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Elevated expression of ELK1 promotes breast cancer cell growth and correlates with poor prognosis of breast cancer patients

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Background: Breast cancer is the most common tumor in women and poses a serious threat to women's physical and mental health. The ETS-like gene 1 (ELK1), upregulated in various malignancies, serves as a transcription regulatory factor. This study primarily investigates the biological functions and prognostic significance of ELK1 in breast cancer.

Materials and methods: The authors conducted an analysis of ELK1 expression in breast cancer and adjacent tissues using data from The Cancer Genome Atlas (TCGA), and validated these findings with clinical specimens. Additionally, the authors employed siRNA transfection, proliferation and apoptosis assays to elucidate the roles of ELK1 in breast cancer cells. Furthermore, we assessed the correlations between ELK1 expression and the tumor microenvironment, as well as tumor-infiltrating immune cells (TIICs), utilizing the ESTIMATE and CIBERSORT algorithms. Finally, we used Kaplan–Meier plots and COX regressions to identify prognostic factors, and developed a predictive alignment diagram to evaluate the prognostic significance of ELK1 in breast cancer.

Results: A marked increase in ELK1 expression is evident in breast cancer tissues ($P < 0.01$). Experimental findings demonstrate that silencing ELK1 suppresses proliferation and promotes apoptosis in breast cancer cells. ELK1 plays a pivotal role in regulating the immune microenvironment of breast cancer. Furthermore, the alignment diagram indicates that ELK1 may serve as an independent prognostic factor for breast cancer patients.

Conclusion: The authors' study reveals that ELK1 exhibits a high expression level in breast cancer tissues and is associated with disease progression and poor prognosis.

Keywords: apoptosis, breast cancer, ELK1, prognosis, proliferation

Introduction

Breast cancer stands as the most prevalent tumor among women, as evidenced by cancer statistics released by the American Cancer Society, indicating that breast cancer accounts for 31% of female cancers. However, there has been a remarkable decline in the mortality rate of breast cancer, dropping by 43% since 1989, with an average five-year survival rate reaching 90%[1]. Unlike other malignancies, early detection of female breast cancer is feasible through self-examination and early screening, thereby enhancing the likelihood of successful treatment and reducing mortality rates associated with breast cancer. Nevertheless, the molecular mechanisms underlying the onset and progression of breast cancer remain incompletely understood, influenced over time by various genes, factors, and stages, exhibiting heterogeneity[2–4]. Furthermore, CA15-3, a widely used clinical serological index for breast cancer, demonstrates suboptimal specificity and sensitivity, lacking the capacity to offer timely alerts regarding the progression of breast cancer[5]. Consequently, delving deeper into novel genes associated with breast cancer holds important significance in elucidating its molecular mechanisms, devising rational therapeutic drugs, and evaluating prognosis.

ELK1 belongs to the E-twenty six (ETS) family, one of the largest families of transcription regulatory factors in the cell. These factors encompass highly conserved DNA binding regions and participate in embryonic development, cell growth, differentiation, and apoptosis via the mitogen-activated protein kinase (MAPK), Ca^{2+}, and TGF-β signaling pathway[6,7]. Research indicates that ETS family proteins can modulate the expression of numerous tumor genes and tumor suppressor genes by directly or indirectly regulating protein interactions[7]. Additionally, studies

HIGHLIGHTS

- We explored the biological functions and prognostic significance of ETS-like gene 1 (ELK1) in breast cancer.
- We combined experiments with bioinformatics analysis to conduct this study.
- ELK1 silencing inhibits proliferation and promotes apoptosis in breast cancer cells.
- Highly expressed ELK1 correlates with tumor immune cell infiltration and poor prognosis.

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Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

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have demonstrated that the silence of ELK1 results in decreased colony formation, cell migration, and invasion, as well as increased apoptosis in bladder cancer cells[8]. Our previous study also shows that ELK1 expression is upregulated in liver cancer cells, and silence of ELK1 reduces the migration and invasion ability of HuH7 cells[9]. Other studies have shown that ELK1 activation in prostate cancer cells contributes to tumor progression, whereas interference with ELK1 can impede tumor growth[10].

