Discover Oncology

Analysis

Synergistic bioinformatics and sophisticated machine learning unveil ferroptosis-driven regulatory pathways and immunotherapy potential in breast carcinoma

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Abstract

Background The intersection of aberrant iron metabolism and the rapidly advancing field of immunotherapy has emerged as a critical focus in breast cancer (BRCA) therapeutics. Ferroptosis, a distinct form of iron-dependent cell death driven by lipid peroxidation, has garnered increasing attention for its pivotal role in cancer progression.

Methods Utilizing extensive datasets from TCGA and GEO, this research extracted a wealth of biological data, including mRNA splicing indices, genomic aberrations, copy number variations (CNV), tumor mutational burden (TMB), and diverse clinical information. Through precise Lasso regression analysis, this research constructed a prognostic model that elucidates the molecular interactions of FRGs in BRCA. Concurrent co-expression network analyses were performed to explore the dynamic interplay between gene expression patterns and FRGs, revealing potential regulatory mechanisms.

Results This research analysis revealed significant overexpression of FRGs in high-risk BRCA samples, highlighting their prognostic relevance beyond traditional clinical parameters. GSVA identified immune response and cancer-related pathways as predominantly active in high-risk groups, suggesting ferroptosis as a central modulator within the tumor microenvironment. Notably, genes such as ACTL8, VGF, and CPLX2 emerged as markers of tumorigenesis, while IL33 and TP63 were identified as potential key regulators of cancer progression, each exhibiting distinct expression profiles across risk levels. Furthermore, this research incorporated gene correlations, CNV profiles, SNP arrays, and drug susceptibility analyses, contributing to the advancement of precision oncology.

Conclusions The integration of bioinformatics and machine learning in this study underscores a strong correlation between FRG expression patterns and BRCA prognosis, affirming their potential as precise biomarkers for personalized immunotherapy.

Keywords BRCA · Ferroptosis · Immunity · m^6a · Immune checkpoint · Drug prediction · CNV · Drug prediction

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1 Introduction

Breast cancer (BC) remains one of the most prevalent and clinically challenging malignancies worldwide, constituting a leading cause of cancer-related morbidity and mortality among women [1]. In 2020, the WHO reported nearly 2.3 million new diagnoses, making BC the most commonly diagnosed cancer globally. Furthermore, the disease is responsible for over 685,000 deaths annually. The epidemiology of BC shows significant geographic, ethnic, and temporal variation. In high-income nations, while incidence rates are higher, mortality has declined due to advances in early detection, treatment, and patient management [2]. In contrast, in lower-resource regions, BC often presents at more advanced stages, contributing to poorer survival outcomes. The risk factors for BC are complex, involving both modifiable and non-modifiable components. Age, genetic predisposition, and family history are well-established non-modifiable risks [3]. Genetic mutations, particularly in BRCA1 and BRCA2, markedly elevate the lifetime risk of developing BC. Additionally, lifestyle factors—such as sedentary behavior, poor diet, and obesity—have become increasingly linked to rising incidence, especially among postmenopausal women [4]. Historically, axillary lymph node metastasis has been a key prognostic biomarker for BC, but its predictive power is limited [5]. For example, a study showed that approximately 30% of untreated patients without nodal involvement developed metastatic disease within 10 years, while about 50% of patients with nodal involvement were cured with local treatment. Other established prognostic factors include tumor size and grade, though these also have notable limitations [6]. Tumor grading suffers from issues of reproducibility, and many tumors are ambiguously classified as grade 2, reflecting the inherent heterogeneity of the disease. In the era of personalized medicine, these traditional biomarkers are no longer sufficient for optimal management, particularly for early-stage BC [7]. Consequently, there has been growing interest in the identification and validation of molecular biomarkers that can more precisely predict prognosis and treatment response. Recent efforts have focused on multi-parameter, multi-analyte, and multi-gene approaches, offering more nuanced and individualized insights into BC prognosis and therapy.

Ferroptosis, a distinct form of iron-dependent cell death driven by lipid peroxidation, has garnered increasing attention for its pivotal role in cancer progression. Extensive research has underscored the significance of ferroptotic regulatory genes in shaping the oncogenic microenvironment, highlighting their potential as therapeutic targets [8]. The induction of ferroptosis has emerged as a powerful strategy to enhance chemotherapeutic efficacy across various cancer types, thereby expanding the repertoire of treatment options. Mechanistically, ferroptosis is initiated by a depletion of glutathione, impaired activity of glutathione peroxidase 4 (GPX4), and the disruption of metabolic balance governed by GPX4 [9]. These perturbations lead to the accumulation of reactive oxygen species (ROS) derived from iron-rich lipids, culminating in cell death. Phenotypically, ferroptosis is marked by notable changes in mitochondrial morphology, including reduced organelle size, cristae disintegration, and membrane convolutions, while preserving nuclear integrity [10]. This regulated cell death is accompanied by increased iron deposition, elevated lipid peroxidation, and alterations in the gene networks responsible for iron homeostasis. The strategic exploitation of ferroptotic pathways represents a breakthrough in overcoming chemoresistance, ushering in a new era of cancer therapy [11]. Ferroptosis is orchestrated by a network of genes that not only regulate cell fate but also intersect with inflammatory response pathways. This genetic synergy opens new avenues for therapeutic intervention, potentially revolutionizing cancer treatment [12]. Moreover, aberrant iron metabolism is increasingly recognized as a key driver of oncogenesis. Cancer cells often exhibit a phenomenon known as "iron addiction," a dependency on iron that constitutes a major vulnerability in cancer biology [13]. The targeting of ferroptosis, therefore, represents a promising strategy to overcome the robust drug resistance mechanisms typical of conventional chemotherapy. Recent advances have highlighted innovative approaches to induce ferroptosis, including the use of an Fe^{2+}-anchored metal–organic framework designed to selectively deliver Fe^{2+} ions into cancer cells [14]. This technique triggers the Fenton reaction, generating a surge of ROS that drives cells toward ferroptotic death, as demonstrated in BRCA-positive cells. However, cancer cells, particularly BRCA-positive ones, exhibit adaptive mechanisms to counteract ferroptosis [15]. One such mechanism involves prominin2, a pentaspanin membrane protein that modulates lipid dynamics within cellular membranes, reducing ferroptotic sensitivity. This complex interplay, involving prominin2, multivesicular body-derived exosomes, and ferritin, underscores the intricacy of cancer cell survival strategies and highlights the need for novel approaches to targeting ferroptotic pathways for more effective cancer therapies [16]. Therefore, it is of great significance to explore the genes related to ferroptosis associated with BRCA.

The interplay between BRCA and its immune microenvironment (TME) is a pivotal determinant of tumor progression, metastasis, and therapeutic response. The TME, composed of immune cells, stromal components, and

extracellular matrix proteins, plays a critical role in shaping tumor biology, modulating both local immune surveillance and systemic immune responses [17]. In BRCA, the TME is marked by a complex and dynamic immune landscape, where immune cells can either promote or inhibit tumor progression, depending on the disease context and stage. Tumor-associated immune cells, including macrophages, T lymphocytes, dendritic cells, and neutrophils, contribute to the establishment of an immunosuppressive environment that facilitates tumor growth and metastasis [18]. For instance, the accumulation of regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) within the TME is closely linked to poor prognosis in BRCA patients. These cells enable immune evasion by suppressing the activity of cytotoxic T cells and natural killer (NK) cells, thus allowing tumor cells to escape immune surveillance [19]. In contrast, a robust presence of activated CD8 + T cells and NK cells correlates with improved clinical outcomes, highlighting the importance of immune cell infiltration and functional status in determining tumor behavior [20]. Through high-throughput data analysis, empowered by bioinformatic tools, this research have conducted a comprehensive exploration of the gene networks across various disease models, providing valuable insights into the molecular mechanisms underlying BRCA [21]]. The BRCA Initiative, with its vast repository of high-throughput transcriptomic data and detailed clinical annotations, offers a powerful platform to investigate altered transcriptional landscapes and the associated molecular pathways in BRCA [22]. The findings emerging from this bioinformatics-driven research shed light on the pathophysiology of BRCA from multiple angles, offering new perspectives on its molecular underpinnings. These discoveries are visually represented in Fig. 1, which encapsulates the integration of bioinformatics and cancer biology, presenting a cohesive overview of this investigative journey.

2 Materials and methods

2.1 Data acquisition and ferroptosis-related genes

Gene expression profiles and associated clinical data for BRCA were sourced from The Cancer Genome Atlas (TCGA), encompassing 1,109 BRCA cases and 113 normal samples. Additionally, mRNA expression data were retrieved from the Gene Expression Omnibus (GEO), specifically from Series GSE45255 on platform GPL96-57554, which provided expression profiles for 139 BRCA cases. FRGs were identified and extracted from the FerrDb [23] database yielding a comprehensive list of 382 FRGs. Although a notable discrepancy exists between the number of BRCA and normal samples, this approach is consistent with methodologies employed in previous studies. This research primary focus was on analyzing the BRCA cohort, thereby mitigating the potential impact of this sample size imbalance. The adherence to established research practices ensures the reliability and robustness of this research's findings.

2.2 Delineation of differentially expressed genes (DEGs) associated with ferroptosis and analysis of mutation rates

To ensure the accuracy of the mRNA data, transcriptional datasets were processed and organized through Perl scripting, followed by the mapping of identifiers to their corresponding gene names. A comparative analysis between BRCA and normal sample groups revealed significant alterations in the expression of FRGs. Genes with a false discovery rate (FDR) below 0.05 and an absolute log2 fold change (|log2FC|) of 1 or greater were classified as DEGs. The relevance of these DEGs in the context of ferroptosis was further investigated. The mutation frequencies of these DEGs were evaluated using the cBioPortal platform. Correlations between DEG expression in this research's prognostic model and CNVs were assessed through Spearman's correlation analysis ($P < 0.05$), with results visualized using the Corrplot package in R [24]. Furthermore, the association between DEG expression in the prognostic model and drug sensitivity was evaluated using the Pearson method ($P < 0.05$), with data sourced from the CellMiner database. The drug sensitivity of these FRGs was also analyzed using the OncoPredict package.

