (version 4.2.1; R Core Team) and SPSS (version 22.0; IBM Corp.) software. The normality of continuous variables was assessed using the Shapiro-Wilk test; normally distributed data are expressed as the mean $\pm$ standard deviation and compared using the two-tailed unpaired Student's t-test, while non-normally distributed data are presented as the median (interquartile range) and compared using the Mann-Whitney U test. For multiple comparisons, the Benjamini-Hochberg false discovery rate (FDR) method was applied, with adjusted P-values (q-values) <0.05 considered to indicate a significant difference. Spearman's rank correlation coefficient was employed for correlation analyses. ROC curves were generated using the pROC R package, with the area under the curve (AUC) reported alongside its 95% confidence interval calculated via the percentile bootstrap method. In survival analysis, nomogram construction and immune infiltration comparison, the data were divided into high-expression and low-expression groups based on the median expression level of KLF2. For comparisons of categorical clinical characteristics between KLF2 high- and low-expression groups, the Pearson's χ^2 test was used. Univariate and multivariate Cox proportional hazards regression models were fitted using the survival R package; the proportional hazards assumption was verified by Schoenfeld residual tests, and results were presented as hazard ratios (HR) with 95% confidence intervals. A nomogram was developed based on the multivariate Cox model and validated internally using bootstrap

resampling to generate calibration curves and calculate the concordance index (C-index). $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Screening of differentially expressed genes in SOC. The GSE54388 dataset was retrieved from the GEO database, comprising samples from 16 patients with SOC and 6 normal individuals. The samples were categorized into an SOC group (test) and a normal control group (ref) for analysis. Initially, normalization of the data for both groups was performed using the limma package. The boxplot (Fig. 1A) indicates a high degree of normalization across samples, allowing for meaningful comparisons. The PCA plot (Fig. 1B) reveals that PC1 and PC2 exhibit no overlap, with distances within groups being relatively close. This highlights the distinct and comparable differences between the two groups. The criteria for identifying DEGs were set at $|\log_2FC| > 1$ and $P < 0.05$, resulting in the identification of a total of 1,483 DEGs, as depicted in the volcano plot (Fig. 1C) and the heatmap (Fig. 1D).

WGCNA. To construct a scale-free co-expression network, the appropriate soft-thresholding power for the adjacency matrix was selected as recommended by WGCNA. Powers ranging from 1 to 30 were tested, and the corresponding scale-free fit indices and mean connectivity were calculated for each (Fig. 2A). Optimal levels for both scale-free fit and mean connectivity were achieved at a power of 16, which was chosen for subsequent module construction. Based on this selected power, a weighted gene co-expression network was established, categorizing 9,312 genes into 26 distinct modules, as illustrated in the gene dendrogram (Fig. 2B). The upper portion of the diagram displays the hierarchical clustering of genes, while the lower section indicates module assignments, with distinct colors representing individual modules. Modules identified through the Dynamic Tree Cut method are labeled accordingly. Due to correlations observed among certain modules, closely related modules were merged into a single module, labeled as 'Merged'. Additionally, a heatmap illustrating all gene clusters was produced (Fig. 2C). Module eigengenes were correlated with clinical traits using Pearson correlation, revealing the blue and green modules as having the most significant associations (Fig. 2D). Following merging and deduplication, a total of 1,477 genes from these two modules were retained for further analysis.

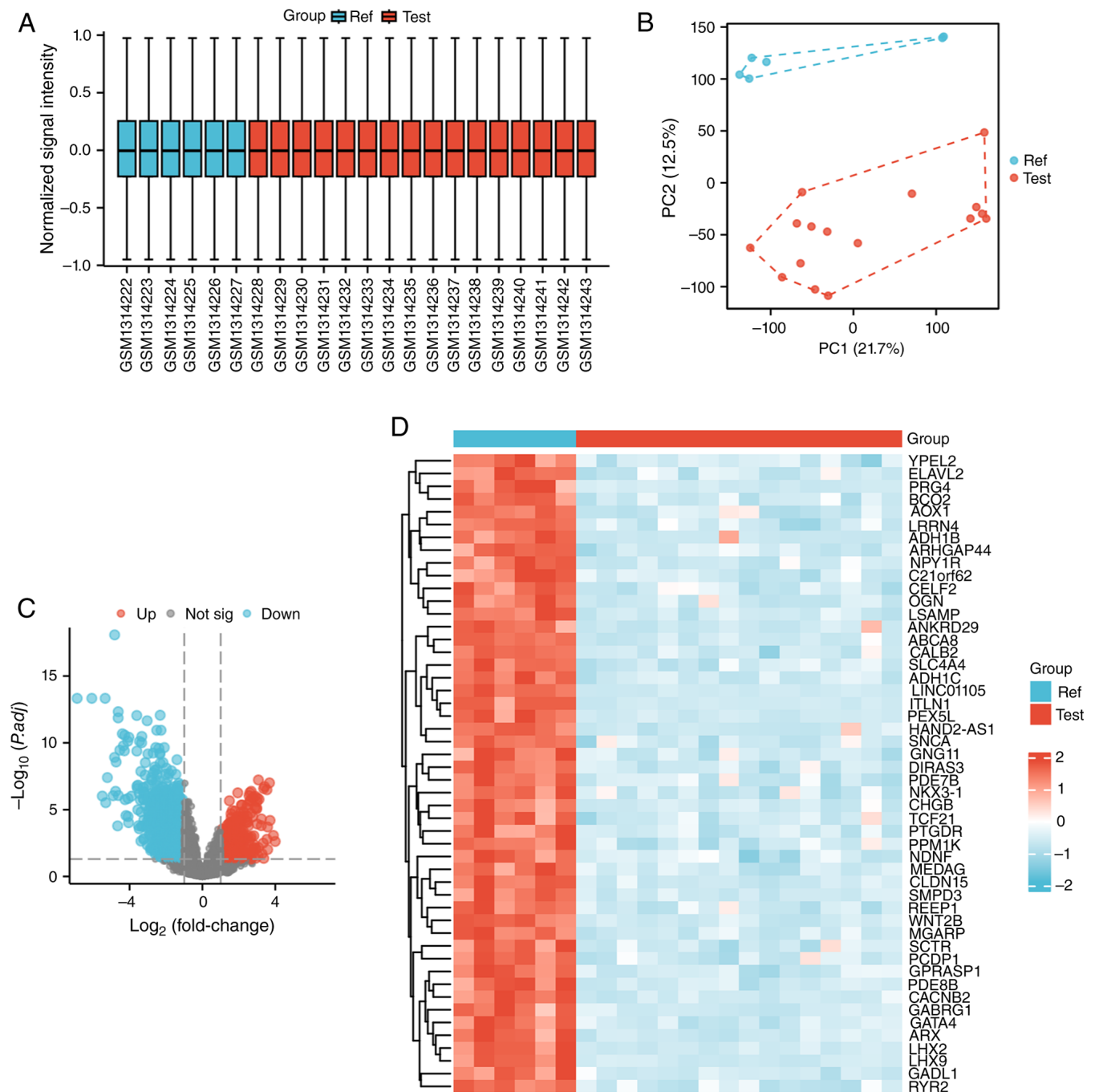


Figure 1. Differential gene screening in GSE9750. (A) Boxplot showing normalized signal intensity distributions of the GSE54388 dataset after quantile normalization. Red boxes represent serous ovarian cancer samples (test; n=16); blue boxes represent normal ovarian surface epithelium samples (ref; n=6). (B) PCA plot of the normalized expression data. PC1 and PC2 explain 25.5 and 12.5% of the variance, respectively. (C) Volcano plot of differentially expressed genes. Red dots represent significantly upregulated genes ($\log_2FC > 1$; $P < 0.05$), blue dots represent significantly downregulated genes ($\log_2FC < -1$; $P < 0.05$) and gray dots represent non-significant genes. (D) Heatmap of differentially expressed genes ($\log_2FC > 1$; $P < 0.05$) in GSE54388. PCA, principal component analysis.