In the present research, we integrate cytological experiments with bioinformatics analysis to investigate the impact of ELK1 on breast cancer cell growth and evaluate its prognostic significance for breast cancer patients.

Material and methods

Analysis of ELK1 expression between breast cancer tissues and controls in TCGA database

mRNA expression levels and clinical data from 1113 female breast cancer and 113 normal breast samples were retrieved and analyzed from the TCGA database (https://portal.gdc.cancer.gov/) (access date: 24 August 2023). Clinical variables for breast cancer patients encompassed age, sex, survival duration, mortality status, and TNM stage.

The non-expressed genes were excluded, and the multiple expression levels of genes were averaged. ELK1 gene was chosen for visualization, and the Wilcox Test along with the "Beeswarm" package in R software were employed to analyze differences in ELK1 mRNA expression between breast cancer and normal/adjacent cancer tissues. A false discovery rate (FDR) less than 0.05 and $|Log_2FC|$ greater than 2 were established as screening criteria, with P less than 0.05 indicating statistical significance.

Correlation analysis between ELK1 expression and tumor-infiltrating immune cells (TIICs) in breast cancer

Samples were divided into high and low ELK1 expression groups according to the median value (50%) of ELK1 expression. We examined the variation in TIICs between the high and low expression groups of ELK1 using "CIBERSORT" analysis tool (https://cibersort.stanford.edu/). The tumor microenvironment analysis primarily focused on assessing disparities in stromal and immune cell compositions, and scores with the "Estimate" package, illustrated via a violin plot. Furthermore, the correlation between differential ELK1 expression and immune cell populations was assessed through Spearman correlation analysis, visually depicted using a lollipop diagram.

Correlation analysis of ELK1 and related genes of immune checkpoint

We conducted a correlation analysis between ELK1 and immune checkpoint-related genes. Immune checkpoint genes were screened to identify those associated with ELK1, employing a correlation test with a significance threshold of P less than 0.001. Positive and negative correlations are depicted in red and blue, respectively.

Kaplan–Meier plotter survival analysis

For Kaplan–Meier survival analysis, ELK1 (210850_at) was input into the "Gene symbol" column. The survival endpoint options include post-progression survival (PPS), distant metastasis-free survival (DMFS), or overall survival (OS), with the "Auto select best cutoff" option enabled. Kaplan–Meier survival plots were generated accordingly. Additionally, hazard ratios and log-rank P values were computed and presented on the main plot.

Analysis of independent prognostic value of ELK1 and generation of Alignment diagram

To assess the independent prognostic significance of ELK1, we conducted a prognostic analysis employing the "Survival" package. Univariate and multivariate Cox regression analyses were employed to examine the associations between the ELK1 gene, patient age, TNM stage, and patient prognosis. The outcomes were presented using a forest plot. Subsequently, we explored the correlation between the ELK1 gene and identified independent prognostic factors of breast cancer through chi-square testing, followed by the generation of a predictive Alignment diagram.

Clinical specimens, tissue microarray and cell lines

Fresh-frozen breast cancer tissues stored at – 80°C were obtained from patients who underwent surgery at our hospital. Ethical approval for specimen usage was granted by the hospital's Ethics Committee. Tissue microarrays were procured from Shanghai Outdo Biotech Co., Ltd. Additionally, cell lines in this study were sourced from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China), having undergone mycoplasma testing and authentication.