2.3 Tumor stratification based on DEGs

To categorize tumors based on the identified DEGs, this research performed cluster analysis using the Limma and ConsensusClusterPlus packages. This approach led to the identification of two distinct clusters, designated as Cluster 1 and Cluster 2, based on prognosis-related FRGs. To investigate the relationship between these FRGs and patient survival, this research utilized the Survminer package. This analysis provided insights into the survival patterns associated with

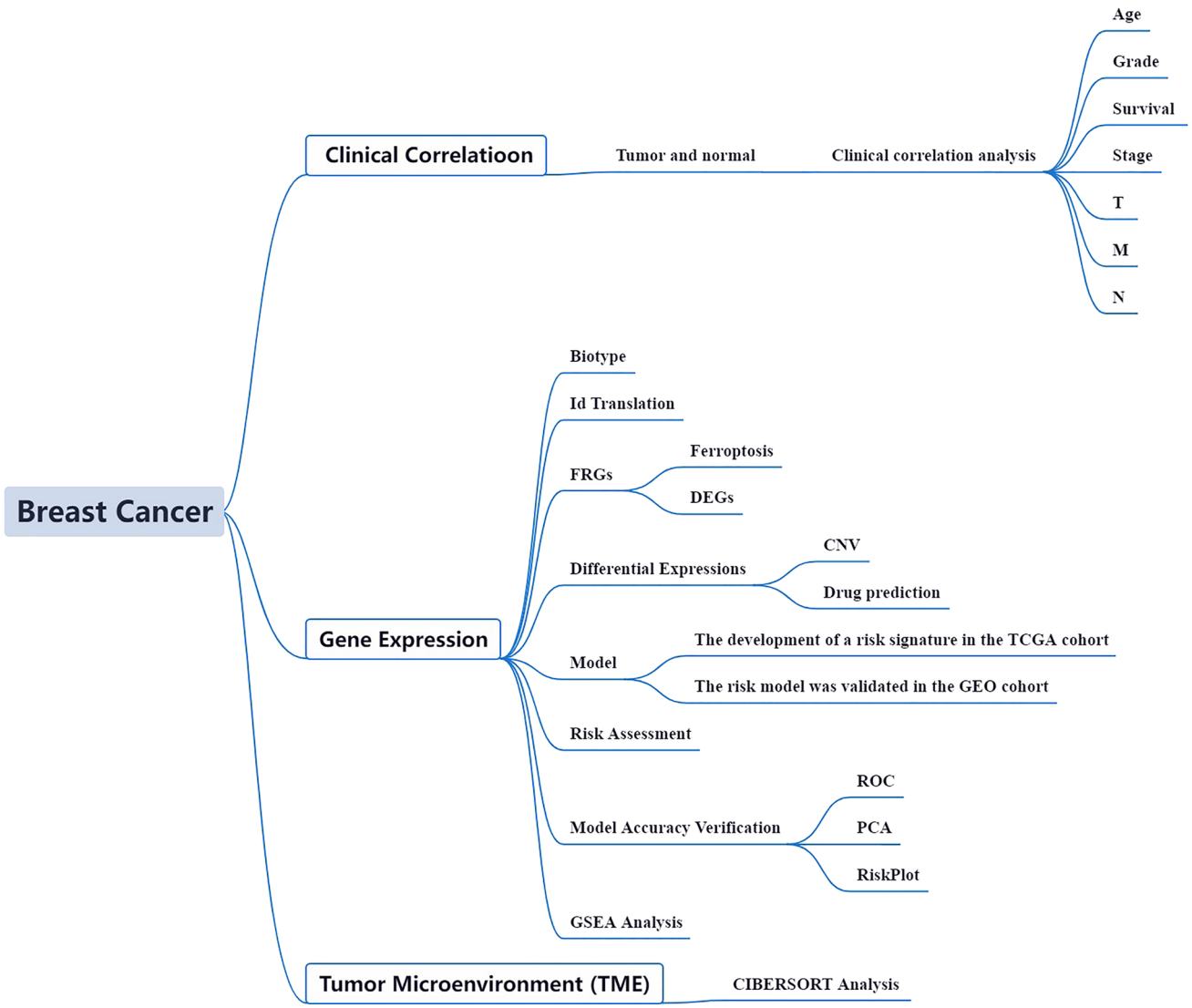

Fig. 1 Framework. The data of BRCA patients were obtained from TCGA and GEO databases, and then the FRGs were matched to carry out difference analysis and risk model construction, respectively. TCGA data set was used as the main body and GEO data was used to verify the model with good grouping, and FRG related to the prognosis of BRCA patients were obtained. Then, GO, KEGG and GSEA analyses were performed with multiple databases to obtain the functions related to FRG. Last, the immune cells and function were analyzed

FRGs and their prognostic potential in predicting patient outcomes. Additionally, the Limma package was employed to assess gene expression variations across different tumor subtypes and tissue types. This analysis facilitated the identification of genes exhibiting significant expression changes, thereby uncovering the molecular heterogeneity present within distinct tumor subtypes and tissue environments.

2.4 Development of a prognostic signature for FRGs

To develop a robust and accurate consensus signature, this research integrated 22 machine learning algorithms and 101 algorithmic combinations, including Lasso, Stepwise Generalized Linear Models (StepGLM, both directions), Support Vector Machine (SVM), Ridge, and Elastic Net (Enet) with varying alpha values (0.1 to 0.9). Additional methods incorporated into the analysis were Partial Least Squares Regression Generalized Linear Model (plsRglm), Random Forest (RF), Generalized Boosted Models (GBM), Linear Discriminant Analysis (LDA), XGBoost, and Naive Bayes. The performance and stability of all models were evaluated using two independent validation datasets, TCGA and GEO. This research employed the glmnet package to perform Lasso regression, optimized through rigorous cross-validation.

After identifying the most promising machine learning methods, this research proceeded to develop a prognostic signature for FRGs. This was achieved using the glmnet and survival R packages, employing Lasso-penalized Cox regression and Univariate Cox regression analyses. The selection parameters were set as nfolds = 10 and maxit = 1000. The risk score for each BRCA patient was calculated using the formula: Σ(coefficient of each DEG × expression level of the corresponding DEG). This score allowed for stratification of patients into low-risk and high-risk groups. Lasso regression analysis was performed to define the low- and high-risk cohorts, and the results were visualized through informative plots. Confidence intervals and hazard ratios were computed, followed by the construction of a forest plot using the pheatmap package. Survival curves were plotted to compare outcomes between the two risk groups. To assess the prognostic accuracy of the model, a ROC curve was generated with the timeROC package, evaluating its capacity to predict survival in BRCA patients. The risk score's performance was further analyzed through correlation with survival status and risk categories associated with FRGs. An independent prognostic analysis was conducted to validate the model's robustness across various clinical factors. This research explored the relationship between clinical characteristics and the risk prediction model, as well as the interaction between FRGs and patient cohort stratifications. Additionally, PCA and t-SNE were performed using the Rtsne and ggplot2 packages to evaluate the model's ability to accurately segregate patients into distinct risk groups. By integrating these predictive factors, this research developed a comprehensive framework to forecast 1-, 3-, and 5-year OS rates for BRCA patients, thus enhancing the clinical applicability and precision of the prognostic model.

2.5 Functional enrichment analysis and evaluation of immune activity across subgroups

To investigate the biological functions and pathways associated with differentially expressed FRGs, this research performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses. Using R, this research examined the biological processes (BP), molecular functions (MF), and cellular components (CC) impacted by these differentially expressed FRGs. Gene Set Enrichment Analysis (GSEA) was employed to identify alterations in functional pathways across different sample groups. The resulting enrichment scores and visualizations facilitated a comprehensive assessment of dynamic biological activities and pathway alterations within distinct risk subgroups. Based on the analysis, samples were categorized into 'High' (H) or 'Low' (L) risk groups. To evaluate immune cell enrichment and immune activity within these subgroups, this research applied single-sample Gene Set Enrichment Analysis (ssGSEA). Additionally, this research explored the interactions between FRGs, immune checkpoint molecules, and mRNA chemical modifications, such as N6-methyladenosine (m6A), N1-methyladenosine (m1A), 7-methylguanosine (M7G), and 5-methylcytosine (m5C). This analysis also included the identification of regulators associated with these epitranscriptomic modifications, thereby deepening this research's understanding of their relationship with immune activities.

2.6 Establishing causality via Mendelian randomization

In this research's endeavor to ascertain the non-confounded relationship between genetic predispositions and BRCA incidence, a Mendelian randomization study was conducted, leveraging the TwoSampleMR package within R. This analysis aimed to explore the potential causal linkage between FRGs gene expression—designated as the exposure variable—and BRCA, identified as the outcome of interest. (1) IV Selection: This research pinpointed FRGs gene expressions closely linked to the exposure, employing a stringent significance cutoff of $P < 5 \times 10^{-8}$ to ensure the relevance of the chosen genetic instruments. (2) Ensuring Independence of IVs: To ascertain the independence of SNPs serving as risk factors, this research utilized the PLINK clustering methodology to examine LD. SNPs were screened for LD, with those exhibiting an LD coefficient (r^2) greater than 0.001 or a physical proximity of under 10,000 kilobases being excluded. This step was crucial to uphold the independence of SNPs and mitigate the risk of pleiotropy that could confound causal inferences. (3) Statistical Robustness of IVs: The integrity of each instrumental variable was scrutinized through the calculation of the F-statistic ($F = \beta^2/SE^2$), where β represents the effect size of the allele, and SE is the standard error. Instrumental variables demonstrating an F-value less than 10 were disregarded to reduce the likelihood of bias introduced by latent confounders.

3 Results

3.1 Profiling of differentially expressed FRGs

In this research's analysis, this research utilized the limma and pheatmap packages in R to scrutinize the gene expression differences, applying a FDR below 0.05 and an absolute log2 fold change (|log2FC|) of 1 or greater as selection criteria. This analytical cycle facilitated the construction of a heatmap. This research identified 208 DEGs associated with ferroptosis in BRCA specimens compared to normal tissue: 107 genes were upregulated, while 101 genes were downregulated (Fig. 2a). Further investigation through PPI analysis revealed key central hub genes, including JUN, UBC, RELA, MAPK1, MAPK3, and SRC, highlighting their potential as critical prognostic markers for BRCA (Fig. 2b). Additionally, this research constructed a comprehensive correlation matrix for all FRGs, offering a visual representation of their complex interactions (Fig. 2c).

Mutation analysis revealed a predominance of truncating and missense mutations within these genes. Notably, PIK3CA emerged as a gene of particular interest, exhibiting a remarkable mutation frequency of 38%, underscoring its potential role in the pathogenesis of BRCA (Fig. 2d). This comprehensive examination of differential gene expression, protein interactions, and mutational patterns provides vital insights into the molecular landscape of BRCA, particularly in the context of ferroptosis-related processes.