Screening of genes related to ferroptosis in SOC. Due to the critical role of ferroptosis in SOC, the relationship between DEGs in SOC and ferroptosis-related genes was further explored. As shown in Fig. 3, by intersecting the 564 ferroptosis-related genes from the MisgDB database, the 1,477 potential SOC-related genes identified through WGCNA and the 1,483 DEGs in SOC, 22 DEGs were isolated. Among these, the 12 genes exhibiting downregulated expression are RGS4, SNCA, KLF2, FABP4, TXNIP, DDR2, VLDLR, CPEB1,

P4HB, LURAP1L, TSC1 and CAV1, while the 10 genes with upregulated expression are MUC1, KIF20A, RRM2, EZH2, NOX4, GPT2, IDH2, SLC7A5, SLC39A7 and KRAS.

Enrichment analysis of differentially expressed genes. To explore the potential biological functions of ferroptosis-related differential genes in SOC, GO enrichment analysis and KEGG pathway analysis were conducted using online software, with results presented in Fig. 4A and B. In the MF component of the

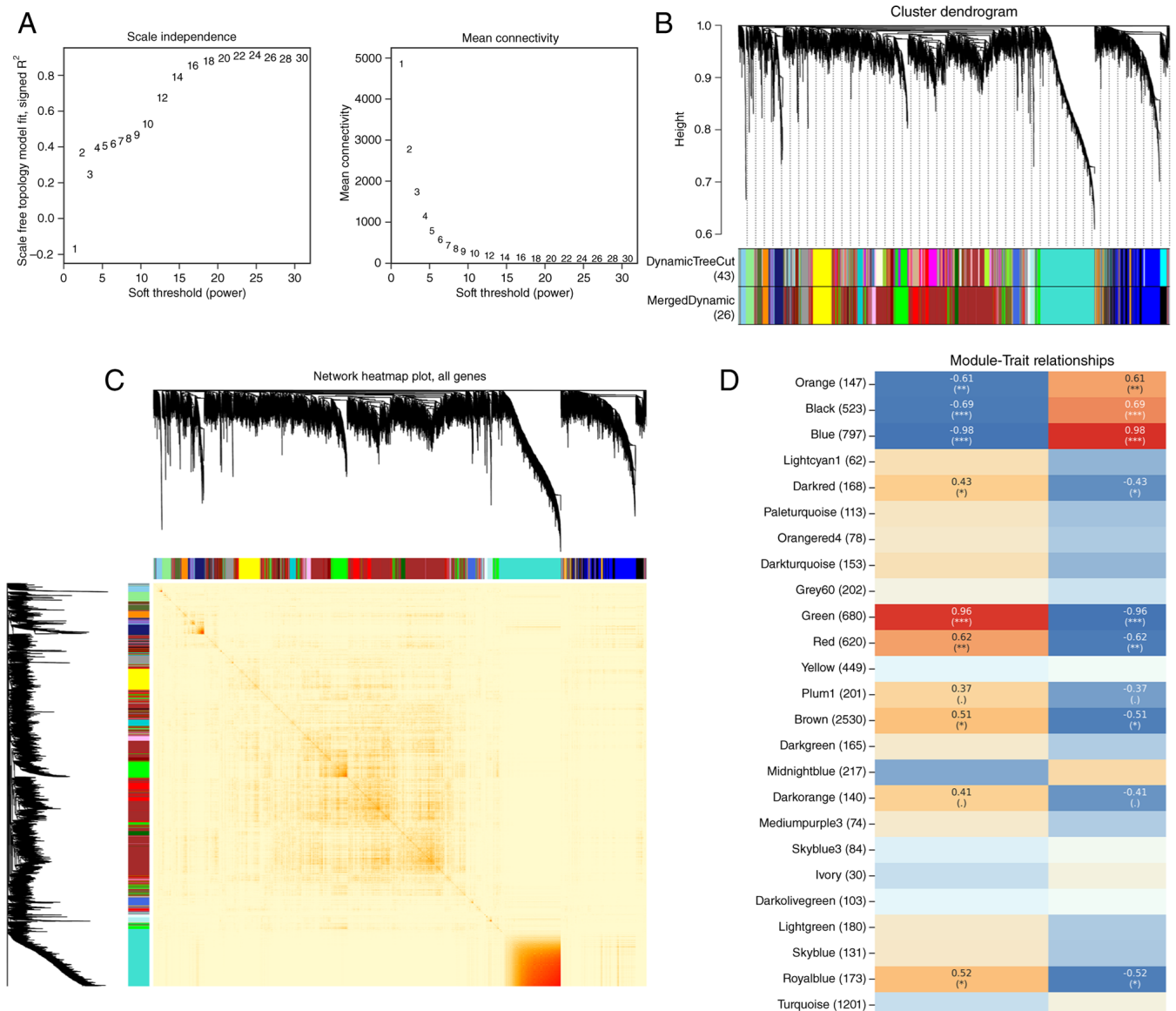


Figure 2. Weighted gene co-expression network analysis. (A) Determination of the soft-thresholding power (β). The left panel shows the correlation coefficients associated with different power levels, while the right panel represents the average connectivity within networks constructed based on these different power values. (B) Gene dendrogram (top) and module assignment (bottom) based on the dissimilarity topological overlap matrix. Each module is represented by a unique color. (C) Heatmap of gene clustering derived from the weighted co-expression network. The color gradient ranges from red (high adjacency, strong co-expression) to yellow/pale yellow (low adjacency, weak co-expression). (D) Module-trait correlations analyzed by the Pearson's method. Red indicates positive correlation and blue indicates negative correlation. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

GO analysis, key activities included 'heat shock protein binding', 'molecular function inhibitor activity' and 'Hsp70 protein binding'. The CC terms mainly encompassed 'cell-substrate junction', 'focal adhesion', 'oxidoreductase complex' and 'lipid droplet'. BP highlighted terms included 'response to oxidative stress', 'cellular response to chemical stress' and 'striated muscle cell differentiation'. Furthermore, the KEGG pathway analysis identified significant focus areas such as 'Alzheimer disease', the 'mTOR signaling pathway', 'central carbon metabolism in cancer', 'glutathione metabolism' and '2-oxocarboxylic acid metabolism'. Notably, the mTOR signaling pathway and glutathione metabolism are directly associated with the regulation of ferroptosis; the mTOR pathway controls lipid peroxidation and iron homeostasis, while glutathione metabolism supplies the substrate (glutathione) for GPX4, a key suppressor of ferroptosis.