Immunohistochemical staining (IHC)

Tissue chips were heated overnight at 65°C and deparaffinized using dimethylbenzene and ethyl alcohol. Antigen retrieval was then conducted in EDTA buffer (pH 9.0) heated to 97°C for 20 min. Following endogenous peroxidase blocking with 2% hydrogen peroxide, specimens were incubated with the ELK1 primary antibody (diluted 1:400, ab32106, Abcam) at room temperature for 60 min. Subsequently, specimens were incubated with a secondary antibody (K5007, Dako) for 30 min. Finally, specimens were stained with 3,3'-diaminobenzidine (DAB) and counterstained with hematoxylin. The expression of ELK1 was assessed based on the staining intensity and the percentage of positive cells, scored as follows: staining intensity - 0 (no staining), 1 (light yellow), 2 (dark yellow), and 3 (brown); percentage of positive cells—0 (<5%), 1 (5–25%), 2 (26–50%), 3 (51–75%), and 4 (76–100%).

Transfection of small interfering RNA (siRNA)

Cells in the logarithmic growth phase were transfected with ELK1 siRNA using Lipofectamine 3000 (CN2481201; Invitrogen) following the manufacturer's protocol. Specific sequence of ELK1 siRNA targeting is 5'-GGCTACGCAAGAACAAGAC-3'. The siRNAs were synthesized by RIBOBIO Biotechnology Co., LTD. (Guangzhou, China).