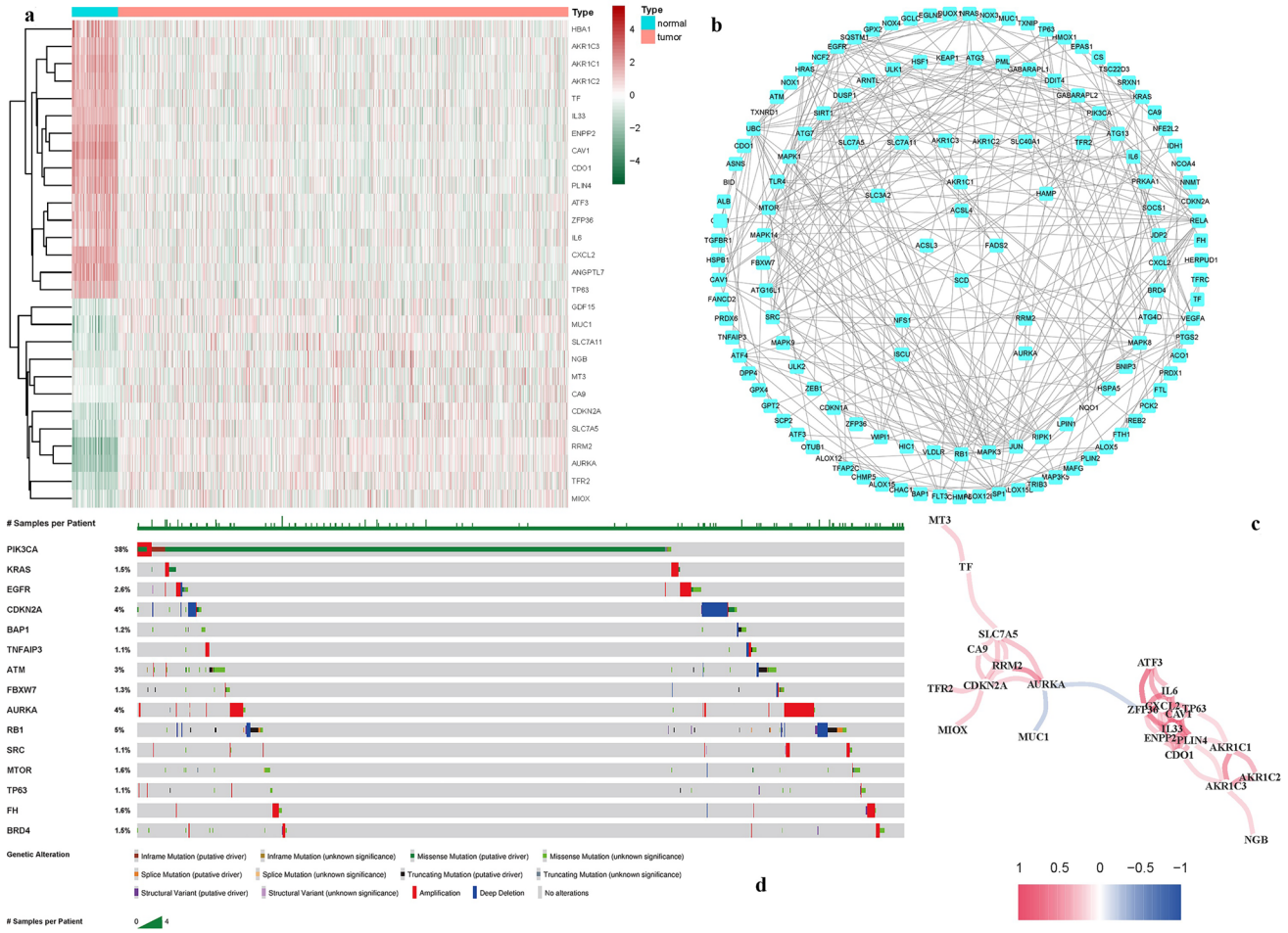

Fig. 2 FRGs' expressions and interactions. **a** Heatmap (green: low expression level; red: high expression level) of the genes participating in autophagy between the normal (N, brilliant blue) and the tumor tissues (T, red). P values were showed as:*P < 0.05; **P < 0.01; ***P < 0.001. **b** PPI network showing the interactions of the FRGs (interaction score = 0.7). **c** The correlation network of the genes participating in autophagy (red line: positive correlation; blue line: negative correlation. The depth of the colors reflects the strength of the relevance). **d** Mutations (The most common types of mutations observed were truncating and missense mutations, with PIK3CA exhibiting the highest mutation rate at 38%)

3.2 Interplay between ferroptosis regulatory genes and clinicopathological/molecular characteristics in breast cancer

This research's predictive analysis focused on drug response highlighted genes with significant expression variances, suggesting their potential impact on therapeutic efficacy. Notably, an in-depth correlation analysis between the expression of DEGs in this research's prognostic model and CNVs revealed a subset of genes influenced by CNVs. This finding underscores a link between genetic alterations in BRCA and key gene dysregulation, indicating potential avenues for targeted therapy.

3.3 Stratification of breast cancer patients based on oncological profiles

Utilizing advanced clustering techniques, this research categorized 1109 BRCA patients from TCGA database into two distinct clusters (Fig. 3a). This classification was achieved through the employment of limma and pheatmap packages, aligning DEG expression data with clinical information and culminating in the creation of a heatmap. Notably, Cluster 1 was characterized by a higher survival rate (Fig. 3b), suggesting its utility as a significant prognostic marker for individuals with BRCA (Fig. 3c). This stratification offers a refined understanding of the oncological heterogeneity within BRCA, potentially guiding more tailored and effective treatment strategies. We incorporated 101 machine learning algorithms

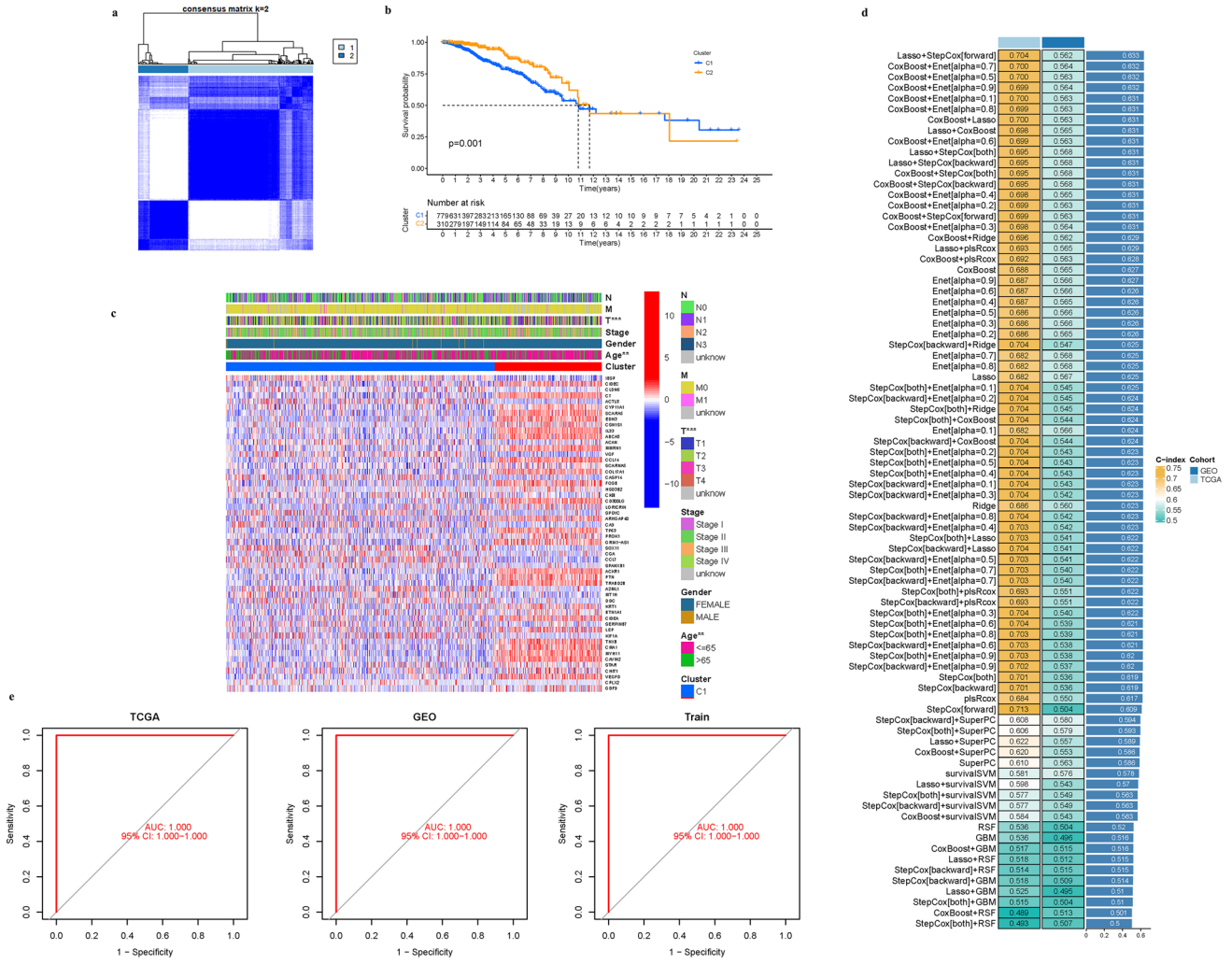

Fig. 3 **a** 1109 BRCA patients were grouped into two clusters according to the consensus clustering matrix (k = 2). **b** Heatmap. Heatmap and the clinicopathologic characters of the two clusters classified by these DEGs (T, N, and Stage are the degree of tumor differentiation. **c** Kaplan–Meier OS curves for the two clusters

and 89 algorithm combinations. It can be seen from the results that LASSO regression has the highest accuracy in this analysis. In addition, TCGA, GEO and trian datasets were used to validate the machine learning results. This shows that the results are stable and credible (Fig. 3d, e).

3.4 Establishment of a prognostic gene framework in the TCGA breast cancer cohort

By integrating both univariate and multivariate Cox regression analyses, this research identified six FRGs—ACTL8, IL33, VGF, COL17A1, TP63, and CPLX2—as pivotal prognostic biomarkers for BRCA (Fig. 4a). These genes were subsequently employed to construct a novel gene signature (Fig. 4b, c), which exhibited a robust association with patient prognosis. Specifically, the risk score derived from this signature displayed a striking inverse correlation with patient survival, such that higher risk scores were consistently associated with poorer survival outcomes (Fig. 4d). Importantly, patients characterized by high-risk FRG profiles demonstrated significantly reduced survival rates, further validating the prognostic potential of this gene signature (Fig. 4e). To assess the predictive capability of this research's model, this research evaluated its accuracy in forecasting 1-, 3-, and 5-year overall survival rates for BRCA patients, with the results illustrating its strong prognostic power (Fig. 4f). Moreover, this research employed sophisticated dimensionality reduction techniques, including PCA and t-SNE, to effectively stratify BRCA patients into two distinct risk groups based on their gene signature profiles (Fig. 4g, h). This clear bifurcation not only demonstrates the model's capacity to delineate prognostic subgroups, but also highlights its potential to facilitate more personalized therapeutic strategies, tailored to the individual risk profiles of patients. Further survival analysis of BRCA patients revealed significant differences in Disease-Specific Survival (DSS), Progression-Free Interval (PFI), and Overall Survival (OS) between the identified high- and low-risk groups, underscoring the clinical relevance of the FRGs in predicting disease outcomes (Fig. 4i). These findings collectively suggest that the FRG signature offers a powerful tool for prognostication in BRCA, with the potential to guide more effective, individualized treatment regimens.