Construction of protein networks and identification of key genes. To investigate the interactions between SOC and ferroptosis-related differential genes, a protein interaction network analysis was performed using the STRING database (Fig. 5A). The four genes with the highest connectivity, KLF2, fatty acid binding protein 4 (FABP4), caveolin 1 (CAV1) and α -synuclein (SNCA), were identified (Fig. 5B).

TCGA database validation of screened key genes and analysis of their diagnostic value. Validation analysis was conducted using SOC tumor samples from TCGA database and normal tissue samples from the GTEx database. Results indicated that CAV1, SNCA, KLF2 and FABP4 were all downregulated in patients with SOC, with significant differences compared with the control group ($P < 0.05$), as illustrated in Fig. 6A. To assess



Figure 3. Venn diagram of serous ovarian cancer differentially expressed genes from GSE54388 dataset, serous ovarian cancer-related genes from WGCNA analysis and ferroptosis-related genes. WGCNA, weighted gene co-expression network analysis.

the diagnostic value of these four genes for SOC, ROC curve analysis was performed on the expression data from TCGA database. The findings revealed that the areas under the ROC curves for CAV1, SNCA and KLF2 were 0.954, 0.981 and 0.990, respectively, indicating high diagnostic value. FABP4 exhibited an AUC of 0.819, suggesting moderate diagnostic value (Fig. 6B).

Assessment of key gene expression in clinical samples. Subsequently, blood samples were collected from clinical cases for experimental validation. cDNA was extracted from whole blood samples of 20 adult female patients with high-grade SOC (mean age, 54.6 ± 14.33 years) and 20 healthy adult female volunteers (mean age, 51.3 ± 14.31 years), and RT-qPCR was utilized to measure the expression levels of CAV1, SNCA, KLF2 and FABP4. The sex composition of the two groups was comparable, and there was no significant difference in age (SOC: 54.6 ± 14.33 years; control: 51.3 ± 14.31 years; $t=0.73$; $P=0.48$). The results presented in Table II indicate that, compared with the control group, the experimental group displayed downregulation of SNCA, KLF2 and FABP4, with KLF2 exhibiting a significant difference ($P<0.05$), while differences for SNCA and FABP4 were not significant ($P>0.05$). Additionally, CAV1 expression was higher in the experimental group compared with that in the control group, but this difference was also not statistically significant ($P>0.05$).

In summary, among the four key genes, only KLF2 demonstrated significant downregulation in the RT-PCR assessment ($P<0.05$), whereas SNCA, FABP4 and CAV1 did not meet significance ($P>0.05$). Moreover, KLF2 exhibited a relatively high diagnostic accuracy (AUC=0.990) and consistent

downregulation in both TCGA dataset and clinical blood sample assessment. Consequently, KLF2 was prioritized for further investigation due to its potential critical role in SOC.

Expression of key genes was verified in tissues. The expression of KLF2 in SOC pathological tissues and healthy ovarian tissues was validated using the HPA database through immunohistochemistry imaging. As illustrated in Fig. 7, the results demonstrated that the expression levels of KLF2 in SOC tissues (low, moderate and strong groups) were lower than in normal ovarian tissues, aligning with previous findings.

Prognostic value analysis of KLF2 in SOC. To study the role of KLF2 in SOC, patients with SOC from TCGA database were divided into a high-expression group (top 50% of samples with the highest expression, $n=191$) and a low-expression group (remaining 50% of samples, $n=190$). The results revealed that low KLF2 expression was only associated with residual tumor ($P<0.05$; Table III) while no significant associations were observed with clinical stage, tumor status, primary therapy outcome or age (all $P>0.05$). Subsequently, the impact of KLF2 on SOC prognosis was investigated. To assess whether the expression of KLF2 influences patient survival, Kaplan-Meier survival analysis revealed that low KLF2 expression was associated with a poor prognosis in patients with SOC (HR, 0.61; 95% confidence interval 0.38-0.97; $P=0.038$; Fig. 8A). These findings suggest that KLF2 serves as a prognostic factor for SOC. KLF2 expression levels were incorporated with clinical variables to construct a nomogram for predicting patient survival at 1, 3 and 5 years. The predictive capability of KLF2 expression approached that of clinical staging, widely recognized as a fundamental tool for evaluating cancer progression (Fig. 8B). Corresponding calibration plots further illustrated high agreement between the predicted model of the nomogram and the actual model for 1-, 3- and 5-year survival probabilities, highlighting the prognostic ability of the nomogram (Fig. 8C). Collectively, these findings indicate that KLF2 may function as a prognostic biomarker in SOC.

Correlation between KLF2 expression and immune characteristics in SOC. Previous research has shown that KLF2 levels are diminished in autoimmune diseases (31). The present study identified that KLF2 is abnormally downregulated in SOC, leading to the speculation that KLF2 may participate in regulating the immune response within this context. To investigate the correlation between KLF2 expression levels and the immune response in SOC, immune infiltration across varying KLF2 expression levels was analyzed using TCGA database. As depicted in Fig. 9A, infiltration levels of B cells, CD8 T cells, cytotoxic cells, dendritic cells (DC), eosinophils, immature DCs, macrophages, mast cells, neutrophils, natural killer (NK) CD55dim cells, NK cells, plasmacytoid DCs, T cells, T helper cells, central memory T cells (Tem), T follicular helper cells, $\gamma\delta$ T cells, Th1 cells, Th2 cells and regulatory T cells in patients with SOC with low KLF2 expression were substantially lower than in those patients with high KLF2 expression. A further analysis of the correlation between KLF2 expression levels and immune infiltration in SOC revealed that KLF2 expression levels exhibited moderate to strong positive correlations with the infiltration of 10 different types of