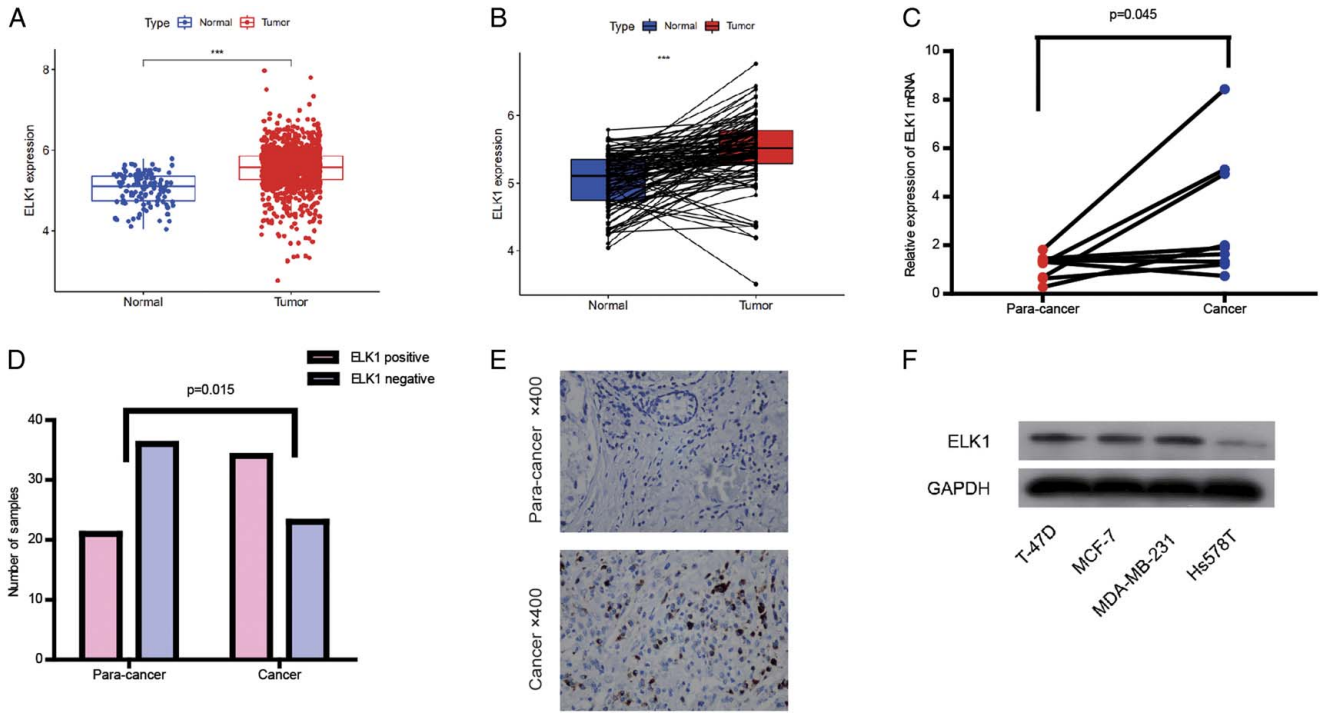

Figure 1. Expression levels of ELK1 in breast cancer. (A) ELK1 expression was significantly upregulated in 1113 female breast cancer samples compared to 113 normal breast tissues. (B) Differential expression of ELK1 between breast cancer and matched para- cancer tissues in The Cancer Genome Atlas database. (C) ELK1 mRNA Expression levels detected through polymerase chain reaction in breast cancer and their para-cancer tissues. (D) Comparison of ELK1 protein expression between breast cancer and paired normal tissues. (E) Representative images of breast cancer and paired normal tissues stained with IHC. Magnification: 400×. (F) ELK1 protein expression in 4 breast cancer cell lines. ***$P < 0.01$. ELK1, ETS-like gene 1.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from breast cancer tissues and cells using AIDzol reagent (341733AX; Aidlab), and quantified with One Step TB Green PrimeScript RT-PCR Kit (RR066A; TaKaRa). PCR reactions were proceeded under the following conditions: 42°C for 5 min, 95°C for 10 sec, 40 cycles of 95°C for 3 sec, and 60°C for 30 sec. Levels of ELK1 expression were calculated by the $2^{-\Delta\Delta CT}$ method. The PCR primer sequences were as follows:

ELK1 F, 5′-AACTTTCAGGAGACCCGTCC-3′,
R, 5′-CCATCCCAAGGGGTACTGTTA-3’;
β-actin F, 5’-ACCCACACTGTGCCCATCTAC-3’,
R, 5’-TCGGTGAGGATCTCATGAGGTA-3’.

Western blotting (WB)

Protein was extracted using Enhanced RIPA lysate (AR0102, Boster) and quantified with BCA protein assay kit (P0010, Beyotime Biotechnology). Subsequently, protein separation was performed using SDS-PAGE (P1200, Solarbio) and then transferred to the PVDF membranes (IPVH00010, Billerica). After blocking non-specific antigen, the membranes were incubated overnight at 4°C with the following primary antibodies: GAPDH (1:3000; ET1601–4; Huabio), ELK1 (1:500, ab32106, Abcam), and PTEN (1:1000, ab170941, Abcam). The next day, the target proteins were detected using a secondary antibody (1:5000; A0216; Abcam) after washing with tris-buffered saline with tween (TBST). Lastly, developing was performed using BeyoECL Star (P0018AS; Beyotime) and automatic imager.

Cell proliferation assay

One hundred microlitres suspension with 2×10^3 cells was dispensed into 96-well plate, with five replicate wells per group. Then cells were cultured at 37°C for ~4 h until adherent. At this point, 10 µl Cell Counting Kit-8 (CCK-8) solution (C0042, Beyotime, China) was added to each well and incubated for an additional 2 h. Blank control wells, consisting of the equivalent volume of cell culture medium and CCK-8 solution without cells, were also included. Lastly, the absorbance was measured at 450 nm (A450). The following 4 days, CCK-8 solution was added, and absorbance was measured at the same time.

Flow cytometry apoptosis experiment

The apoptosis of breast cancer cells was tested by flow cytometry. Adherent cells were digested into single-cell suspensions with trypsin, and washed twice with pre-cooled PBS. The cell suspension was then centrifuged, the supernatant discarded, and the cells re-suspended in 195 µl of Binding Buffer. Subsequently, 5 µl of Annexin V-FITC and 10 µl of PI Stain (HY-K1073, MedChemExpress) were added to the cells, and the mixture was gently mixed before being incubated in the dark for 15 min. Cell fluorescence was analyzed using a flow cytometer (Beckman Coulter).

Table 1

Relationships between ELK1 expression and clinical features.