3.5 External validation of the prognostic risk signature in an independent BRCA cohort

To externally validate the prognostic risk signature derived from this research's study, this research engaged with a cohort from the GEO, encompassing 139 BRCA patients. Consistent with this research's observations in the TCGA dataset, a significant portion of the identified FRGs exhibited an inverse correlation with the risk profile (Fig. 5a). High-risk FRG signatures were closely associated with poorer survival outcomes (Fig. 5b). The efficacy of this research's risk signature in prognosticating survival was further confirmed over 1-, 3-, and 5-year periods within this independent cohort (Fig. 5c–e). Utilizing the limma and pheatmap packages, this research aligned the risk gene expression data with clinical parameters and iterated through the process to construct a heatmap.

Additionally, this research developed a comprehensive nomogram that integrated clinicopathological factors with the FRG-based risk assessment. This nomogram demonstrated notable accuracy and reliability, underscoring its potential as a valuable tool in guiding therapeutic decision-making for BRCA patients (Fig. 6a, b). The external validation in the GEO cohort not only reinforces the robustness of this research's prognostic model but also highlights its applicability across diverse clinical settings, enhancing its potential for clinical translation in the management of breast cancer. In addition, there was little difference in OS between BRCA patients associated with FRGs after 1 and 3 years. A greater reduction in OS was observed after 5 years (Fig. 6c). Among the clinically relevant factors, Risk score was the most relevant. Gender had the lowest correlation, whereas other factors had similar correlations (Fig. 6d).

3.6 Pathway and functional enrichment analysis

Through GO exploration, this research discerned pivotal genes intrinsically linked with diverse biological processes, molecular machinations, and cellular milieus. In tandem, the KEGG exploration unveiled the engagement of hyper-expressed genes within salient signal conduits, notably chemical carcinogenesis, reactive oxygen species dynamics, and autophagy circuits (Fig. 7a, b).

3.7 Insights from GSEA

GSEA's lens brought to the fore that the lion's share of this research's FRG prognostic imprint governs both immune and tumorigenic trajectories, spanning focal adhesion to the JAK-STAT signaling spectrum and beyond. A tabulation

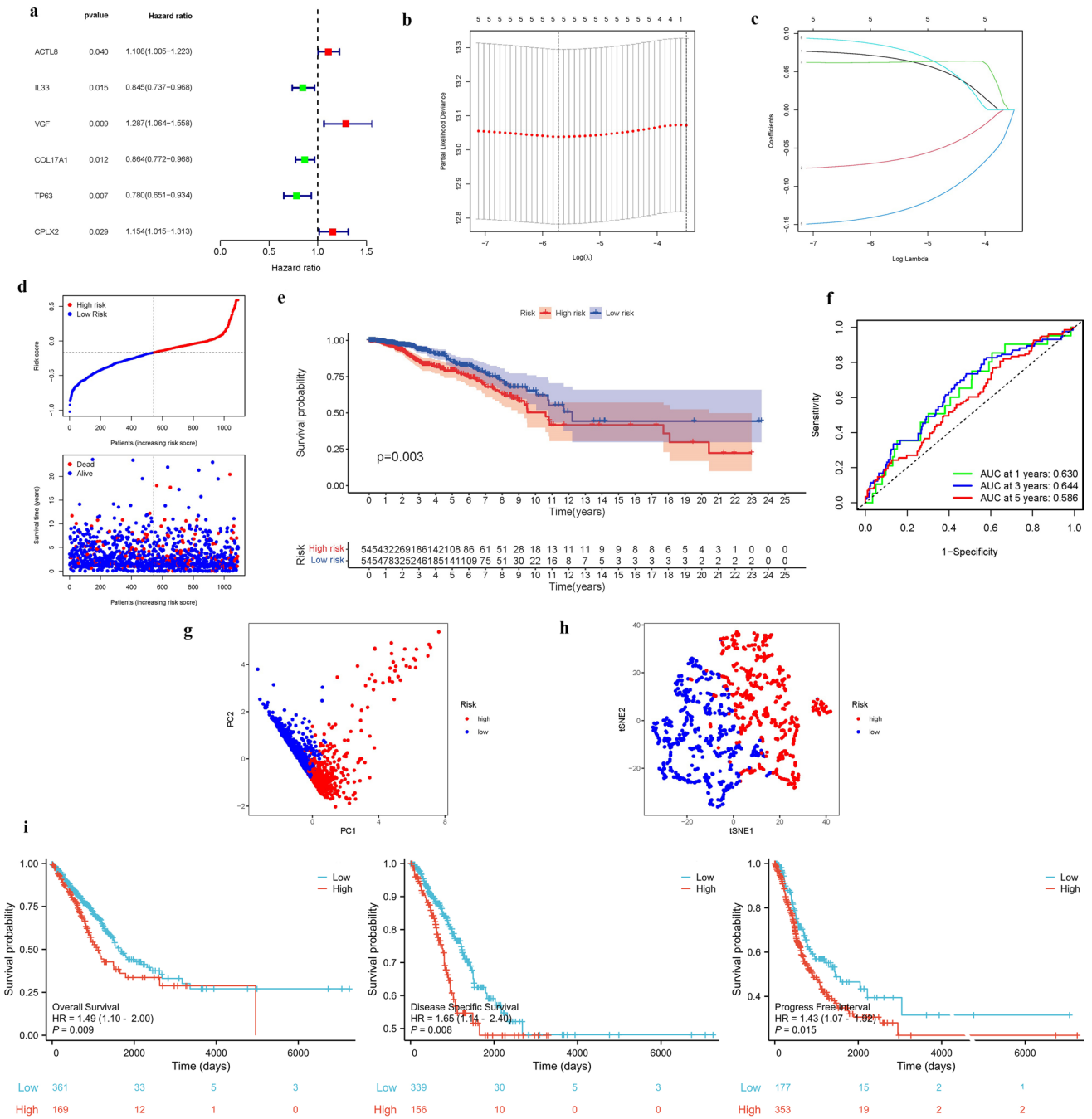

Fig. 4 **a** A Univariate Cox regression analysis of OS for each FRGs, and 6 genes with P < 0.01. **b** Lasso regression of the 6 OS-related genes. **c** Cross-validation for tuning the parameter selection in the Lasso regression. **d** The survival status for each patient (low-risk population: on the left side of the dotted line; high-risk population: on the right side of the dotted line). **e** Kaplan–Meier curves for the OS of patients in the high- and low-risk groups. **f** The AUC of the prediction of 1, 2, 3-year survival rate of BRCA. **g** PCA plot for BRCAs based on the risk score. **h** t-SNE plot for BRCAs based on the risk score. **i** Survival analysis of BRCA

of the top six enriched functionalities or pathways for each cluster can be perused in Fig. 8. Within this tapestry, the "JAK-STAT signaling" paradigm emerged as supremely enriched, with certain genes manifesting positive correlations with either high (H) or low (L) expressions. Such intricate enrichments furnish invaluable perspectives on potential modulatory schemes entangled in the evolution of BRCA (Table 1).

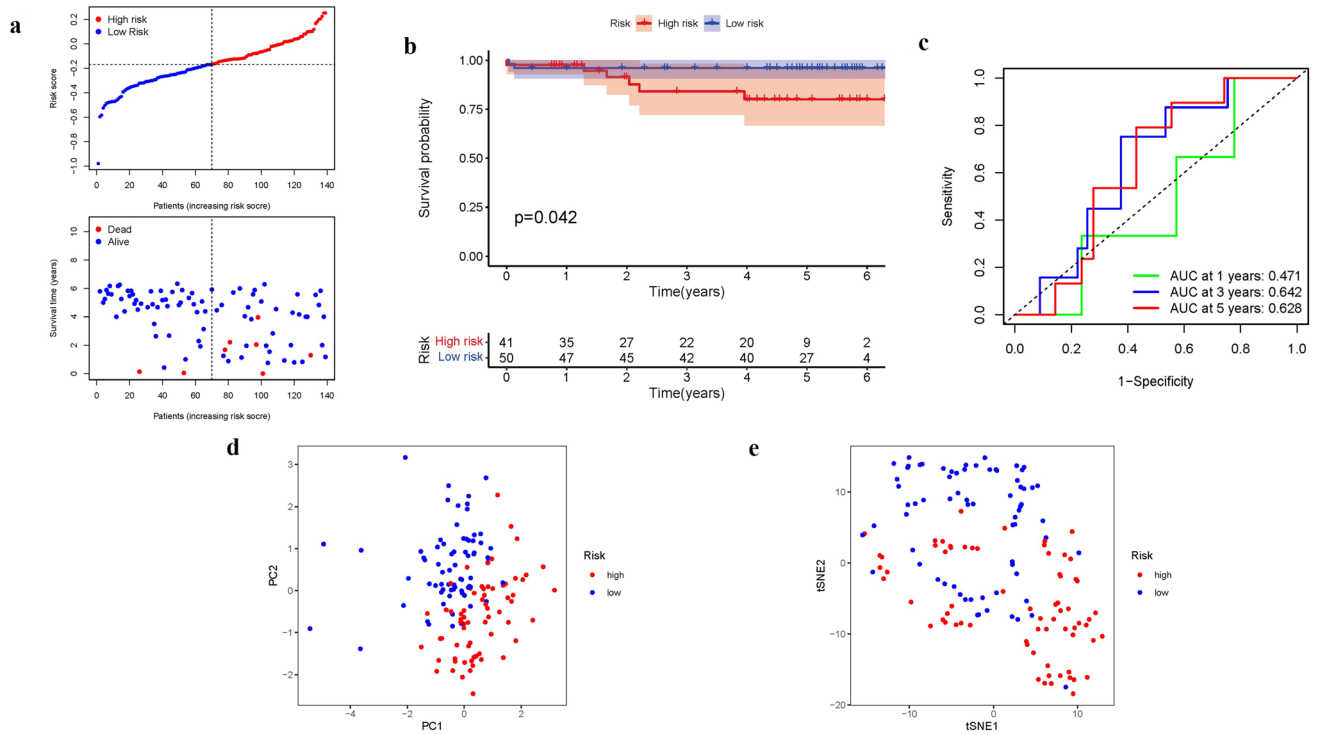

Fig. 5 In the GEO cohort, the risk model was verified. **a** Survival status. **b** Kaplan–Meier curve. **c** The AUC of the survival rate. **d** PCA plot. **e** t-SNE plot

3.8 Comparative analysis of immune activity and dissecting the interplay between FRGs, immunological checkpoints, and m6A epitranscriptomics

Utilizing the single-sample gene set enrichment analysis (ssGSEA) framework, this research unearthed discernable disparities in immune cell penetration and functionalities between the high-risk and low-risk BRCA cohorts (Fig. 9a, b). Notably, the high-risk stratification was characterized by an augmented immune cell incursion, hinting at profound ramifications for the immunological schema in BRCA pathogenesis (Fig. 9c, d). In this research's exploration, a subset of FRGs emerged as differentially expressed, thereby underlining their intricate liaison with immunological checkpoints and the realm of mRNA epitranscriptomic alterations, prominently m6A (Fig. 9e). This differential articulation of certain m6A-modified genes propounds their plausible roles either as tumoral antagonists or oncogenic instigators within the BRCA milieu (Fig. 9f).