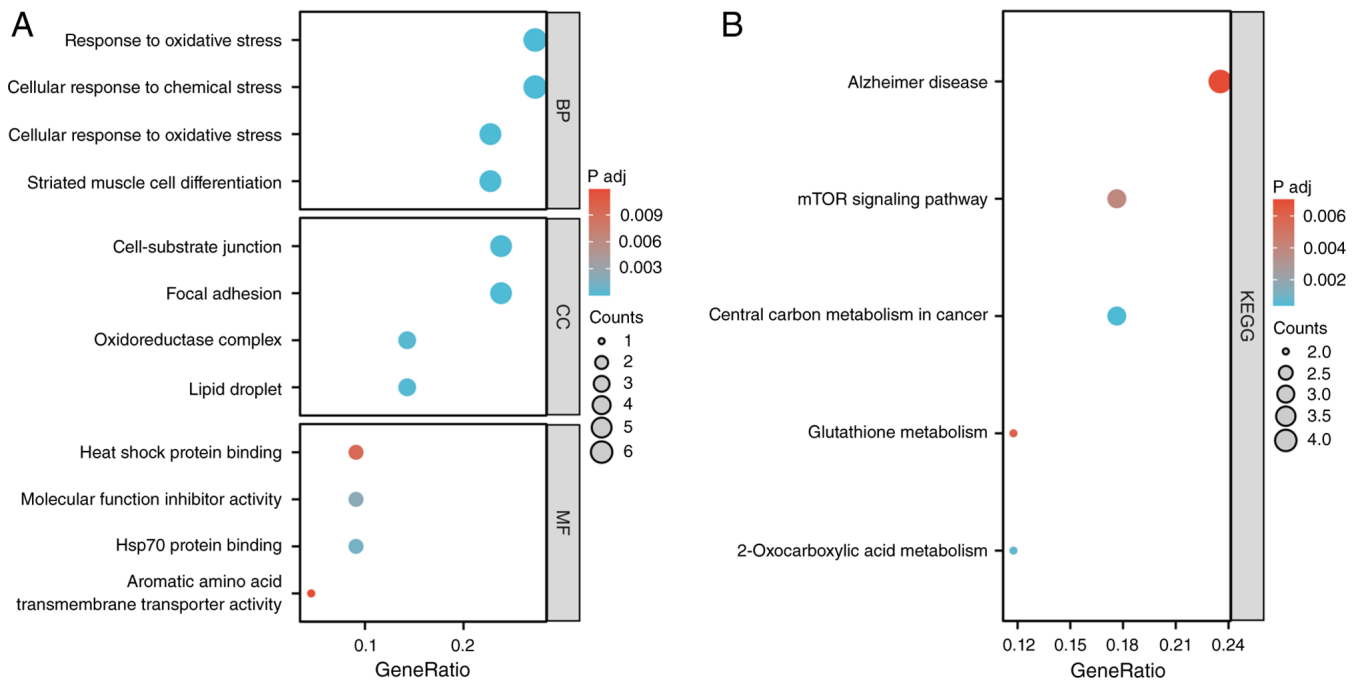


Figure 4. Enrichment analysis of ferroptosis-related genes in serous ovarian cancer. (A) GO enrichment analysis. The bar plot shows the top enriched terms in three categories: BP, CC and MF. The x-axis represents the gene ratio (number of enriched genes/total genes in the term), and the y-axis represents the GO terms. Red indicates highly significant ($P < 0.01$), and blue indicates less significant. (B) KEGG pathway enrichment analysis. The bubble plot shows enriched pathways. The x-axis represents the gene ratio, the y-axis represents KEGG pathways, bubble size indicates the number of enriched genes and bubble color represents the adjusted P-value (red, lower P-value). GO, Gene Ontology; BP, Biological Process; CC, Cellular Component; MF, Molecular Function; KEGG, Kyoto Encyclopedia of Genes and Genomes.

immune cells ($p \geq 0.3$), whereas the remaining 14 showed only weak-to-no associations ($p < 0.3$) (Fig. 9B). These data suggest that KLF2 may be linked to the tumor immune microenvironment (TIME) in SOC.

Prediction and estimation of target miRNAs, TF and compounds. Finally, the NetworkAnalyst database was utilized to predict miRNAs that target key genes, leading to the construction of a potential miRNA-hub gene network designed to elucidate the molecular mechanisms of KLF2. This network comprises 109 connections (Fig. 10A), corresponding to 109 predicted related miRNAs, such as miR-92a-3p and miR-15b-3p. To further examine the role of KLF2 among TFs, predictions were made regarding the TFs associated with KLF2, identifying NANOG, TP53, HNRNP and FOXO1 as key regulators (Fig. 10B). Given that hub genes may exert notable influence, interactions between hub genes and small-molecule compounds were also explored to identify potential therapeutic agents. Utilizing the NetworkAnalyst online tool, compounds targeting KLF2 were predicted, resulting in the construction of an mRNA-chemical network. Within this network, KLF2 was linked to 79 small-molecule compounds (Fig. 10C), including simvastatin and FTI 277.

Discussion

SOC represents the most prevalent malignant tumor of the ovaries, exhibiting high incidence, mortality and prevalence rates (32,33). The lack of typical signs and symptoms in early-stage disease, combined with the aggressive progression of SOC from early to advanced stages within 1 year, results in

>70% of patients being diagnosed at an advanced stage (34). Despite substantial advancements in SOC treatment, the prognosis remains poor due to persistent tumor cell proliferation (35). Therefore, identifying potential biomarkers for early diagnosis and targeted intervention is critical for improving patient outcomes.

In recent years, ferroptosis has emerged as a prominent area of research, particularly in oncology. Defined and named by Dixon *et al* (12) in 2012, ferroptosis is an iron-dependent form of cell death characterized by the accumulation of intracellular ROS, distinguishing it from apoptosis. Dysregulation of intracellular iron levels has been shown to impair macrophages and epithelial cells, and to influence numerous risk factors and pathological processes associated with SOC, including lipid peroxidation, oxidative stress and inflammation (36). The present study investigated the role of ferroptosis-related genes in SOC. Through bioinformatics analysis, the present study further intersected relevant genes from the GSE54388 dataset, WGCNA analysis and the FerrDb database, identifying 22 DEGs. These genes illuminate the complex interplay between ferroptosis and the pathophysiology of SOC. Pathway analyses via GO and KEGG enrichment highlighted significant involvement of ferroptosis-related biological processes, including oxidative stress response, glutathione metabolism and the mTOR signaling pathway, all of which are known to contribute to inflammation and cellular senescence, critical processes in the context of SOC (37,38).

Research indicates that inducing oxidative stress can influence ovarian cancer cell death (39), while the modulation of ferroptosis by inhibition of the mTOR pathway has been shown to regulate tumorigenesis (40). PPI network analysis