Clinical features	*N*	ELK1 positive	ELK1 negative	Positive rate (%)	$\chi 2$	*P*
Para cancer	57	21	36	36.8	5.937	0.015*
Breast cancer	57	34	23	59.6		
Age						
≤55	26	17	9	65.4	0.653	0.419
>55	31	17	14	54.8		
Stage						
I + II	38	24	14	63.2	0.583	0.445
III + IV	19	10	9	52.6		
Lymphatic						
Positive	26	19	7	73.1	4.520	0.033*
Negative	31	14	17	45.2		
ER						
Positive	42	24	18	57.1	1.222	0.269
Negative	15	11	4	73.3		
PR						
Positive	37	24	13	64.9	1.192	0.275
Negative	20	10	10	50.0		
Her-2						
Positive	47	27	20	57.4	0.144	0.704
Negative	10	7	3	70.0		
Ki-67						
Positive	51	31	20	60.8	0.005	0.945
Negative	6	3	3	50.0		

*Statistical significance ($P < 0.05$).

ELK1, ETS-like gene 1; ER, estrogen receptor; Her-2, human epidermal growth factor receptor-2; N, number; PR, progesterone receptor.

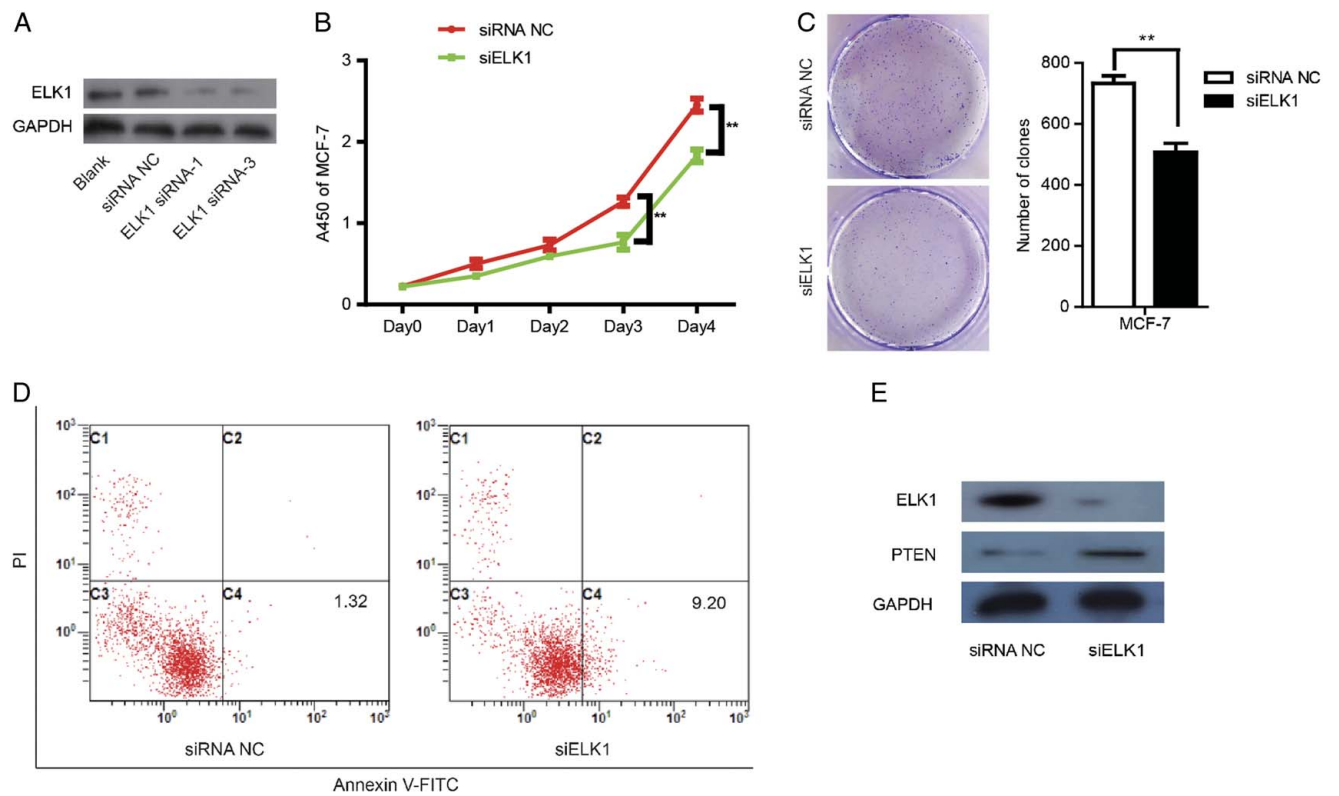

Figure 2. Silencing of ELK1 inhibits the proliferation and promotes apoptosis of MCF-7 cells. (A) Confirmation of ELK1 silencing by small interfering RNA (siRNA) using western blotting after 48 h of transfection. (B, C) Silencing of ELK1 inhibits the proliferation rate of MCF-7 cells by cell counting kit-8 and plate cloning experiments. (D) Silencing of ELK1 promotes apoptosis of MCF-7 cells by flow cytometry experiment. (E) Silencing of ELK1 upregulates the level of phosphatase and tensin homology (PTEN) expression. Mean ± SD, $n \geq 3$, $^{**}P < 0.01$. ELK1, ETS-like gene 1.

Statistical methods

Data analyses were performed using several R software packages, including Limma, Beeswarm, Survival, and Cluster Profiler. Paired or independent sample *t*-test was used to evaluate differences between groups. The χ^2 or continuity correction test was used to investigate the relationship between ELK1 and clinical features. Survival analysis was conducted using COX regression. *P* less than 0.05 was regarded as statistical significance.

Results

Upregulation of ELK1 expression in breast cancer