3.9 Gene regulatory networks and drug sensitivity analysis

To elucidate the underlying mechanisms of FRGs, this research established an extensive gene regulatory network (Fig. 10a). Within this network, IL1RL1, CPLX1, CPLX3, CPLX4 demonstrated robust interactions with FRGs, known for its roles in inflammation and immunity. The intricate relationships within the gene regulatory network. Furthermore, this research's investigation into the relationship between DEG expression and drug sensitivity revealed genes with strong correlations to drug responses, paving the way for future pharmacological research and development (Fig. 10b). Consequently, this research hypothesize that FRGs potentially contributes to the pathogenesis of BRCA by modulating IL1RL1, CPLX1, CPLX3, CPLX4, and associated downstream genes involved in immune and inflammatory pathways. Furthermore, Procarnazine, Olaparib, Lsotretinoin, Dabrafenib, Simvastatin emerges as a promising therapeutic candidate. These findings offer expansive avenues for future research endeavors in this domain.

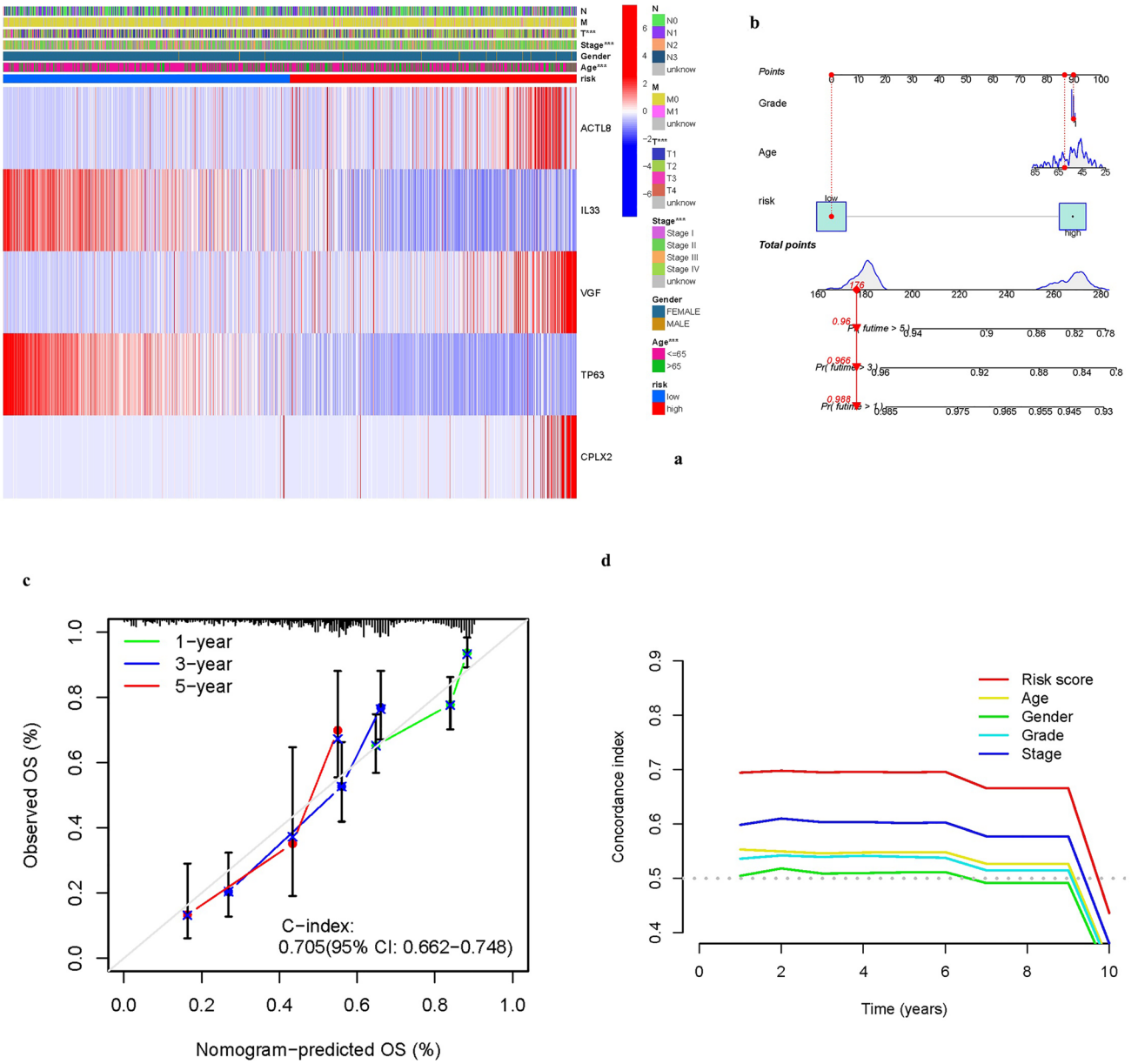

Fig. 6 **a** Heatmap highlighting the relationships between clinicopathologic characteristics and risk categories (green: low expression; red: high expression) illustrating the relationships between clinicopathologic characteristics and risk groups (*P < 0.05; **P < 0.01; ***P < 0.001). **b** Nomogram and assessment of the risk model

3.10 Analysis of immune infiltration and Mendelian randomization analysis

To further solidify this research's understanding of these immune cell dynamics, this research engaged the CIBERSORT platform to validate the incursion patterns of these sentinel immune cells. Positive associations with FRGs were observed for B cells memory, Macrophages M0, Macrophages M2, NK cells resting, T cells follicular helper, T cells regulatory (Tregs). Conversely, negative correlations with FRGs were noted for T cells gamma delta, T cells CD8, T cells CD4 memory resting, B cells naive, Dendritic cells resting, Mast cells resting, Monocytes, Plasma cells. In addition, there was no significant difference between CPLX2 and these immune cells in these FRGs. However, the other five FRGs were all significantly associated with the vast majority of immune cells (Fig. 11). In examining the direct linkage between the FRGs (ACTL8, IL33, TP63, CPLX2) and BRCA incidence, a forest plot was utilized for visual illustration, revealing a general symmetry in the data. Through sensitivity analysis employing the "leave-one-out" technique, it

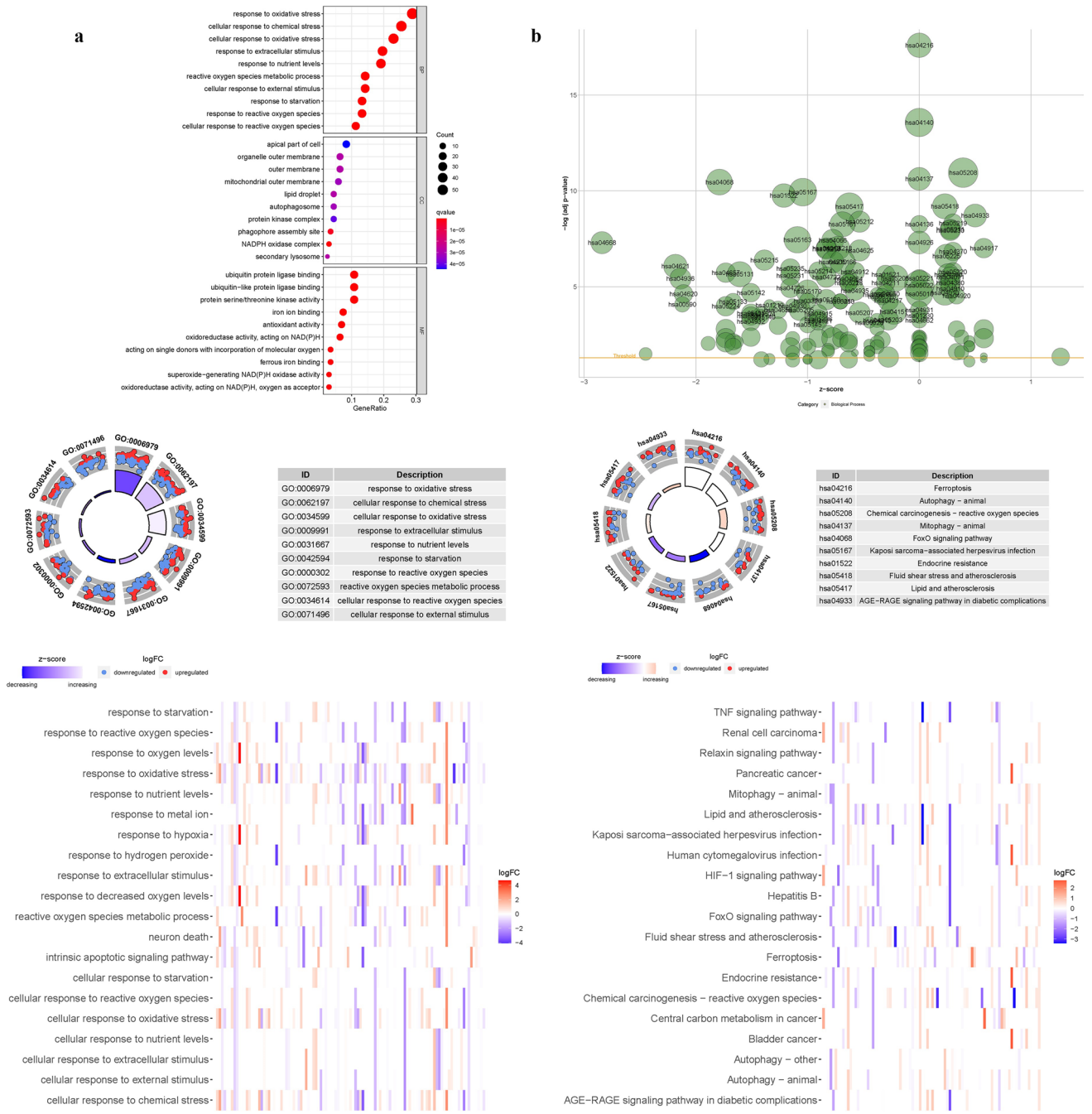

Fig. 7 Enrichment analysis for FRGs. **a** The GO circle illustrates the scatter map of the selected gene's logFC. **b** The KEGG circle illustrates the scatter map of the logFC of the indicated gene. The greater the Z-score value, the greater the expression of the enriched pathway

was determined that the omission of any individual SNP had a minimal effect on the results of the inverse variance-weighted (IVW) analysis, indicating that the remaining SNPs closely mirrored the overall dataset's findings. To further authenticate this research's outcomes, MR-Egger regression analysis was conducted, bolstering the integrity and reliability of this research's results and the chosen analytical framework (Fig. 12a, d).