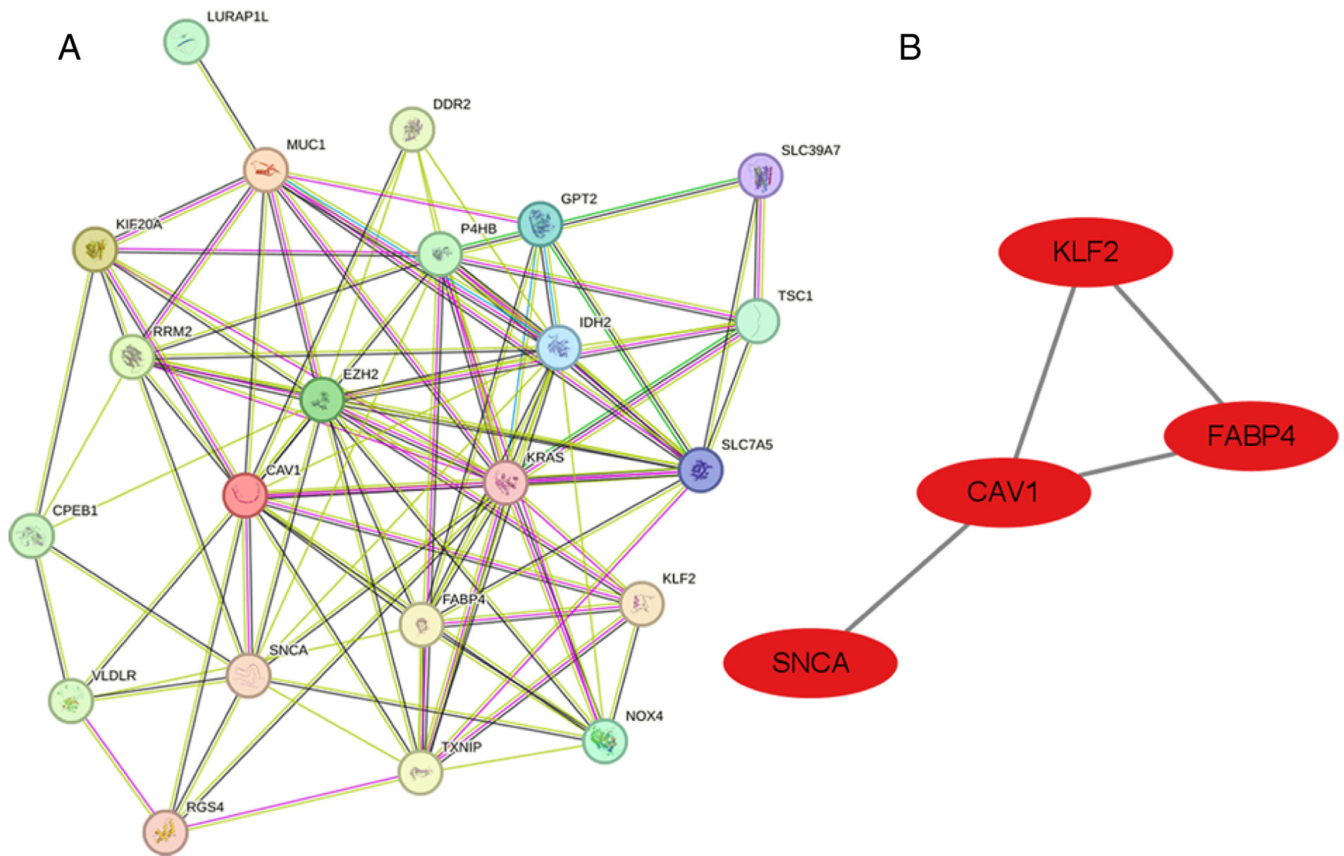


Figure 5. Protein interaction network analysis. (A) STRING network diagram. (B) Key module identified by MCODE in Cytoscape (v3.9.1), with top connectivity hub genes: KLF2, FABP4, CAV1 and SNCA (highlighted in red). CAV1, caveolin 1; SNCA, α -synuclein; KLF2, Kruppel-like factor 2; FABP4, fatty acid binding protein 4.

performed in the present study further elucidated the interconnectivity among the screened differential genes, revealing KLF2, FABP4, CAV1 and SNCA as central nodes. The contemporary approach of integrating multiple datasets alongside clinical sample data to elucidate disease-related genes and their functions has become increasingly commonplace in cancer research (41-43). In the present study, validation of the downregulation of KLF2, FABP4, CAV1 and SNCA in SOC was achieved through data from TCGA database, with ROC analysis indicating robust diagnostic potential for all four genes. Notably, KLF2 exhibited an AUC of 0.990, emphasizing its significant diagnostic value for SOC. RT-qPCR analysis of patient blood samples demonstrated elevated CAV1 expression in the experimental group compared with that in the controls, although this difference was not significant. This finding contrasts with TCGA data indicating CAV1 downregulation in SOC tissues, which may be attributed to several factors, including variations in tissue and blood expression profiles (44,45), sample heterogeneity (46) and a limited sample size (47). Meanwhile, RT-qPCR assessment confirmed significant downregulation of KLF2 in SOC, suggesting its clinical relevance as a biomarker for the disease. Collectively, these findings indicate the potential clinical relevance of the ferroptosis-related gene KLF2 in SOC.

KLF2 is a member of the zinc finger TF family that serves a critical role in embryonic lung development, endothelial cell function, and the maintenance of quiescence in T cells and

monocytes (48). Numerous studies have established a link between KLF2 and various malignancies, with downregulation observed in cancers such as gastric cancer (49), clear cell renal cell carcinoma (50), colorectal cancer (51) and breast cancer (52). In the present study, the downregulation of KLF2 expression in SOC was validated using immunohistochemistry data from tissue sections available in the HPA database. Supporting the findings, Wang *et al* (53) reported marked and specific downregulation of KLF2 in ovarian tumors. A previous study indicated that KLF2 is primarily associated with IL6 in advanced epithelial ovarian cancer, where elevated IL6 levels associate with a poor prognosis (54). The present study findings revealed an association between low KLF2 expression and residual tumor. Specifically, reduced KLF2 levels were more frequently observed in patients with larger residual tumors, suggesting that low KLF2 expression may be indicative of more aggressive tumor biology and a higher likelihood of incomplete surgical resection. The investigation further revealed that low KLF2 expression is associated with unfavorable outcomes in patients with SOC, with its predictive capability approaching that of clinical staging. These results suggest that KLF2 may function as a prognostic biomarker for SOC.

Tumorigenesis and development are often accompanied by notable immune cell infiltration. The TIME is recognized for its pivotal role in cancer progression, tumor growth, angiogenesis, and the overall response to treatment and

Table II. Comparison of key gene expression between experimental group and control group (2^{-ΔΔCq}).

Gene name	Experimental group (n=20)	Control group (n=20)	t	P-value
CAV1	1.458±0.904	1.101±0.444	1.585	0.121
SNCA	1.045±0.554	1.118±0.525	0.425	0.673
KLF2	0.766±0.385	1.144±0.542	2.547	0.015
FABP4	1.202±0.824	1.293±1.044	0.298	0.767

CAV1, caveolin 1; SNCA, α-synuclein; KLF2, Kruppel-like factor 2; FABP4, fatty acid binding protein 4.

