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Potential role of CFLAR in enhancing 5-FU sensitivity and modulating immune cell infiltration in breast cancer

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Abstract

Background Breast cancer (BRCA), the most common malignancy among women, is a highly heterogeneous disease. Chemoresistance is a major factor leading to treatment failure in BRCA. However, mechanisms underlying the development of chemoresistance remain unclear.

Methods In this study, we performed a comprehensive bioinformatic analysis to examine the role of cell death-associated genes in BRCA treatment. Specifically, we focused on caspase 8 and Fas-associated protein with death domain-like apoptosis regulator (CFLAR), which was identified as a co-differentially expressed cell death-associated molecule with potential prognostic values. We then validated these findings through in vitro experiments in BT- 549 and MDA-MB- 231 breast cancer cells.

Results Based on bioinformatics analysis, CFLAR expression was found to be downregulated in patients with BRCA, whereas its high expression was significantly associated with improved prognosis. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis indicated that aberrantly expressed CFLAR was potentially associated with oxidative phosphorylation, T cell receptor signaling, and NADH dehydrogenase (ubiquinone) activity. In vitro experiments demonstrated that overexpression of CFLAR inhibited the generation of reactive oxygen species (ROS), consequently promoting 5-fluorouracil (5-FU) sensitivity in BT- 549 and MDA-MB- 231 breast cancer cells. The expression of CFLAR was positively correlated with the abundance of several tumor-infiltrating immune cells, especially CD8 +T cells, further supporting the role of CFLAR in immune regulation.

Conclusion In conclusion, this study reveals the novel roles of CFLAR in enhancing chemotherapy sensitivity and patient outcome in BRCA and underscores its potential as a therapeutic target. These results supported CFLAR as a therapeutic target and prognostic biomarker in BRCA patients.

Keywords Breast cancer, CFLAR, ROS, Immune infiltration, 5-Fluorouracil, Chemoresistance

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Introduction

Breast cancer (BRCA), the most common malignancy in women, is a highly heterogeneous disease [1, 2]. Meanwhile, it is also one of the most common malignancies [3]. Triple-negative breast cancer (TNBC), one of the common subtypes, has the highest mortality rate [4] and the lowest mean time to relapse [5, 6]. The poor prognosis of TNBC is mainly attributed to its molecular features and the increasing prevalence of drug resistance. Therefore, identifying novel therapeutic targets is necessary for improving the clinical outcomes of patients with BRCA, especially those with TNBC.

Caspase 8 and Fas-associated protein with death domain-like apoptosis regulator (CFLAR), as known as c-Flip, is a key cell death regulator that mediates resistance to apoptosis. Different splicing methods result in both long (c-FLIPL) and short (c-FLIPS) forms of CFLIP, both of which combine with Fas-associated death domain (FADD). Several studies have demonstrated the important role of aberrant CFLAR expression in regulating tumorigenesis and treatment in renal cancer [7] and lung cancer [8]. Treatment of ethanolic asiasari radix extract (ARE) could induce reactive oxygen species (ROS)-dependent downregulation of p53 and its target gene, CFLAR, and promote cell apoptosis in G361 human melanoma cells [9]. In pancreatic cancer, overexpression of CFLAR protected cancer cells from 5-FU-mediated cytotoxicity [10]. Recent studies have shown that CFLAR interacts with p130 Cas to enhance cell migration and invasion by promoting p130 Cas phosphorylation and stabilizing focal adhesion complexes, thereby contributing to tumor metastasis [11]. Increased CFLAR expression is associated with poor prognosis in breast cancer patients and mediates apoptotic resistance in TNBC by inhibiting caspase- 8 and caspase- 3 activation, leading to therapeutic resistance [11, 12]. Collectively, these findings suggest that CFLAR significantly impacts breast cancer progression and treatment resistance. However, the precise roles of CFLAR in the progression and treatment of BRCA remain unclear. These gaps highlight the need for further research to explore the underlying roles of CFLAR in breast cancer.

The observations here also raise the possibility that CFLAR may interact with anti-breast cancer immune response. The immune response plays an important role in the occurrence and development of BRCA [13, 14]. At present, studies are focused on novel immunoregulation therapies, such as those involving myeloid suppressor cells and regulatory T cells [15–17]. Notably, blocking immune checkpoints and targeting programmed cell death- 1/programmed cell death ligand- 1 (PD- 1/PD-L1) can benefit the survival of some patients with BRCA [18, 19]. However, identifying novel biomarkers for predicting the potential response of patients to immunotherapy is challenging.

In this study, we aimed to investigate the biological significance of CFLAR in breast cancer and its role in modulating therapeutic sensitivity. We demonstrated that downregulated CFLAR indicated a favorable prognosis in patients with BRCA. Overexpression of CFLAR increased the sensitivity of BRCA cells to 5-fluorouracil (5-FU) by regulating ROS homeostasis. These results indicate that CFLAR is a novel biomarker for assessing prognosis and 5-FU sensitivity in BRCA.

Materials and methods

Data collection and gene expression validation

As shown in Additional file 1, we obtained two BRCA-GEO datasets, GSE20437 [20] and GSE21422 [21], and screened for differentially expressed genes (DEGs) based on the screening criteria of P-values of < 0.05 and $|\log FC|$ values of > 0.5. Venn diagrams were constructed to identify differentially expressed cell death-associated genes between GEO datasets (GSE20437 and GSE21422) and cell death-associated datasets (Additional file 2) [22].

Bioinformatic analysis

The prognosis of patients with BRCA, including overall survival (OS) and recurrence-free survival (RFS), was assessed using the Kaplan–Meier plotter [23] and Biomarker Exploration of Solid Tumors (BEST) database [24]. The Xiantao tool (https://www.xiantao.love/products) was used to assess the prognostic values and expression profiles of CFLAR patients in BRCA patients. In addition, the expression of CFLAR in normal and tumor samples was evaluated using the TNMplot [25] and GEPIA2 [26] tools. The expression of CFLAR in patients treated with various therapeutic strategies was evaluated using ROC Plotter [27]. The Kaplan–Meier plotter was used to analyze the prognosis of patients treated with various therapeutic strategies. GO and KEGG enrichment analyses were performed using the BEST database. The correlation of CFLAR expression with immune cell infiltration and treatment response was assessed using the Tumor Immune Estimation Resource 2.0 (TIMER2.0) [28]. Single-cell RNA sequencing (scRNA-seq) of GSE136206 dataset [29] was processed using the Tumor Immune Single-Cell Hub (TISCH) [30].