Expression levels of ELK1 mRNA in breast cancer and normal breast tissues were obtained from TCGA, and ELK1 expression was markedly elevated in breast cancer tissues compared to control tissues ($P < 0.001$) (Fig. 1A-B).

To corroborate the results from TCGA, we examined the differences in ELK1 expression at both mRNA and protein levels. ELK1 expression was assessed in 9 paired breast cancer tissues, revealing a substantial increase in breast cancer compared to para-cancer tissues ($P < 0.01$) (Fig. 1C). IHC staining of the tissue chip demonstrated predominant nuclear localization in breast cancer cells, with higher ELK1 protein levels observed in tumor tissues compared to para-cancer tissues (Fig. 1D, E). In addition, we investigated the association between ELK1 expression and various clinical features. The analysis revealed a significant correlation between ELK1 expression and lymph node involvement ($P = 0.033$), while no significant associations were found with age, TNM stage, or expression levels of ER, PR, HER-2, and Ki-67 (Table 1).

After that, we measured the ELK1 expression in four types of breast cancer cells. As shown in Figure 1F, ELK1 expression was notably lower in Hs578T cell compared to the other three cell lines. Consequently, we opted to utilize MCF-7 and MDA-MB-231 cells for our in-vitro experiments.

ELK1 silencing suppresses proliferation and induces apoptosis in breast cancer cells

To investigate the impact of ELK1 on proliferation and apoptosis in breast cancer cells, we conducted CCK-8 assay, plate cloning experiment, and flow cytometry apoptosis assay. ELK1 expression was suppressed in MCF-7 and MDA-MB-231 cells using ELK1-specific siRNA. Confirmation of ELK1 knockdown at the protein level was achieved through western blot analysis (Figs. 2A, 3A). The CCK-8 indicated that the proliferation rate of MCF-7 cells with siELK1 transfection was noticeably decreased compared to the negative control group on the third ($P = 0.008$) and fourth day ($P = 0.005$). The plate cloning experiment corroborated these findings, showing a decrease in the number of cell clones in the