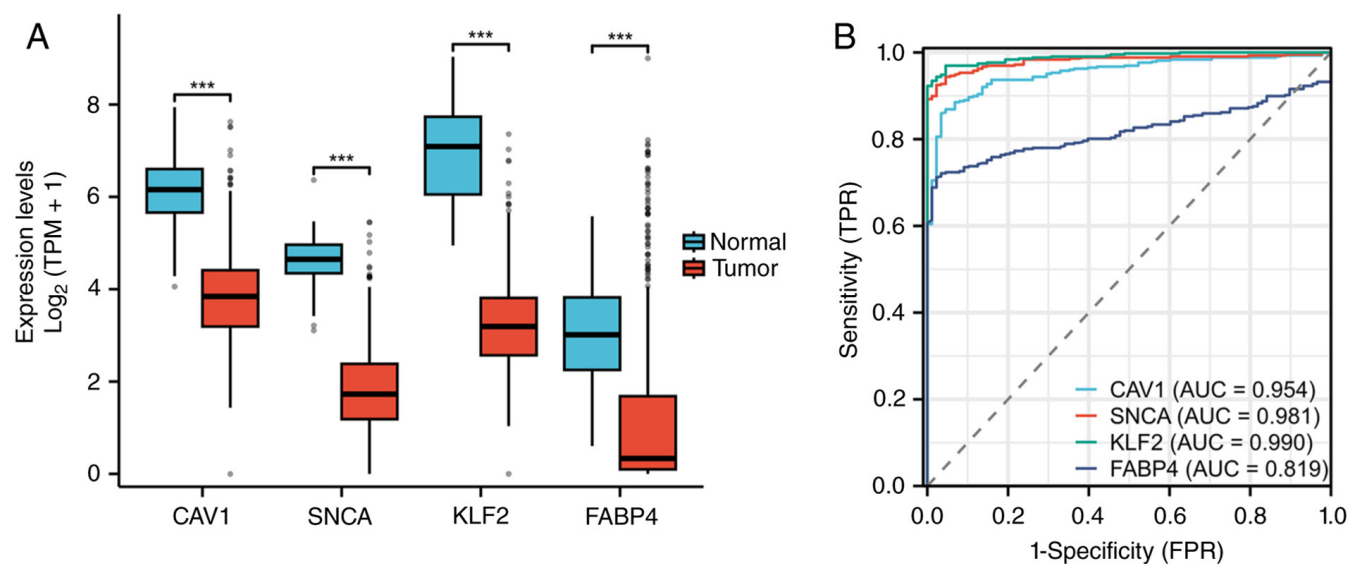


Figure 6. Analysis of key gene expression and its diagnostic value in TCGA database. (A) Boxplots showing expression levels [log₂(TPM+1)] of four hub genes (CAV1, SNCA, KLF2 and FABP4) in normal (blue; n=88 from GTEx) and SOC tumor tissues (red; n=427 from TCGA). ***P<0.001. (B) Receiver operating characteristic curve analysis of CAV1, SNCA, KLF2 and FABP4 expression in SOC and adjacent tissues. The AUC is shown in the graph. TCGA, The Cancer Genome Atlas; SOC, serous ovarian cancer; AUC, area under the curve; CAV1, caveolin 1; SNCA, α-synuclein; FABP4, fatty acid binding protein 4; FPR, false positive rate; KLF2, Kruppel-like factor 2; TPM, transcripts per million; TPR, true positive rate.

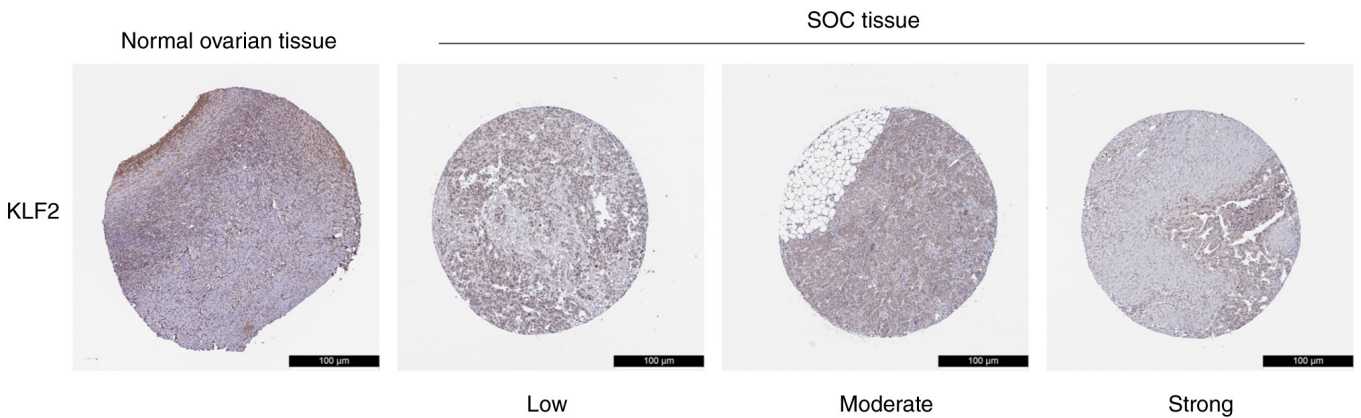


Figure 7. Immunohistochemical validation of KLF2 protein expression using the HPA database. Representative images of KLF2 staining in normal ovarian tissue (left panel) and SOC tissue (right panel). Scale bar, 100 μm. The images used in this study are available from the HPA (v24.proteinatlas.org) at <https://v24.proteinatlas.org/ENSG00000127528-KLF2/tissue>. Image credit: HPA (27). The HPA is licensed under the Creative Commons Attribution-ShareAlike 4.0 International License (28). SOC, serous ovarian cancer; KLF2, Kruppel-like factor 2; HPA, Human Protein Atlas.

prognosis (55,56). A previous study indicated that KLF2 influences the functional differentiation and regulation of immune cells, including monocytes and T lymphocytes (57). The present research explored the relationship between alterations in the ferroptosis-related gene KLF2 and the TIME in SOC. The results demonstrated that KLF2 positively

Table III. Association between KLF2 expression and the clinicopathological features of serous ovarian cancer cases in The Cancer Genome Atlas.

Characteristic	Low expression of KLF2 (n=190)	High expression of KLF2 (n=191)	P-value
Clinical stage			0.230
I and II	16 (4.2)	8 (2.1)	
III	144 (38.1)	152 (40.2)	
IV	28 (7.4)	30 (7.9)	
Tumor status			0.705
Tumor-free	37 (10.9)	35 (10.4)	
With tumor	130 (38.5)	136 (40.2)	
Primary therapy outcome			0.296
PD	12 (3.9)	15 (4.9)	
SD	10 (3.2)	12 (3.9)	
PR	17 (5.5)	26 (8.4)	
CR	117 (37.9)	100 (32.4)	
Ethnicity			0.298
Asian	8 (2.2)	4 (1.1)	
Black or African American	15 (4.1)	10 (2.7)	
White	162 (44.1)	168 (45.8)	
OS event			0.159
Alive	80 (21)	67 (17.6)	
Dead	110 (28.9)	124 (32.5)	
Histological grade			0.709
G1 and G2	24 (6.5)	22 (5.9)	
G3 and G4	160 (43.1)	165 (44.5)	
Venous invasion			0.312
No	24 (22.9)	17 (16.2)	
Yes	31 (29.5)	33 (31.4)	
Lymphatic invasion			0.145
No	28 (18.8)	20 (13.4)	
Yes	46 (30.9)	55 (36.9)	
Age, years			0.437
≤60	108 (28.3)	101 (26.5)	
>60	82 (21.5)	90 (23.6)	
Anatomical neoplasm subdivision			0.063
Bilateral	118 (32.9)	139 (38.7)	
Left	35 (9.7)	21 (5.8)	
Right	25 (7)	21 (5.8)	
Residual tumor			0.042
No	41 (12.2)	27 (8)	
Yes	125 (37.1)	144 (42.7)	
PFI event			0.585
No	49 (12.9)	54 (14.2)	
Yes	141 (37)	137 (36)	

Data are presented as n (%). Patients were stratified into low-KLF2 expression group (n=190) and high-KLF2 expression group (n=191) based on the median expression level. Numbers within each variable row represent patients with available data for that specific variable; totals may differ from the column headers due to missing clinical data. Percentages are calculated as row percentages (proportion of patients with available data for that variable). PD, progressive disease; SD, stable disease; PR, partial response; CR, complete response; OS, overall survival; PFI, progression-free interval; KLF2, Kruppel-like factor 2.