Cell transfection

The two TNBC cell lines (BT- 549 and MDA-MB- 231) used in this study were generous gifts from the Cancer Research Institute, Central South University, China. The cell lines included in our research are commercially

available; for this reason, it is not necessary to obtain ethical approval. A CFLAR-overexpression plasmid was constructed by cloning human CFLAR cDNA into the pcDNA3.1 plasmid (pc3.1-CFLAR) (Tsingke Biotechnology, China). The empty plasmid pcDNA3.1 served as the control. Cells at a density of $\times 10^5$ were seeded in 6-well plates and transfected with plasmids using the Lipofectamine 3000 reagent (L3000015, Invitrogen, USA) according to the manufacturer's protocol.

siRNA transfection

The siRNA sequence for CFLAR was AAGCAGTCT GTTCAAGGAGC [31]. The transfection was carried out when cells were at approximately 70–80% confluence. Cells were seeded in appropriate culture plates and transfected with siRNA using Lipofectamine RNAiMAX (Invitrogen, USA) according to the manufacturer's instructions. After 24–48 h of transfection, the cells were harvested for subsequent analyses.

Western blotting

BT- 549 and MDA-MB- 231 cells were collected and lysed in a lysis buffer. The cells were centrifuged at 12,000 g for 15 min at 4 ℃, and supernatants were collected. Thereafter, 35-μg cell lysates were prepared for SDS-PAGE, and separated proteins were transferred to a PVDF membrane via electroblotting. The membrane was blocked with 5% nonfat dried milk for 2 h at 25 °C and incubated with primary antibodies against CFLAR (EM1708 - 94, HUABIO, 1:1000) and β-actin (sc- 47778, Santa Cruz, 1:3000) overnight at 4 °C. The following day, the membrane was incubated with the corresponding secondary antibodies for 1 h at 25 °C. Target protein bands were detected via enhanced chemiluminescence (ECL), and the proteins were quantified using the Image Lab Software (Bio-Rad) according to the manufacturer's protocol.

Measurement of reactive oxygen species levels

Cellular ROS levels were evaluated using the peroxide-sensitive fluorescent probe DCFDA/H2DCFDA (ab113851, Abcam, United States). CFLAR-overexpressing and CFLAR-knockdown cells, along with their respective control groups, were inoculated in a 6-well plate and treated with 5-FU (HY- 90006, MedChemExpress). According to the manufacturer's introductions, DCFDA Solution was added into the suspended cells and incubated away from light for 30 min. The levels of ROS were determined on a flow cytometer (BD Bioscience, CA, USA).

Colony formation assay

Colony formation assay was performed as described in previous studies [32]. BT- 549 and MDA-MB- 231 cells were transfected and seeded in a 6-well plate (1000 cells per well). After 24 h of incubation, some cells were treated with 5-FU, whereas some were left untreated. After approximately 14 days, the cells were stained with 0.3% w/v crystal violet/methanol for 20 min at 25 ℃, and colonies were counted using ImageJ software [33].

Real-time PCR analysis

Real-time PCR analysis was carried out according to the previous study [34]. Total RNA was extracted from the control group and 5-FU treated BRCA cells of overexpression CFLAR by Trizol, and reverse-transcribed to obtain complementary DNA (cDNA). Quantitative reverse transcription PCR was performed by SYBR Green PCR Master Mix. The relative mRNA expression levels were evaluated by using the $2-\Delta\Delta Ct$ method [35]. The primers utilized in the study are listed in Additional file 3.

Statistical analysis

All experiments were conducted in triplicate ($n=3$), utilizing independent experiments that yielded consistent results. And data were expressed as the mean ± standard deviation (SD). Student's t-test was used to analyze CFLAR expression, ROS levels, colony-forming ability, and cell survival rates. Kaplan–Meier analysis was performed to assess the prognosis of patients with BRCA. Wilcoxon rank-sum test or Kruskal–Wallis test was used to examine the relationship between CFLAR expression and clinicopathological variables. In addition, Spearman correlation coefficients were evaluated to analyze the correlation of CFLAR expression with immune cell infiltration, treatment response, tumor markers, and BRCA subtypes. Significant differences were indicated as follows: *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

Results

CFLAR was downregulated in patients with BRCA

As shown in Fig. 1A, three DEGs (two upregulated genes, SLC11 A2 and CASP6, and one downregulated gene, CFLAR) were identified between the GEO datasets (GSE20437 and GSE21422) and cell death-associated datasets. The expression of these DEGs was validated in