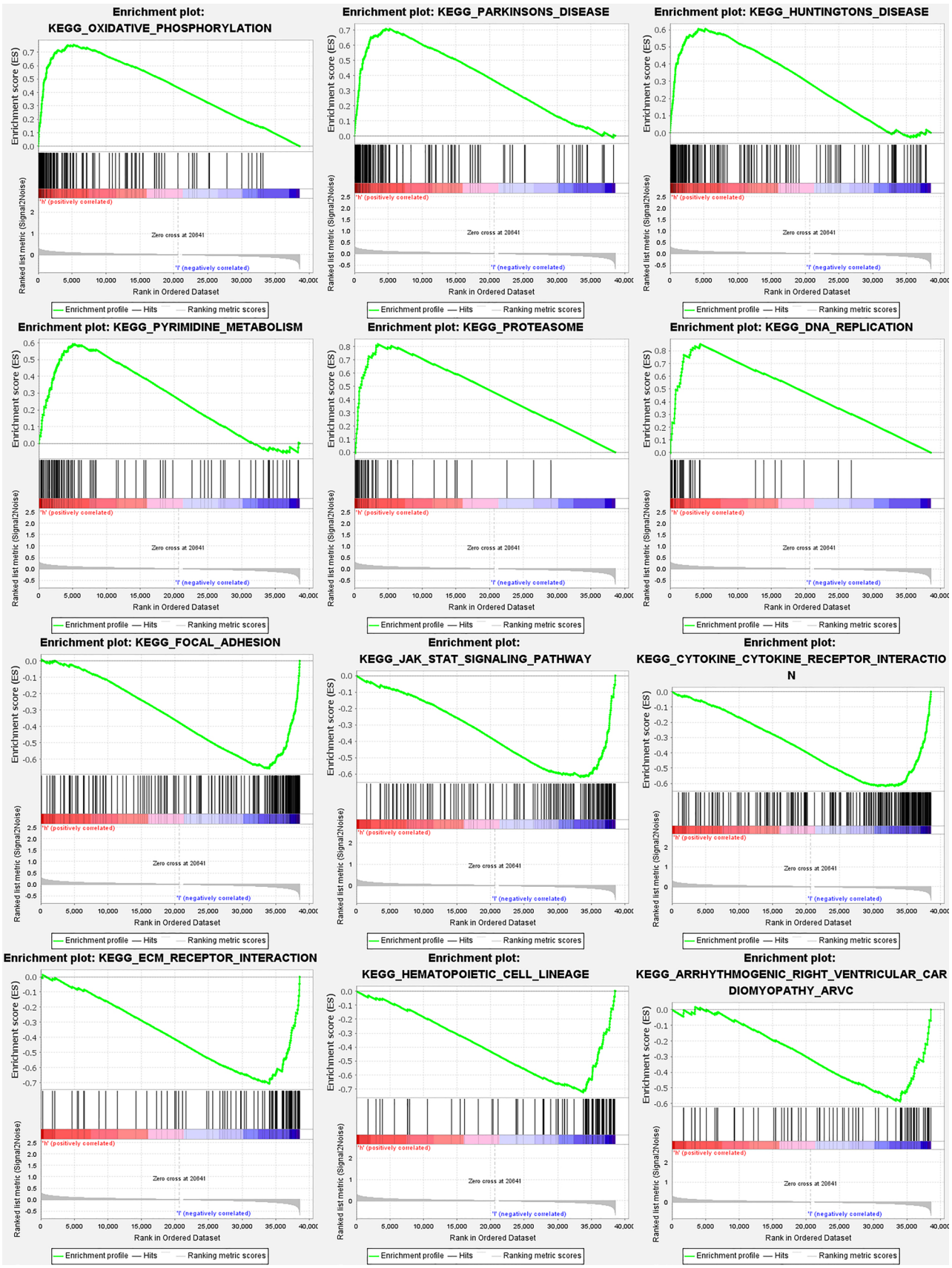

Fig. 8 GSEA analyses. The top six enriched functions or pathways of each cluster were provided to illustrate the distinction between related activities or pathways in various samples. The 'JAK-STAT signaling' was the most enriched

Table 1 The top six enhanced functions or pathways

Name	ES	NES	NOM p-val	FDR q-val
DNA replication	0.85361373	2.217521	0	8.50E−04
Proteasome	0.82036865	2.2445621	0	6.69E−04
Terpenoid backbone biosynthesis	0.7766824	2.0297914	0	0.005566043
Mismatch repair	0.7613202	2.1083157	0	0.002540795
Oxidative phosphorylation	0.7551277	2.3267856	0	0
Homologous recombination	0.74290514	2.178247	0	0.001192128
Hematopoietic cell lineage	− 0.7230753	− 2.273042	0	6.59E-04
Asthma	− 0.7133222	− 1.8212999	0.024590164	0.02349728
ECM receptor interaction	− 0.7068397	− 2.3168972	0	8.24E−04
Allograft rejection	− 0.67772985	− 1.5491151	0.11570248	0.074810006
Intestinal immune network for iga production	− 0.6697375	− 1.7784809	0.040084388	0.02770743
Focal adhesion	− 0.655533	− 2.4876113	0	7.52E−04

4 Discussion

BRCA remains a significant clinical challenge, particularly in its advanced stages [25]. Ferroptosis, a recently identified form of regulated cell death, has been implicated in a range of pathological conditions, including various cancers, strokes, and cellular degeneration in renal failure [26]. Prior studies have underscored the role of ferroptosis in overcoming chemoresistance and facilitating the clearance of aberrant cells, positioning it as a promising therapeutic target [27]. Characterized by the oxidative degradation of polyunsaturated fatty acid-containing phospholipids, the involvement of redox-active iron, and impaired lipid peroxide repair, ferroptosis presents a novel therapeutic avenue for cancer treatment [28]. The therapeutic landscape for cancer is increasingly incorporating agents that target key molecules within the ferroptosis pathway. Despite these advancements, the precise roles and implications of ferroptosis in various pathophysiological contexts, including neurodegeneration, ischemia–reperfusion injury, and malignancies like BRCA, remain incompletely understood [29]. Pharmacological strategies aimed at modulating ferroptosis have shown promise in certain diseases [30]. By integrating transcriptomic data with clinical insights, personalized prognostic models have been developed, providing valuable guidance for cancer management. However, many potential risk markers for cancers, including BRCA, remain underutilized in clinical practice, largely due to challenges in validation and replication. This study focuses on elucidating the complex mechanisms underlying BRCA progression, particularly in relation to alterations in FRGs. We investigate the roles of key proteins and pathways influencing BRCA prognosis, with the goal of identifying potential biomarkers for targeted cancer therapies. This research aims to bridge the gap between emerging scientific insights and clinical application, advancing our understanding of BRCA and its management.

In this study, this research performed an in-depth analysis of 208 DEGs associated with ferroptosis, categorizing them into two distinct clusters within the context of BRCA. The expression patterns of five key FRGs were found to correlate strongly with the prognostic outcomes of BRCA, supporting findings from existing literature. Notably, a subset of these FRGs was significantly overexpressed in high-risk patients, highlighting their potential as predictive biomarkers ($P < 0.05$). This research further explored the functional roles of these FRGs in BRCA and conducted a survival analysis to assess their prognostic significance. This research's results demonstrated that patients with low-risk FRG signatures exhibited significantly better survival outcomes. Among the FRGs analyzed, ACTL8, VGF, and CPLX2 were notably upregulated in the high-risk group, suggesting their potential roles as oncogenic drivers in BRCA progression. Conversely, IL33 and TP63 were significantly downregulated in the low-risk group, indicating their possible tumor-suppressive functions in BRCA. These findings contribute to a more nuanced understanding of the molecular mechanisms governing BRCA progression, emphasizing the complex interplay of FRGs in shaping disease outcomes. This research also observed elevated ACTL8 expression in BRCA patients, which was associated with poor prognosis. Targeted inhibition of ACTL8 effectively suppressed the PI3K/AKT/mTOR pathway in MDA-MB-231 and BT-549 cell lines, leading to reduced cellular proliferation, apoptosis, migration, and invasiveness [31]. In parallel, research by Laura Annaratone highlighted neuroendocrine differentiation in breast cancer, identifying VGF as a key