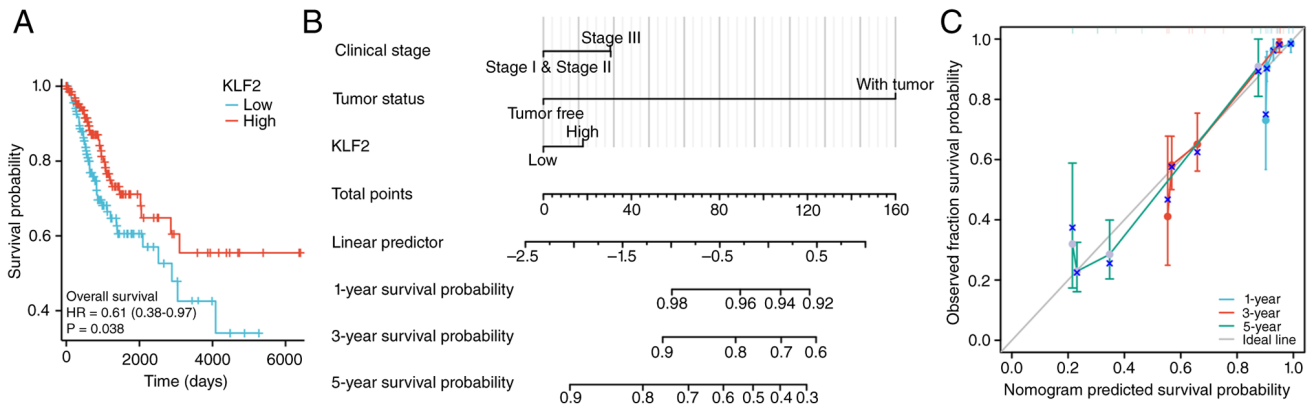


Figure 8. Analysis of the prognostic value of KLF2 in SOC. (A) Kaplan-Meier survival curve of patients with SOC stratified by KLF2 expression level (median cutoff). High expression group (red; n=214) and low expression group (blue; n=213). (B) Nomogram survival prediction chart for predicting the 1-, 3-, and 5-year OS rates in patients with SOC. (C) Calibration curves for the nomogram. The x-axis represents the nomogram-predicted survival probability, and the y-axis represents the actual observed survival probability. The diagonal dashed line indicates perfect prediction. Colored lines represent 1-year (blue), 3-year (red) and 5-year (green) survival, showing good consistency between the nomogram's predictive model and the actual model for 1-, 3-, and 5-year survival probabilities. SOC, serous ovarian cancer; OS, overall survival; KLF2, Kruppel-like factor 2.

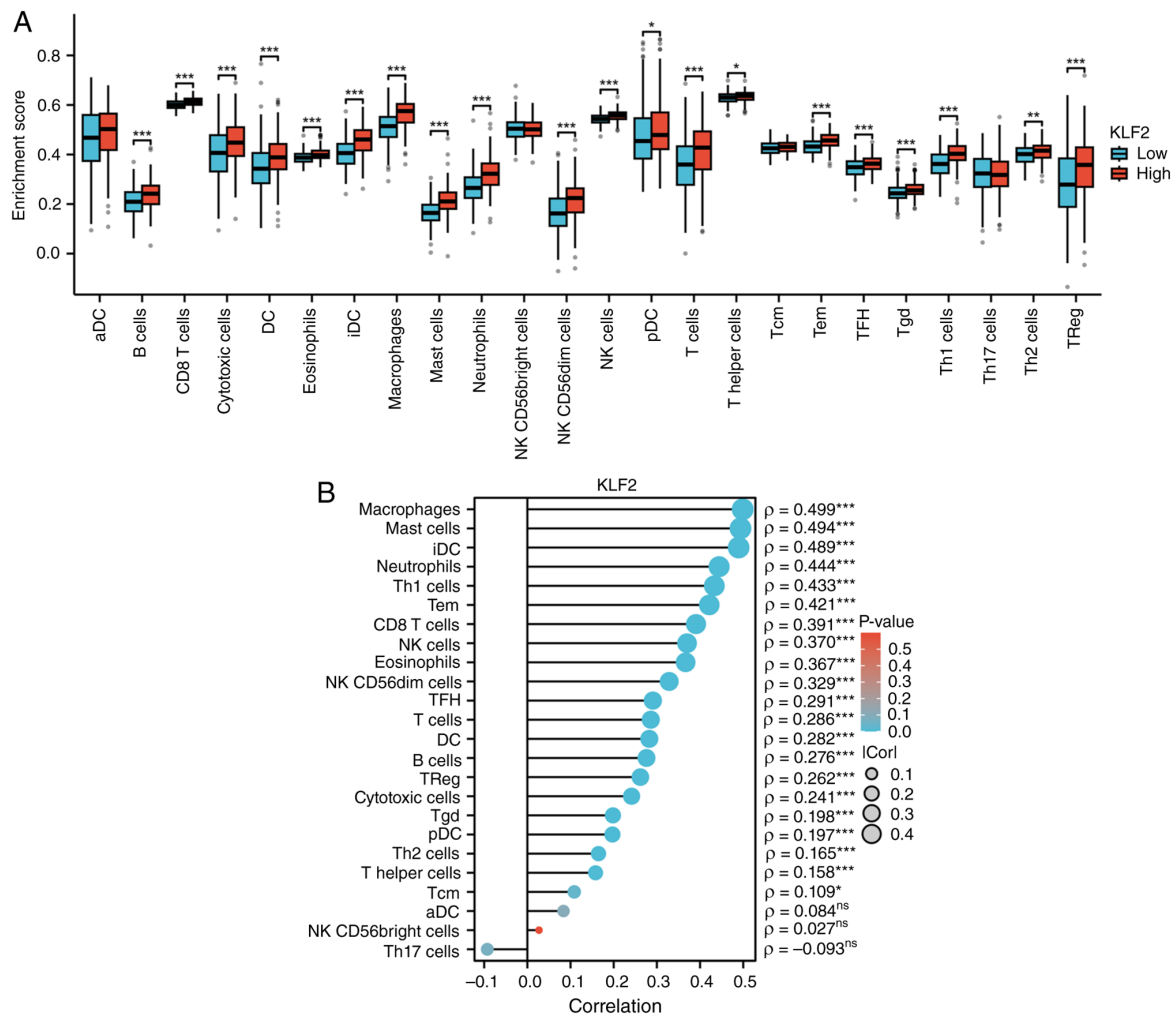


Figure 9. Correlation of KLF2 expression with immune characteristics in serous ovarian cancer. (A) Differential distribution of 24 immune cell types between high and low KLF2 expression groups (based on median expression cutoff). The bar plot shows the relative infiltration scores (single-sample Gene Set Enrichment Analysis-derived) for each immune cell type. Red bars represent the high KLF2 expression group, and blue bars represent the low KLF2 expression group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. (B) Bubble plot showing Spearman correlation coefficients between KLF2 expression levels and immune cell infiltration scores. The x-axis represents the correlation coefficient (ρ), and each bubble corresponds to an immune cell type. Bubble size reflects the absolute correlation magnitude (larger bubbles indicate stronger correlation), and bubble color represents the direction of correlation: Red for positive correlation, blue for negative correlation. The statistical significance is indicated by the color intensity (darker red/blue corresponds to lower P-value). * $P < 0.05$, *** $P < 0.001$. KLF2, Kruppel-like factor 2; aDC, activated DC; DC, dendritic cells; iDC, immature DC; NK, natural killer; pDC, plasmacytoid DC; Tcm, central memory T cells; Tem, effector memory T cells; TFH, follicular helper T cells; Tgd, $\gamma\delta$ T cells; Th, T helper; TReg, regulatory T cells.