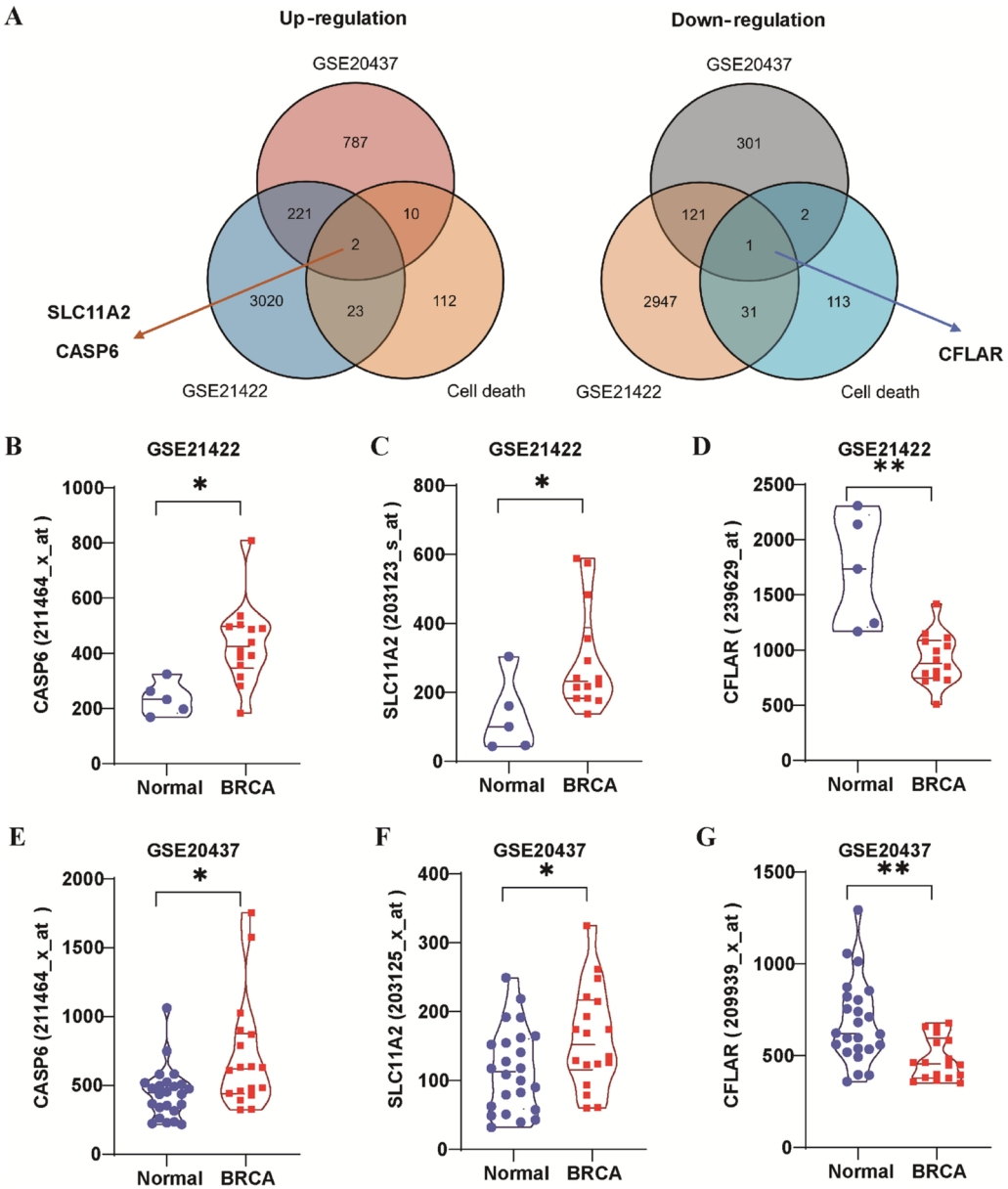

Fig. 1 Differentially expressed cell death-associated genes between two BRCA datasets. **A** Two upregulated genes, SLC11 A2 and CASP6, and one downregulated gene, CFLAR, were identified in BRCA datasets. **B–G** Expression of SLC11 A2, CASP6, and CFLAR in breast cancer tissues. *, $P < 0.05$

the two GEO databases. The expression of CASP6 and SLC11 A2 was significantly high and that of CFLAR was significantly low in patients with BRCA (Fig. 1B–G).

Kaplan–Meier analysis was performed to examine the role of CFLAR in the prognosis of patients with BRCA. Patients with high CFLAR expression had improved OS (Fig. 2A–F) and RFS (Fig. 2G–N). These results were validated in another GEO dataset (GSE9893), in which patients with high CFLAR expression had improved RFS (Fig. 2O). Analysis based on the Xiantao tool indicated that high expression of CFLAR improved the OS of patients with stage-III and -IV BRCA (Fig. 2P).

Gene expression analysis confirmed the downregulated roles of CFLAR in breast patients

CFLAR expression was downregulated in BRCA tissues in TCGA_GTEx-BRCA dataset (Fig. 3A–B). The differential expression of CFLAR between BRCA tissues and the corresponding adjacent normal tissues was validated using the TNMplot and GEPIA2 tools (Fig. 3C–D). Analysis of CPTAC data revealed that the protein