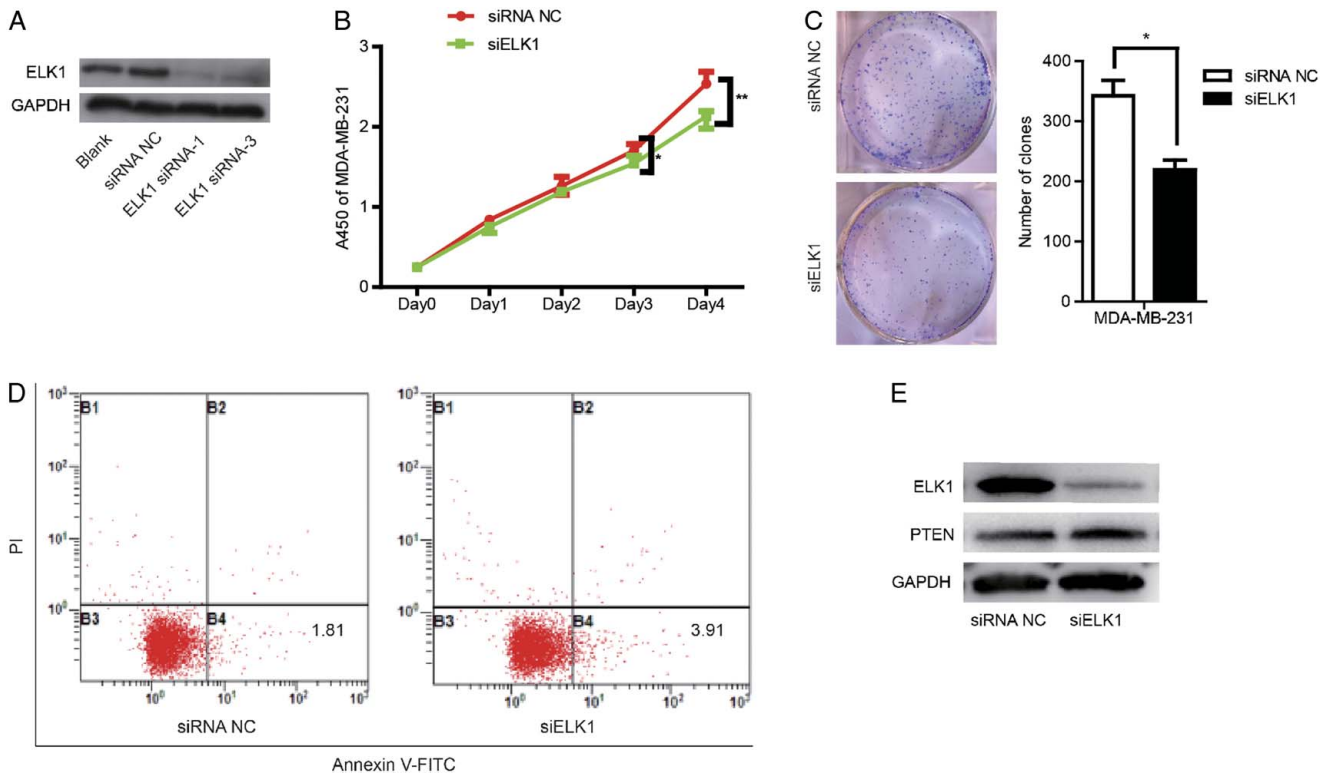

Figure 3. ELK1 interference restrains the proliferation and accelerates apoptosis of MDA-MB-231 cells. (A) Confirmation of ELK1 silencing by siRNA using western blotting after 48 h of transfection. (B, C) ELK1 interference restrains the proliferation rate of MDA-MB-231 cells by cell counting kit-8 and plate cloning experiments. (D) ELK1 interference accelerates apoptosis of MDA-MB-231 cells by flow cytometry experiment. (E) ELK1 interference promotes the expression of phosphatase and tensin homology (PTEN). Mean ± SD, n ≥ 3, *$P < 0.05$, **$P < 0.01$. ELK1, ETS-like gene 1.