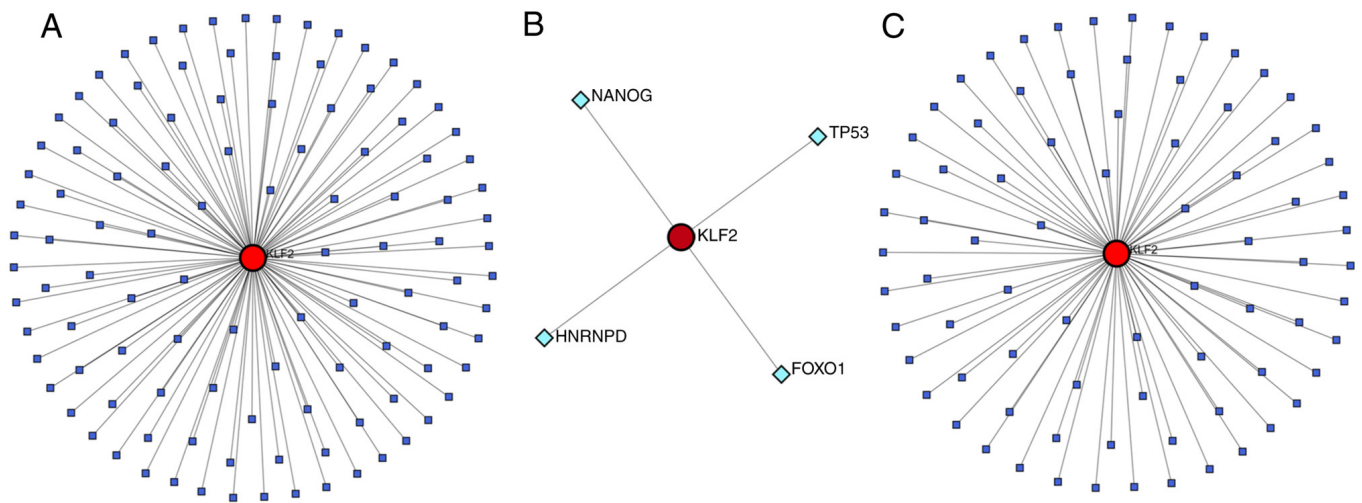


Figure 10. Prediction and estimation of target miRNAs, TFs and compounds. Based on the key gene KLF2, (A) a miRNA-gene interaction network (blue squares represent miRNAs related to KLF2), (B) a TF-gene interaction network (blue squares represent TFs related to KLF2) and (C) a chemical-gene interaction network (blue squares represent compounds related to KLF2) were established. Red circles indicate key genes. TF, transcription factor; miRNA, microRNA; KLF2, Kruppel-like factor 2.

regulates the infiltration of 20 immune cell types, suggesting a potential association with tumor immune evasion in SOC and opening avenues for further mechanistic studies involving KLF2. Additionally, it has been indicated that KLF2 inhibits ferroptosis and mitigates mitochondrial dysfunction in chondrocytes via sirtuin 1/GPX4 signaling, thereby improving osteoarthritis (58). The KEGG enrichment analysis performed in the present study revealed that the 22 ferroptosis-related genes, including KLF2, were markedly enriched in the mTOR signaling pathway and glutathione metabolism, both of which are closely linked to ferroptosis. Although direct evidence connecting KLF2 to glutathione metabolism in SOC is still lacking, these findings support the hypothesis that KLF2 may regulate ferroptosis sensitivity in SOC cells through the mTOR-GPX4 axis or by modulating intracellular glutathione levels. This potential requires validation in future functional studies.

Finally, putative miRNA-key gene, TF-key gene and chemical-key gene networks were constructed in the present study to elucidate the molecular mechanisms and identify potential therapeutic agents targeting KLF2 in SOC. Among the predicted miRNAs, miR-92a-3p and miR-15b-3p are noteworthy as they have been validated to target KLF2 in other types of cancer. Mao *et al* (59) showed that miR-92a-3p can promote gastric cancer progression by targeting KLF2, whereas Zhang *et al* (60) reported that miR-15b-3p suppresses ferroptosis in prostate cancer via KLF2. These findings provide evidence regarding the upstream regulation of KLF2 in SOC. Regarding potential therapeutics, simvastatin has been shown to induce KLF2 expression and exert antitumor effects (52,61,62), and the combination of FTI 277 and GGTI 298 can transcriptionally upregulate KLF2 (63). These observations raise the possibility that pharmacological activation of KLF2 may restore its tumor suppressive function in SOC, although direct evidence is lacking. The network-based predictions of the current study offer testable hypotheses for future experimental validation and drug development. These findings contribute to a theoretical foundation for future

research on SOC treatment and diagnosis. Thus, in SOC, the ferroptosis-related gene KLF2 demonstrates consistent expression stability, as evidenced by both bioinformatics and clinical blood sample validation, while exhibiting high ROC diagnostic efficacy, prognostic relevance and potential relevance in immune modulation, warranting further exploration.

Several limitations of the present study should be acknowledged. First, the bioinformatics analyses were conducted using tumor tissue datasets, whereas RT-qPCR assessment relied on peripheral blood samples. Given that tissue and blood represent biologically distinct compartments, these findings should be interpreted as independent exploratory observations rather than direct validations. Second, the small sample size [comprising only 20 patients with SOC, all of whom were high-grade, the most common and aggressive subtype of SOC (2)] and the single-center design hinder reliable analyses of associations between KLF2 expression and clinical characteristics (such as stage, residual tumor and BRCA gene mutation status), limiting generalizability to low-grade SOC or other subtypes. Third, although bioinformatics generates valuable hypotheses, establishing causal relationships between KLF2 and SOC pathogenesis necessitates functional validation. Future studies should employ paired tissue-blood samples from larger multicenter cohorts, including diverse SOC subtypes, to validate KLF2 expression. Additionally, the use of animal models and mechanistic assays (KLF2 manipulation, ferroptosis induction, rescue experiments) is recommended for functional validation, along with prospective longitudinal studies to assess KLF2 as a circulating diagnostic and prognostic biomarker.

In conclusion, KLF2 is a ferroptosis-associated gene significantly downregulated in SOC and linked to a poor prognosis, indicating its potential as a prognostic biomarker. The expression of KLF2 also correlates with immune infiltration and ferroptosis-related pathways, suggesting its involvement in SOC pathogenesis and its potential as a therapeutic target. However, functional studies are required to establish causal mechanisms.

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Availability of data and materials

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

Authors' contributions

YW, JY, WH and YY were involved in conceiving and designing the study, as well as collecting and acquiring the data. YW, JY and WH wrote, reviewed and edited the manuscript. XLa, YJ and XLi conducted bioinformatics analysis and interpreted the data. SW and XH performed the statistical analysis and participated in interpreting the results. GX and XW supervised the project, conceived and designed the study, and took part in writing and revising the manuscript. All authors were involved in drafting important parts of the manuscript or making critical revisions. GX and XW confirm the authenticity of all the raw data. All authors read and approved the final manuscript, and agreed to take responsibility for all aspects of the work to ensure that any issues related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

The present study received approval from the Ethics Committee of Pidu District People's Hospital [approval no. LWSPZ (2023) no. 09, 2023-4-11]. Patients were recruited based on inclusion and exclusion criteria. Upon identification, the purpose and requirements of the study were explained to each participant, and written informed consent was obtained prior to participation.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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