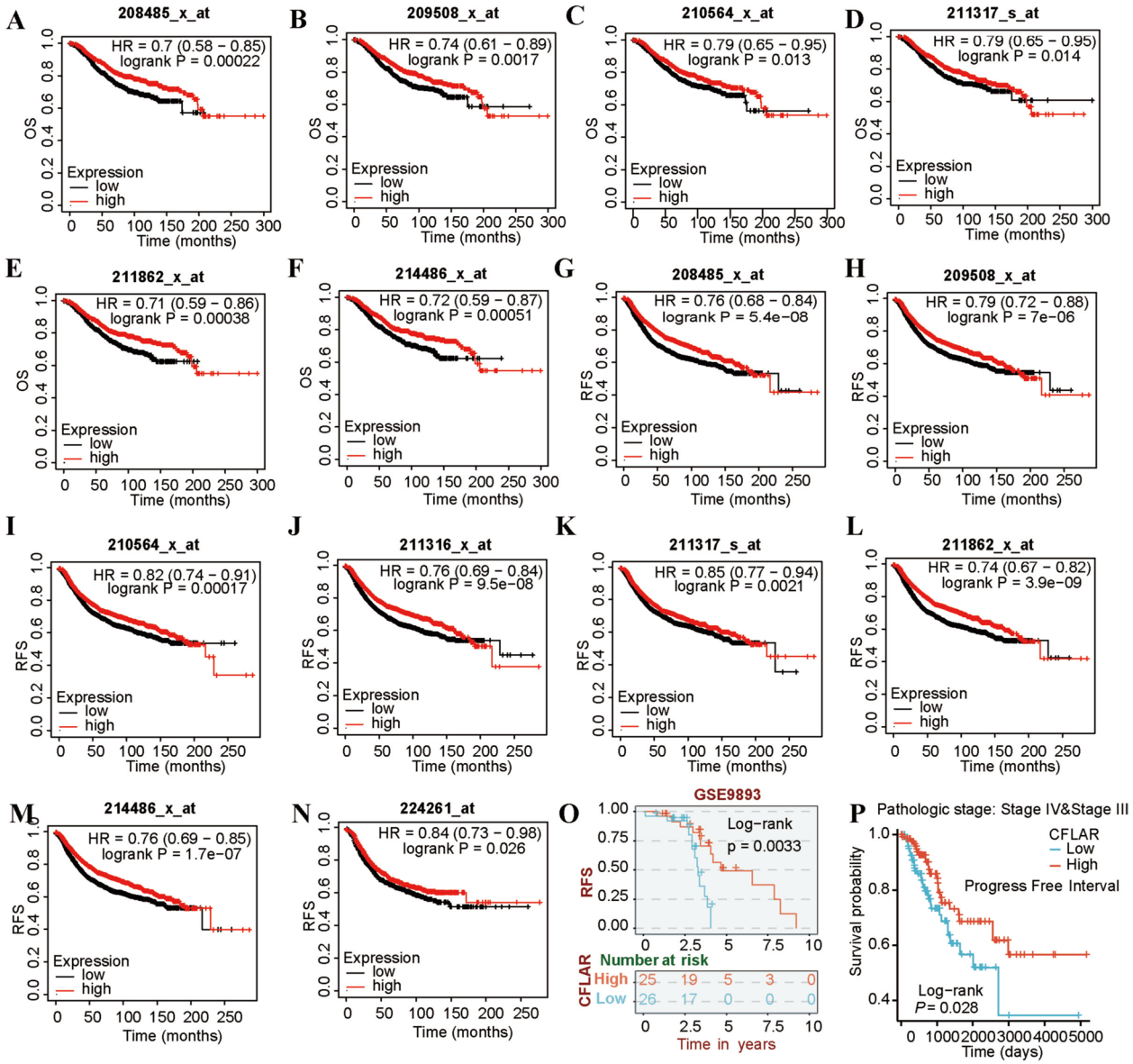

Fig. 2 Prognostic values of CFLAR in BRCA patients. **A–F** Kaplan–Meier plotter indicated the roles of CFLAR on patients' OS. **G–O** Kaplan–Meier plotter and BEST database indicacted the roles of CFLAR on patients' RFS. **P** Xiantao tool indicated the prognostic values of CFLAR in BRCA patients with stage-III and -IV. The transcripts of CFLAR were clearly marked on the corresponding data

expression of CFLAR was significantly downregulated in primary tumor tissues in BRCA (Fig. 3E). BEST database indicated that the expression of CFLAR was low in patients with Ki67-positive BRCA patients from GSE21653 dataset (Fig. 3F).

Overexpression of CFLAR inhibited cell survival and promoted 5-FU sensitivity in BRCA cells

As shown in Fig. 4A–E, the expression of CFLAR was higher in patients with BRCA who responded to anti-cancer drugs, such as trastuzumab, taxanes, anthracycline, cyclophosphamide–methotrexate–fluorouracil (CMF), and fluorouracil–epirubicin–cyclophosphamide (FEC). After treated with tamoxifen, patients with high CFLAR expression had favorable OS (Fig. 4F–G). CFLAR expression was positively correlated with 5-FU sensitivity (Fig. 4H). Subsequently, we examined the effects of CFLAR on 5-FU sensitivity in BT- 549 and MDA-MB- 231 BRCA cells. An overexpression strategy was applied to promote the expression of CFLAR in the two BRCA cells (Fig. 4I). After treatment with 5-FU, overexpression of CFLAR

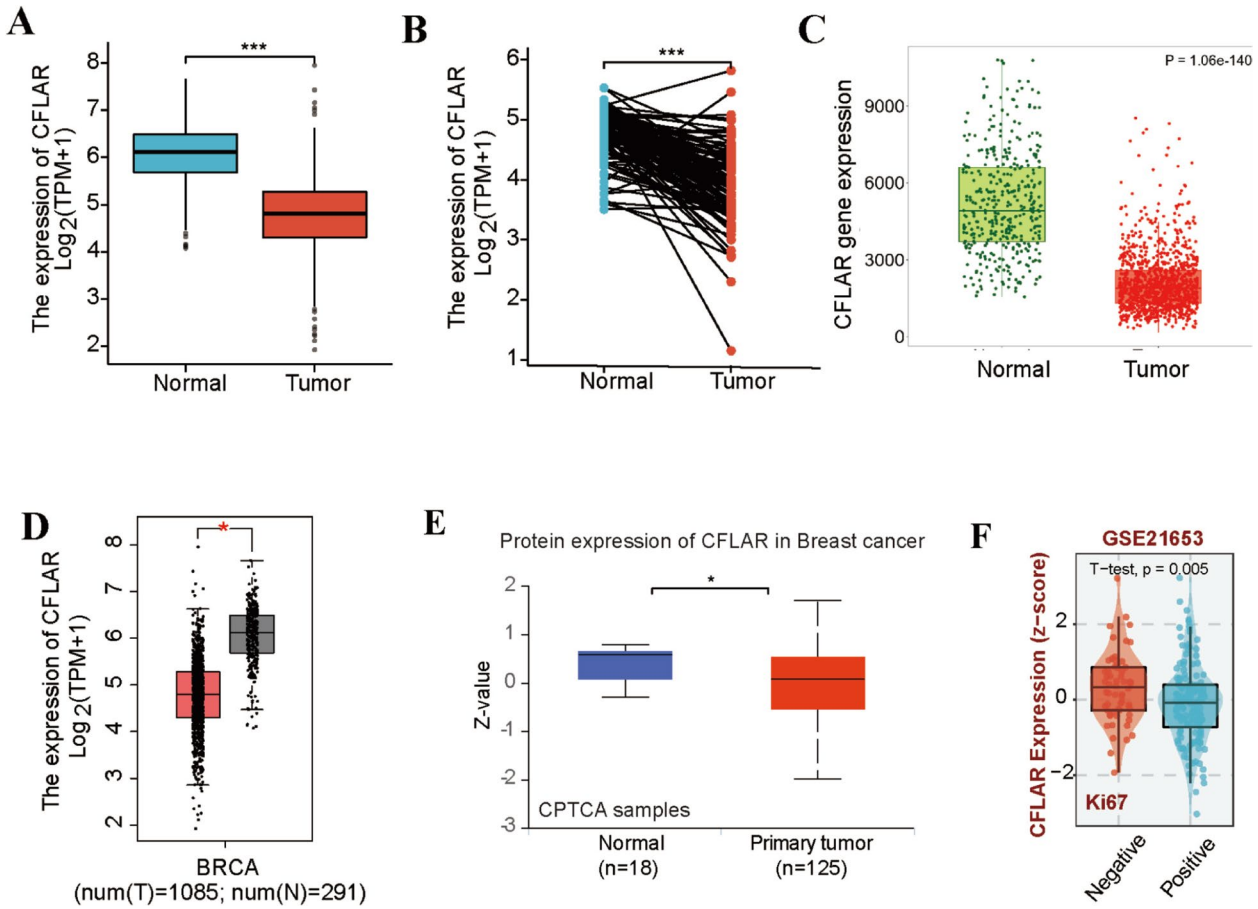

Fig. 3 CFLAR expression was significantly low in breast cancer tissues. **A–B** CFLAR expression in TCGA-BRCA patients. **C** TNM plotter was used to analyze the expression of CFLAR. **D** GEPTA2 was used to analyze the expression of CFLAR. **E** CPTAC was used to analyze the expression of CFLAR. **F** CFLAR expression in patients with Ki67-positive BRCA in the BEST dataset. *, $P < 0.05$; ***, $P < 0.001$