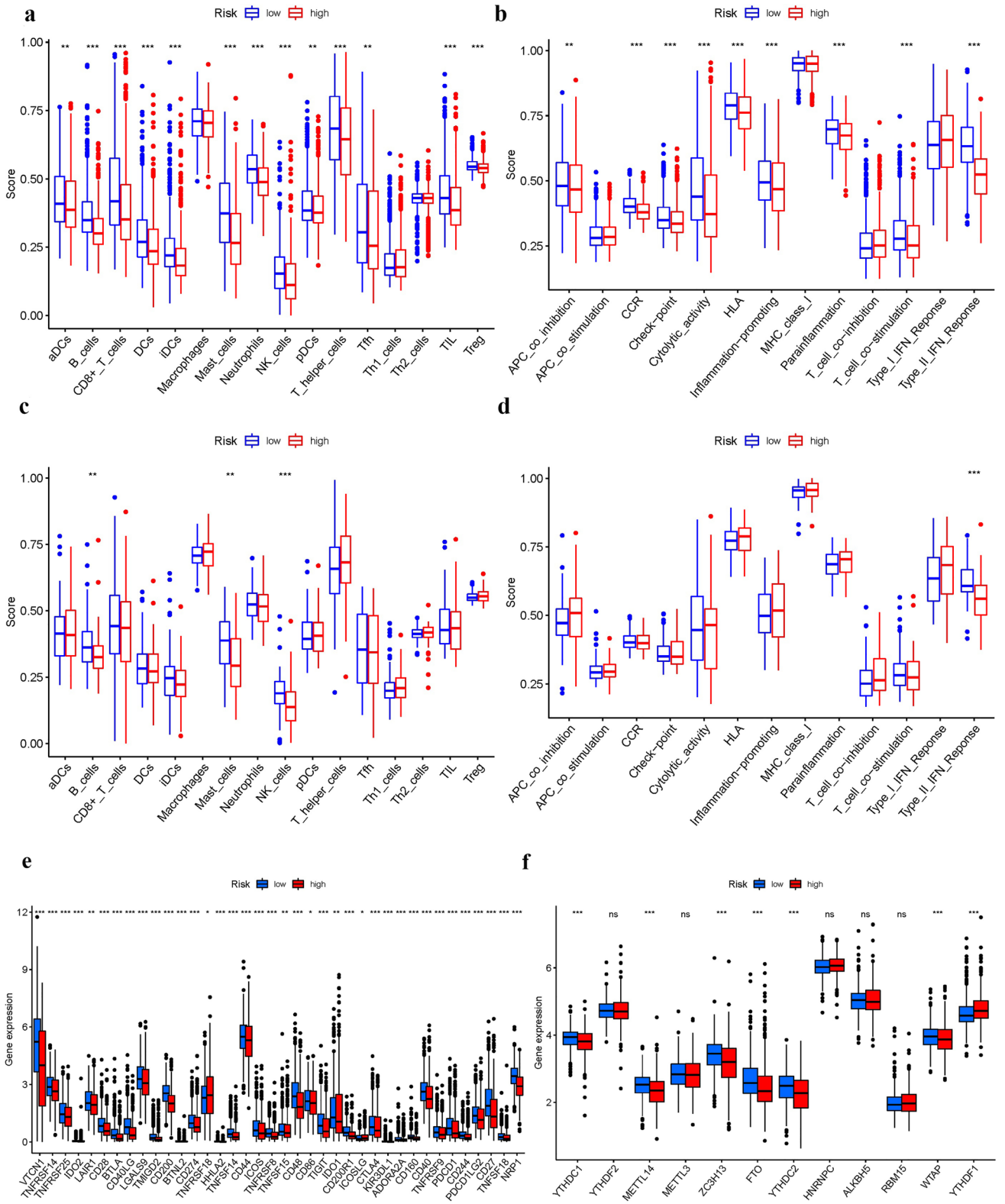

Fig. 9 The ssGSEA scores are compared. **a** + **b** Comparison of the enrichment scores of 16 kinds of immune cells and 13 immune-related pathways in the TCGA cohort between the low-risk (green box) and high-risk (red box) groups. **c** + **d** In the GEO cohort, tumor immunity was compared between the low-risk (blue box) and high-risk (red box) groups. P values were shown as follows: ns not significant. **e** Expression of immune checkpoints. **f** The expression of m^6a-related genes

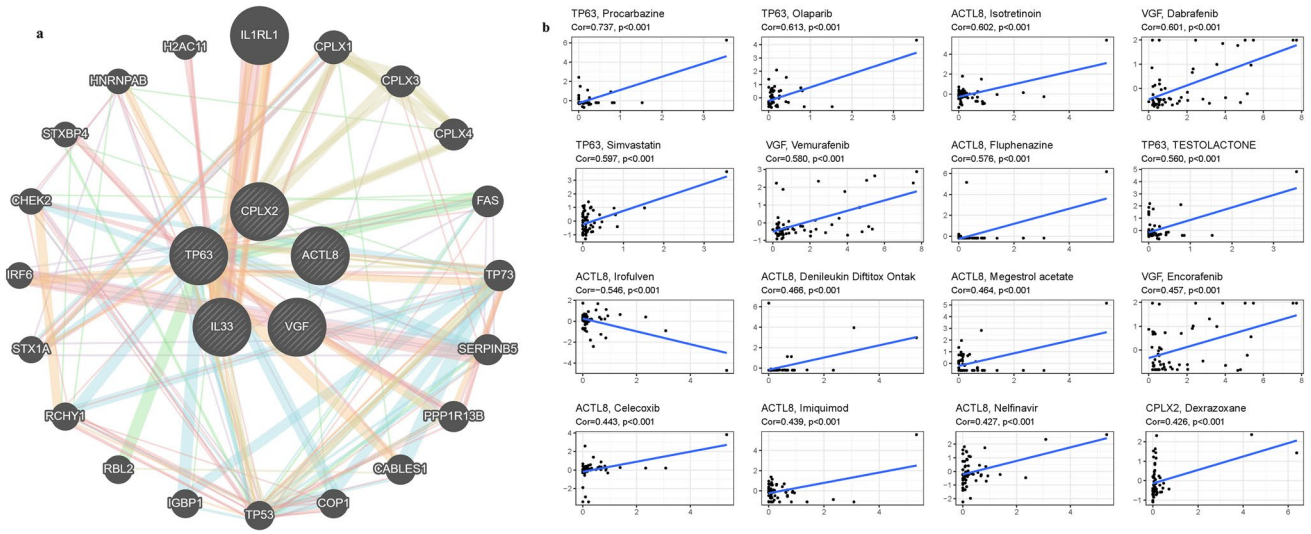

Fig. 10 **a** Gene regulatory networks of FRGs. **b** Correlation analysis between the expression of genes (TP63, ACTL8, VGF, and CPLX2) in prognostic signatures and drug sensitivity