inhibited the clone formation rate and decreased cellular ROS levels in BT- 549 and MDA-MB- 231 cells (Fig. 4J–M). Elevated cellular ROS levels were observed in CFLAR knockdown after 5-FU treatment (Additional file 4A–D). Given the underlying functions of NRF2-HOMX1-SOD1 in redox homeostasis [36], we evaluated the changes of NRF2-HOMX1-SOD1 signaling pathways upon CFLAR overexpression. As shown in Additional file 4E, the levels of NRF2, HOMX1 and SOD1 were all found to be significantly increased in CFLAR-overexpressed cells after treatment with 5-FU. These results suggest that upregulation of CFLAR increases 5-FU sensitivity by regulating ROS levels in BRCA cells, which is consistent with the redox regulation pathway suggested by our bioinformatics analysis.

Role of CFLAR in immune infiltration and immunotherapy

To understand the role of CFLAR in BRCA progression, GO and KEGG pathway analyses were implemented using the BEST database. As shown in Fig. 5A, CFLAR was enriched in KEGG pathways associated with the sensory system, the immune system, and cell metabolism. Specifically, the "sensory system" encompasses pathways involved in detecting and processing environmental stimuli, such as those related to vision, hearing, and other sensory functions. The "immune system" pathways are associated with immune responses, including antigen recognition, immune cell signaling, and activation of immune responses. Moreover, GO analysis indicated that CFLAR was enriched in pathways associated with the regulation of immune response and mitochondria (Fig. 5B). Thus, we evaluated the potential function of CFLAR in regulating immune cell infiltration in TCGA cohort. The results showed that CFLAR expression was positively correlated with the abundance of tumor-infiltrating Tcm cells, T helper cells, Tem cells, T cells, and B cells and negatively correlated with the abundance of $CD56^{bright}$ NK cells and Th2 cells (Fig. 6A–C). Data extracted from the BEST and TIMER databases were analyzed through Spearman correlation analysis. The

Figure 4

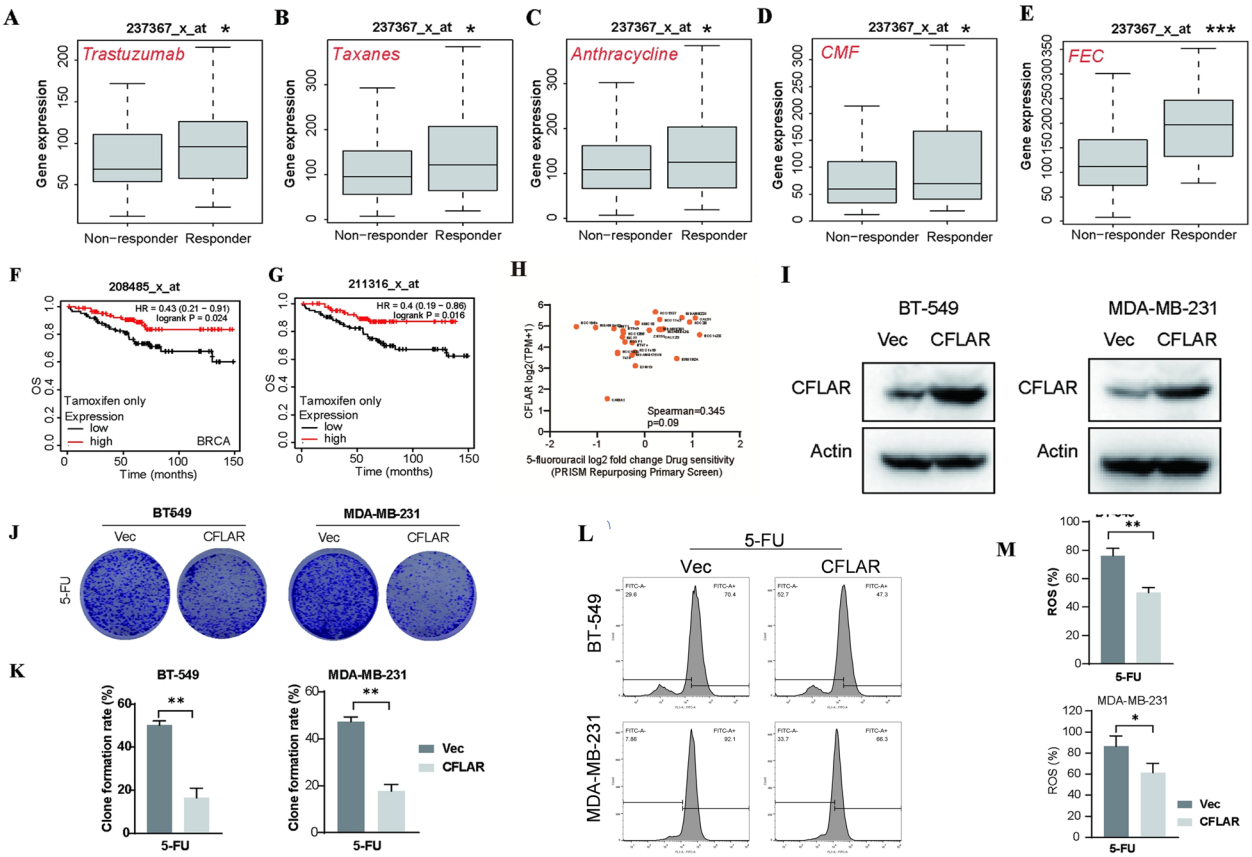

Fig. 4 Role of CFLAR in the treatment of BRCA. **A–E** Expression of CFLAR in patients who responded to trastuzumab, taxanes, anthracycline, CMF, and FEC. **F–G** Prognostic value of CFLAR in patients with BRCA treated with tamoxifen. The transcripts of CFLAR were clearly marked on the corresponding data. **H** Analysis of CCLE data extracted from DepMap revealed that CFLAR expression was correlated with sensitivity to 5-FU. I BT- 549 and MDA-MB- 231 cells were transiently transfected with Flag-CFLAR. **J–K** Colony formation assay revealed that overexpression of CFLAR increased the inhibitory effects of 5-FU (10 μM) on cell growth. **L–M** Overexpression of CFLAR resulted in a decrease in ROS levels after 5-FU treatment. Each bar represented the mean ± SD ($n = 3$). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

results revealed that CFLAR expression was positively correlated with the abundance of CD8 + T cells (Fig. 6D–F). Single-cell sequencing of CFLAR showed that its expression was positively correlated with the abundance of several tumor-infiltrating immune cells in BRCA (Fig. 6G–I), especially CD8 + T cells (Fig. 6J–K). In addition, high CFLAR expression improved the OS of BRCA patients treated with anti-PD- 1 and anti-PD-L1 therapies (Fig. 6L–M). Additional file 5A-D shows that CFLAR expression is positively correlated with CD8 + T cell infiltration in multiple cancer types, including UVM, SARC, and MESO. This pan-cancer analysis highlights the potential regulatory role of CFLAR in modulating tumor immunity, particularly by promoting CD8 + T cell infiltration across.

Discussion

In this study, we examined the role of CFLAR in BRCA progression and drug sensitivity in BRCA using various bioinformatic platforms. Three DEGs, including two upregulated genes (SLC11 A2 and CASP6) and one downregulated gene (CFLAR), were identified between two GEO datasets and cell death-associated datasets. Of the three DEGs, only CFLAR exhibited potential prognostic value in BRCA. In vitro experiments and bioinformatic analysis demonstrated that downregulated CFLAR was critical to the pathological progression of BRCA and 5-FU sensitivity.

Recent studies have reported the therapeutic roles of several types of cell death mechanisms in cancer, including apoptosis, necrosis, ferroptosis, and autophagy [37]. Apoptosis, marked by cell shrinkage, chromatin

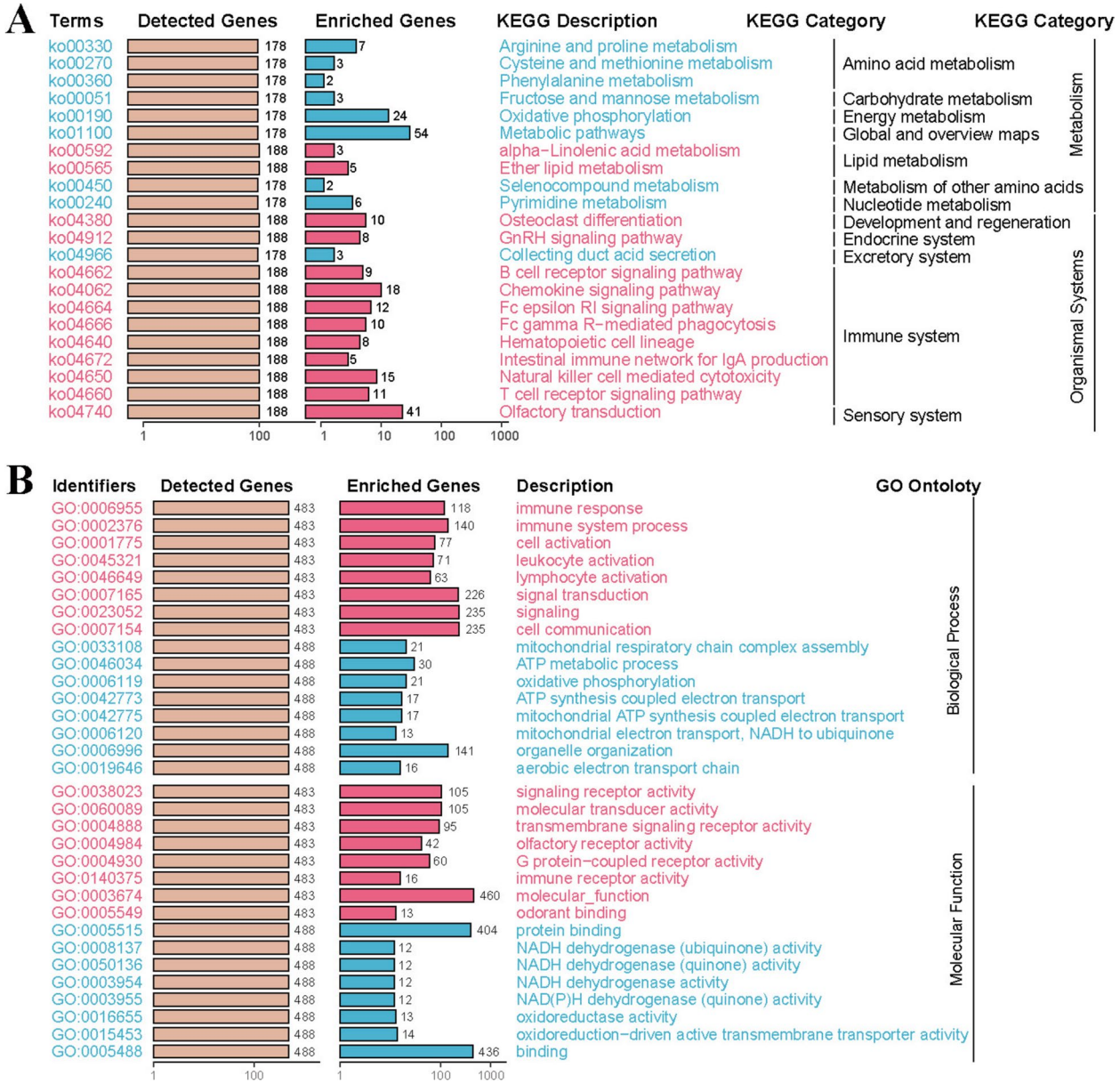

Fig. 5 Signaling pathways associated with CFLAR in BRCA. **A** KEGG pathway enrichment analysis of CFLAR in BRCA. **B** GO functional annotation analysis of CFLAR in BRCA

condensation, and DNA fragmentation, is crucial for tissue homeostasis and eliminating damaged cells [38]. Necrosis, characterized by cell swelling and membrane rupture, can induce inflammation and tissue damage, contributing to tumor progression in certain contexts [39]. Flubendazole can regulate autophagy and apoptosis

(See figure on next page.)