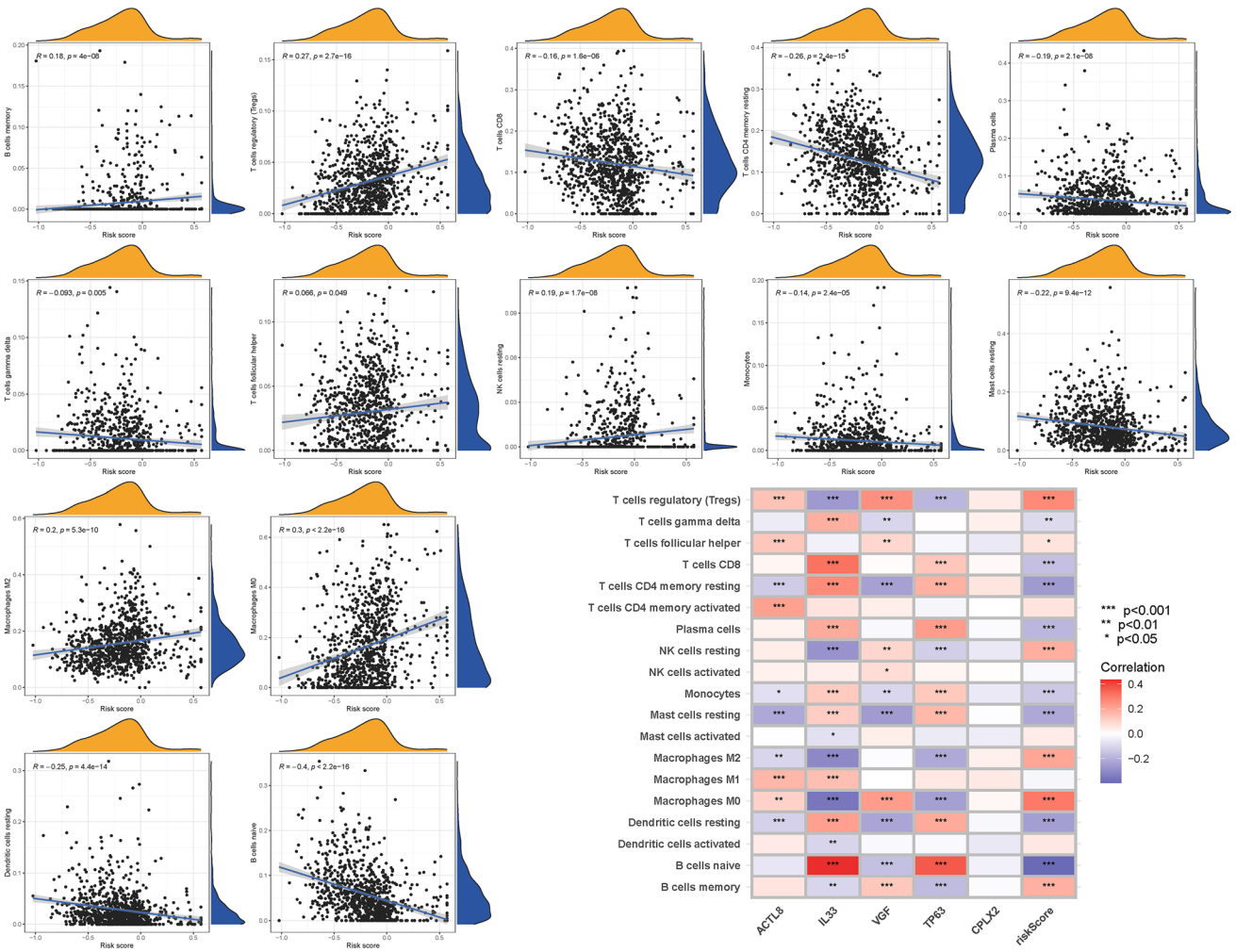

Fig. 11 The CIBERSORT scores are validated

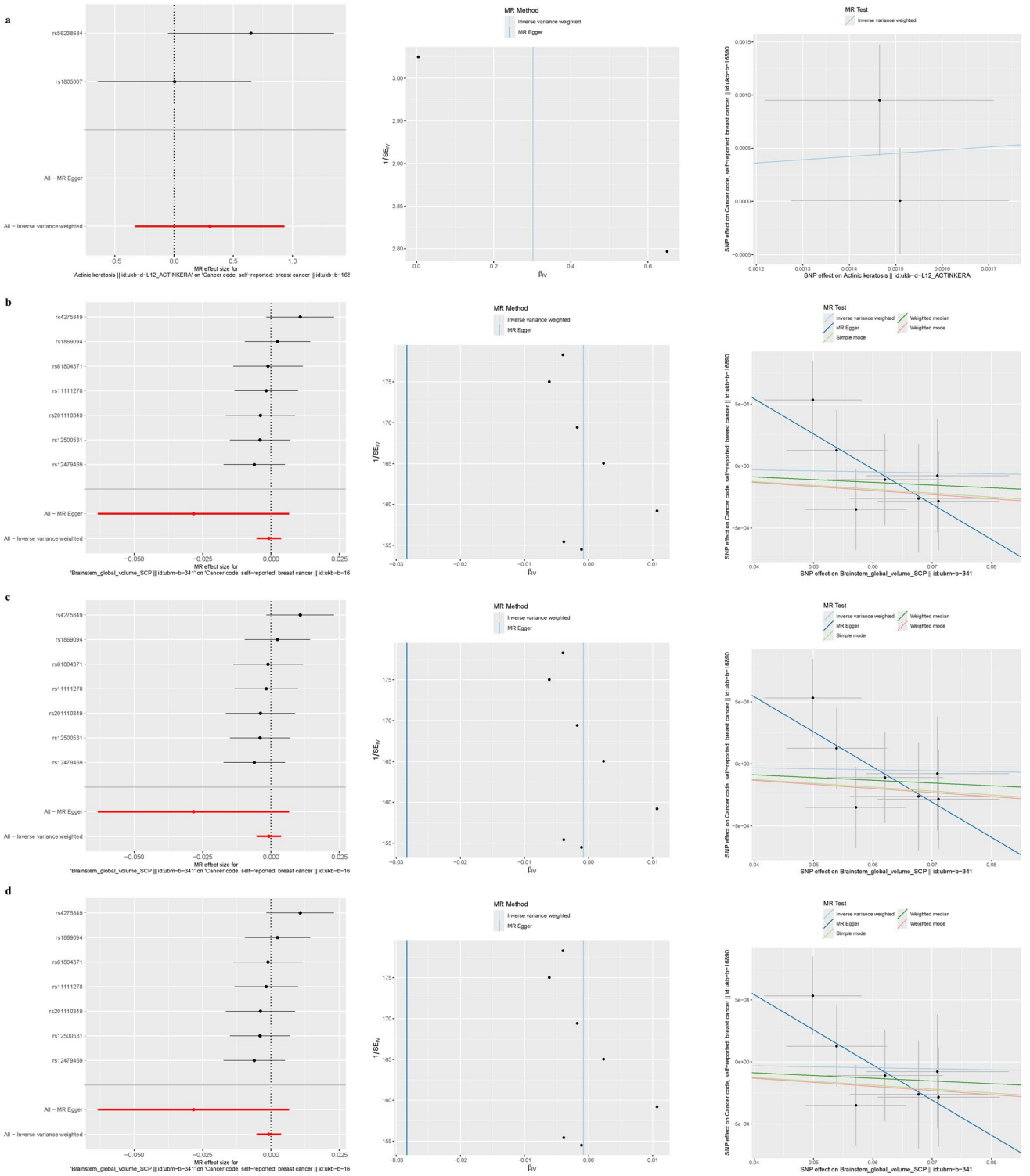

Fig. 12 Mendelian randomization analysis. **a** ACTL8. **b** IL33. **c** TP63. **d** CPLX2

marker in a significant subset of invasive cases [32]. Additionally, this research found that AIB3 plays a crucial role in cellular processes, with its expression levels influencing insulin synthesis and signaling pathways, including CPLX2 [33]. Importantly, stromal fibroblast-secreted IL33 was found to promote BRCA metastasis by modulating the immune response and enhancing Type 2 immune activities [34]. Furthermore, TP63, regulated by the interaction between estrogen receptor signaling and ERK2, was implicated in BRCA growth and invasion [35]. This research's analysis, which

incorporated OS and ROC assessments, along with KM curves from the GSE45255 dataset, supports the potential of an FRG-based signature as a robust prognostic tool. While the role of ferroptosis-related genomic alterations in cancer remains underexplored, this research's findings underscore the necessity for further research to unravel the complex mechanisms through which FRGs contribute to oncogenesis. Moreover, this research's investigation into KEGG pathways revealed the pivotal role of the FoxO signaling pathway. Previous studies, including those by Qin Ma et al. and others, have demonstrated the impact of miRNAs on oligodendrocyte (OL) development, with links to endocytosis, ferroptosis, and the FoxO pathway, highlighting their relevance in OL maturation and function [36–38]. This integration of transcriptomic data with bioinformatics analyses underscores the multifaceted roles of these pathways in cancer biology and opens promising avenues for novel therapeutic interventions.

In the GSEA conducted in this study, the JAK-STAT signaling pathway emerged as a key enriched pathway, underscoring its pivotal role in the pathophysiology of BRCA. Previous studies have highlighted the involvement of interferon (IFN) in promoting ferroptosis in cancer cells through mechanisms such as iron accumulation and lipid peroxidation, with these processes notably mediated by the JAK/STAT pathway [39]. Moreover, the JAK/STAT3 axis is critical in regulating fatty acid metabolism, which is essential for maintaining BRCA stem cells and conferring resistance to chemotherapy [40]. The IL6/STAT3 pathway has also been implicated in BRCA metastasis through the modulation of estrogen receptor signaling [41]. These findings suggest that FRGs may influence BRCA progression by modulating the JAK-STAT signaling network, thus uncovering novel therapeutic opportunities for clinical application. In this study, we developed a prognostic model based on FRGs, which demonstrated strong predictive value for survival outcomes in BRCA [42]. Higher risk scores, as determined by the FRG-based model, were significantly associated with increased mortality and risk, highlighting the potential of FRGs as critical prognostic markers in BRCA. These findings are particularly relevant in the context of recent advances in cancer therapy, such as the emergence of immune checkpoint inhibitors (ICIs). Despite the transformative impact of ICIs, resistance remains a significant challenge, and our study aligns with the growing interest in alternative cell death pathways—such as ferroptosis and necroptosis—as potential strategies to enhance anticancer efficacy [43]. Additionally, this study explored the interplay between insulin regulation, immune checkpoint dynamics, and their impact on cancer cell behavior, including PD-L1 expression in pancreatic ductal adenocarcinoma [44]. Our findings also resonate with the work of Kyrollis Attalla, which investigates the potential of TIM-3 and TIGIT as therapeutic targets in bladder cancer. Crucially, this study provides a comprehensive analysis of the relationship between ICIs and ferroptosis in BRCA, suggesting a complex interaction between FRG alterations and BRCA progression. These insights open new therapeutic avenues in BRCA treatment and emphasize the necessity for further research to unravel the intricate molecular interactions driving these processes. Our findings underscore the importance of continued exploration into the synergies between immune checkpoint regulation and ferroptosis, paving the way for innovative and transformative therapeutic strategies in BRCA management.

Immune cells within the ME play a pivotal role in shaping BRCA progression and therapeutic response. The TME is composed of a diverse array of immune cells, including macrophages, dendritic cells, T lymphocytes, and natural killer (NK) cells, which interact dynamically with tumor cells and stromal components. These immune cells can either promote or hinder tumor growth, depending on their activation state and the overall immune landscape [45]. Regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) are frequently enriched in the BRCA TME, where they foster an immunosuppressive environment that enables tumor evasion of immune surveillance [46]. In contrast, the presence of activated CD8 + T cells and NK cells has been associated with improved clinical outcomes, underscoring the critical importance of immune cell functionality in the antitumor immune response [47]. Furthermore, immune checkpoint molecules such as PD-1/PD-L1 and CTLA-4, which regulate immune cell activation, are often dysregulated in BRCA, contributing to immune escape and disease progression. The intricate balance of immune cells within the TME not only influences tumor behavior but also holds significant implications for the design of immunotherapeutic strategies in breast cancer [48]. In this research, Positive associations with FRGs were observed for B cells memory, Macrophages M0, Macrophages M2, NK cells resting, T cells follicular helper, T cells regulatory (Tregs). Conversely, negative correlations with FRGs were noted for T cells gamma delta, T cells CD8, T cells CD4 memory resting, B cells naive, Dendritic cells resting, Mast cells resting, Monocytes, Plasma cells. In addition, there was no significant difference between CPLX2 and these immune cells in these FRGs. However, the other five FRGs were all significantly associated with the vast majority of immune cells. This intricate interplay between FRGs and various immune cell populations underscores the critical role of inflammation and immune responses in the pathophysiology of BRCA, providing a foundation for targeted therapeutic strategies.

Bioinformatics, functioning as a pivotal cornerstone within the interdisciplinary landscape, plays an instrumental role in deciphering the intricate relationship between FRGs and the enigmatic progression of cancer [49–51]. In this domain, Jin et al. skillfully utilized the extensive TCGA database to map a prognostic landscape defined by

259 key FRGs, providing profound insights into the molecular dynamics of oncogenesis. Parallel to this depth of analysis, Zhang et al. revealed a complex interplay of long non-coding RNAs (lncRNAs) with ferroptotic mechanisms, offering valuable prognostic perspectives for the clinical trajectory of BRCA patients. From this elaborate matrix, eight lncRNAs, closely associated with ferroptosis, were identified as critical markers for diagnosis and prognosis. In a similar vein, Wang et al. developed a comprehensive prognostic model for BRCA, incorporating a group of nine essential FRGs. This framework significantly enhances the precision of patient prognostication. Concurrently, the emerging role of immune checkpoint inhibitors (ICIs) in BRCA treatment represents a burgeoning area of interest, particularly in understanding and predicting therapeutic responses [52]. The complex interplay between FRGs and the immune milieu in BRCA presents a fertile ground for investigation, with the potential to uncover the veiled mechanisms of gene dysregulation and ferroptosis induction in this cancer type. The intricacies of FRGs and their influence on the evolutionary course of BRCA are enshrouded in complexity. This research's study leverages advanced statistical and machine learning techniques to identify key prognostic factors and develop a more accurate and robust gene signature. This facilitates refined stratification of patient risk profiles, essential for personalized therapeutic approaches. A notable strength of this research's analysis lies in its focus on the functional relevance of the identified biomarkers. Through pathway analysis and GSEA, this research provide deeper insights into the biological processes and signaling pathways underlying the prognostic markers. Moreover, this research's exploration of FRGs represents a novel direction in cancer research. Ferroptosis, an underexplored cell death mechanism, holds substantial therapeutic promise. By identifying and validating FRGs as key mediators of cancer progression and therapeutic response, this research's study paves the way for new, targeted treatment strategies. Despite its theoretical base and research technique, this research's study has several limitations. Both in vivo and in vitro studies hold immense potential for unraveling these complexities, suggesting numerous avenues for future research. Additionally, exploring the correlation between prognostic genes and FRG in the context of BRCA is essential. These places provide excellent prospects for future exploration and research.

5 Conclusions

This research's research navigates the complex landscape of cancer, elucidating the critical role of FRGs and their broad prognostic relevance in oncology. Using advanced prognostic models, this research mapped the transcriptional profiles of FRGs, revealing significant expression differences between cancerous and normal tissues. Crucially, this research's study identifies a robust association between FRG expression patterns and immune cell infiltration within the TME, suggesting that FRGs could serve as valuable biomarkers for predicting response to immunotherapy across multiple cancer types. Consequently, FRGs emerge as vital prognostic markers with significant implications for survival outcomes across a wide array of cancers.

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Data availability and limitations All the data can be obtained from the open source website we provide, and the conclusion can be drawn through the analysis of the relevant software. "The datasets generated and/or analysed during the current study are available in the [TCGA and GEO] repository, [https://portal.gdc.cancer.gov/; https://www.ncbi.nlm.nih.gov/geo/]". The citation guidelines: www.kegg.jp/kegg/kegg1.html.

Declarations

Ethics approval and consent to participation This manuscript is not a clinical trial, hence the ethics approval and consent to participation is not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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