Fig. 6 Role of CFLAR in immune infiltration and immunotherapy. **A** Correlation between CFLAR expression and the abundance of 24 types of tumor-infiltrating immune cells. Absolute values of Spearman correlation coefficient (R) were measured based on the size of the dots. **B** Heatmap through TIMER2.0 to pan-cancer evaluate the association between CFLAR expression and immune cell infiltration. **C** Scatter plots demonstrating the correlation between CFLAR expression and T cell infiltration. **D–F** CFLAR expression was positively correlated with CD8 +T cell infiltration in BRCA. **G–K** Single-cell sequencing of CFLAR in BRCA. **L**–**M** Correlation between CFLAR expression and prognosis in patients treated with anti-PD- 1 and anti-PD-L1 therapies. *$P < 0.05$

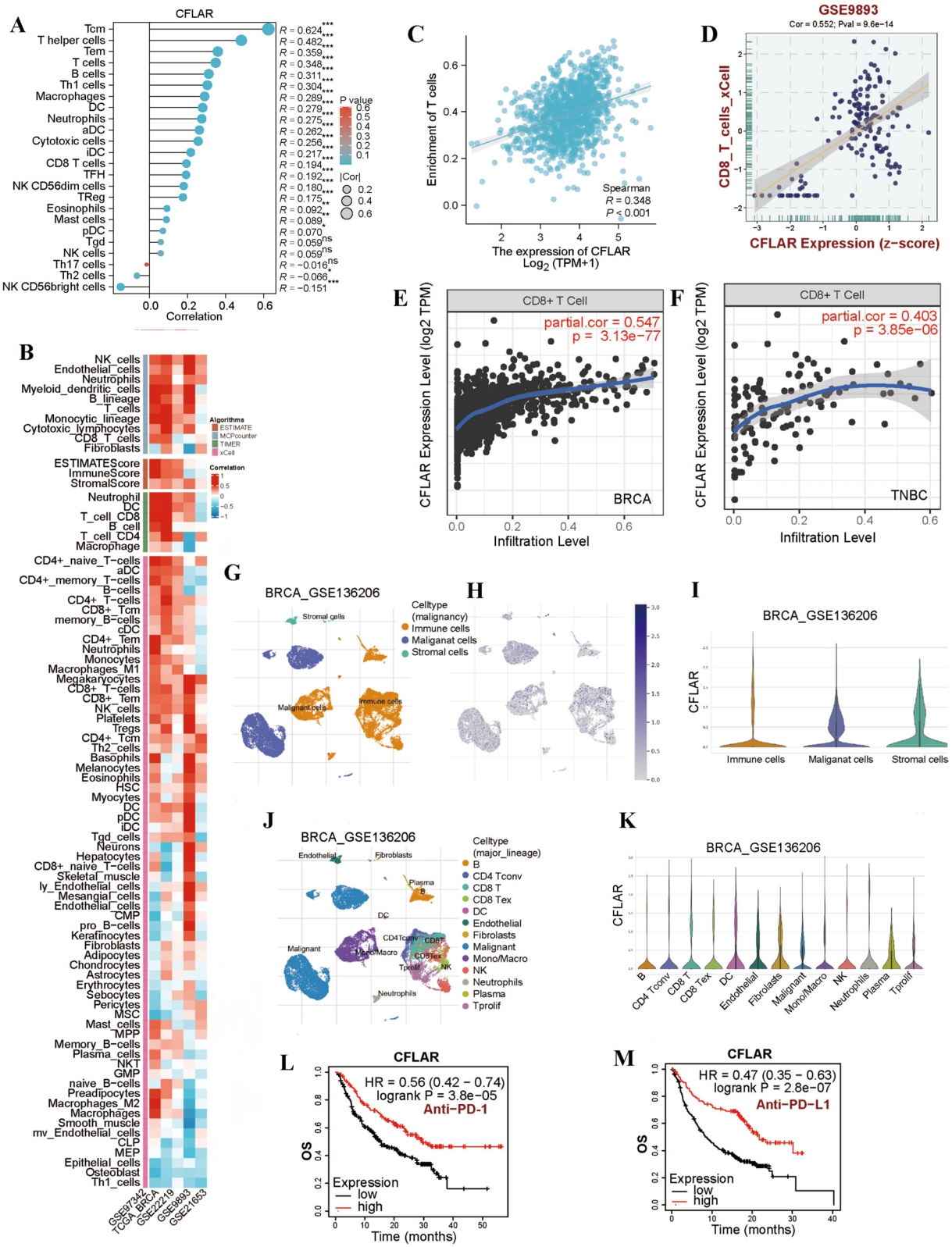

Fig. 6 (See legend on previous page.)

by targeting EVA1 A, thus affecting the proliferation and migration of TNBC cells [40]. Under hypoxic conditions, cell apoptosis-associated signaling can affect tumorigenesis in BRCA [41]. Ketoprofen suppresses the growth of TNBC cells by inducing apoptosis and inhibiting autophagy [42]. Therefore, investigating the therapeutic effects of cell death mechanisms may help to develop promising strategies for the treatment of BRCA. In particular, the precise role of CFLAR, a cell death-associated gene, in prognosis and treatment resistance in BRCA warrants further investigation. In this study, CFLAR was identified as a prognostic marker and potential therapeutic target for BRCA. The immune cell infiltration of CFLAR across various cancers was assessed using the Genomic Data Commons (GDC) Portal to prove the regulatory effects of CFLAR on tumor immunity.

Previous studies have shown that CFLAR is highly expressed in a variety of cancers, including pancreatic cancer [43] and colorectal cancer [44]. Other studies have also demonstrated that CFLAR expression is low in soft tissue sarcoma (STS) [45]. Here, we found that CFLAR was downregulated in BRCA, and its low expression was associated with a poor prognosis. miR- 27a ameliorates chemoresistance in BRCA cells by disrupting ROS homeostasis [46]. Accordingly, we observed overexpression of CFLAR attenuated 5-FU resistance by decreasing cellular ROS levels and inhibiting oxidative stress responses. This may be through activation of the NRF2-HMOX1-SOD1 pathway, which plays a key role in redox homeostasis [47]. Chemotherapy are one of the conventional treatment strategies for BRCA patients [48]. And we found that CFLAR expression was higher in patients treated with trastuzumab, taxanes, anthracycline, CMF, and FEC. Moreover, high CFLAR expression improved the survival time of patients treated with tamoxifen. Although the correlation between CFLAR expression and 5-FU sensitivity was not statistically significant, it may have a certain clinical significance. Moreover, we found that overexpression of CFLAR remarkably promoted 5-FU-mediated cell clonal inhibition. These results supported that CFLAR might be a critical chemotherapeutic target for human cancers [10]. In colorectal cancer cells, CFLAR overexpression inhibits 5-FU-induced cell death [49]. Silencing of SART1 could down-regulate the caspase 8 inhibitor, CFLAR, subsequently inducing apoptosis and reversing 5-FU resistance [50].

The tumor immune microenvironment (TIME) is closely associated with the clinical prognosis of patients with cancer [51–53]. Activation of CD8 + T cells can enhance the anti-tumor efficacy of immunotherapy by regulating ferroptosis [54]. A study reported that PD-L1 blockade resulted in an increase in lipid ROS and a decrease in tumor weight [55]. A systematic meta-analysis showed that high CD8 + T cell infiltration levels were associated with a better prognosis in BRCA [56]. Moreover, in pancreatic ductal adenocarcinoma (PDAC), monocytes expressing CFLAR upregulate PD-L1 through NF-κB activation and promote immune escape in the tumor microenvironment [57]. Notably, patients with non-small cell lung cancer (NSCLC) with reduced CFLAR expression in monocyte MDSCs showed an improved response against PD- 1 therapy, suggesting that CFLAR inhibition can work synergistically with existing immunotherapies [58]. Here, this study demonstrated that CFLAR expression was associated with the abundance of various tumor-infiltrating immune cells, including Tcm cells, T helper cells, Tem cells, B cells, $CD56^{bright}$ NK cells, and Th2 cells. Especially, CFLAR expression was positively correlated with the abundance of CD8 + T cells, which is similar to the previous reports [59, 60]. Moreover, the diagnostic and prognostic biomarker CFLAR has been proven to positively modulates the infiltration of CD8 + T cells and M1 macrophages in TIME [45]. Thus, our results collectively suggested that CFLAR plays a potential role in the immune response of BRCA and potentially functions as a novel immunotherapeutic target.

This study elucidates the prognostic significance of CFLAR in BRCA and its role in enhancing 5-FU sensitivity and immune cell infiltration. However, the study has several limitations. GEO dataset analysis (GSE20437 and GSE21422) may be affected by platform batch effects and sample selection bias. The TCGA validation, due to its limited sample size and retrospective design, introduced confounding factors such as treatment history and tumor heterogeneity into the following analysis. In this study, in vitro cell experiments may not be able to accurately mimic the interaction between cells and extracellular mechanisms in vivo and the dynamic changes of the immune system, etc. Therefore, further in vivo studies or clinical trials are needed to determine the specific mechanism of CFLAR in BRCA.

TP53 mutations and HER2 overexpression were generally associated with higher tumor aggressiveness and worse prognosis [61, 62]. Apart from these biomarkers, we also found that low expression of CFLAR was associated with poorer prognosis in breast cancer patients. Aberrantly expressed CFLAR more

likely to influence BRCA prognosis and chemotherapy resistance by regulating the interaction of cell death and immune microenvironment, which is different from the traditional mechanism of genetic mutations or surface markers. In particular, CFLAR is closely related to the infiltration of immune cells (such as CD8 + T cells) in the tumor immune microenvironment, showing its potential in immunotherapy.

Conclusion

In conclusion, this study demonstrated that CFLAR was downregulated in BRCA and its low expression indicated a poor prognosis. Overexpression of CFLAR sensitized BRCA cells to 5-FU. In addition, CFLAR expression is positively correlated with the abundance of CD8 + T cells, affecting the anti-BRCA immune response. While these findings are promising, further research, including clinical validation, is required to confirm CFLAR's potential as a prognostic and therapeutic marker in BRCA. Altogether, this study highlights the involvement of CFLAR in both ROS regulation and immune modulation in BRCA.

Abbreviations

BRCA	Breast cancer
CFLAR	Caspase 8 and Fas-associated protein with death domain-like apoptosis regulator
PD- 1	Programmed cell death- 1
PD-L1	Programmed death ligand- 1
ROS	Reactive oxygen species
DEGs	Differentially expressed genes
OS	Overall survival
RFS	Recurrence-free survival
PRI	Progression-free interval
SD	Standard deviation
ECL	Enhanced chemiluminescence
MDSCs	Myeloid-derived suppressor cells
UVM	Uveal melanoma
SARC	Sarcoma
MESO	Mesothelioma

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40001-025-02532-4.

Additional file 1. The main characteristics of two GEO datasets for breast cancer

Additional file 2. The cell death-associated signal molecules

Additional file 3. The primers used for qRT-PCR

Additional file 4.BT- 549 and MDA-MB- 231 cells were transiently transfected with siCFLAR.The flow cytometry analysis shows that CFLAR knockdown enhances the promoting effect of 5-FU on cell growth. Quantitative analysis of ROS levels in BT- 549 and MDA-MB- 231 cells showed that ROS production in CFLAR-knockdown cells increased significantly after 5-FU treatment.The levels of NRF2, HOMX1 and SOD1 in CFLAR-overexpressed cells treated with 5-FU. Each bar represented the mean ± SD. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Additional file 5. The regulatory role of CFLAR in tumor immunity across various cancers.Spearman correlation of CFLAR expression with immune cell fractions across various cancer types. The color scale reflects the strength of the correlation, from - 1.0to + 1.0.CFLAR expression is positively correlated with CD8 + T cell infiltration in UVM, SARC, and MESO. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

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Author contributions

Acquisition of Data: SY and FW. Analysis and Interpretation of Data: SY and WC. Conception and Design: LX and DZ. Data Curation: SL. Development of Methodology: DZ. Implementation of experiments: WC. Writing the manuscript: LX and PJ. All authors contributed to the article and approved this submitted version.

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Availability of data and materials

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not available.

Competing interests

The authors declare no competing interests.

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