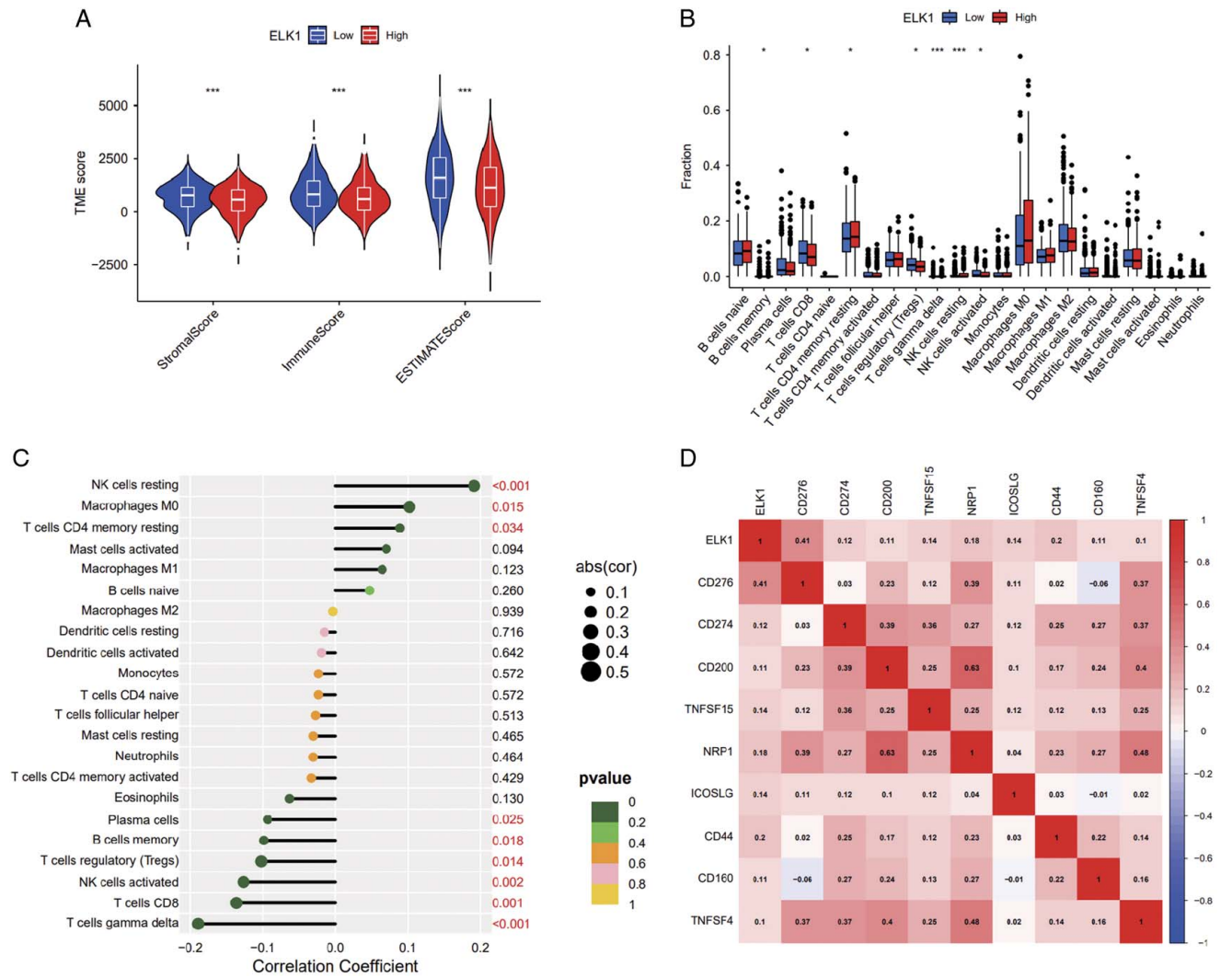

Figure 4. Analysis of tumor-infiltrating immune cells predicted by CIBERSORT. (A) The difference in tumor-infiltrating immune cells within the tumor microenvironment between the high and low ELK1 expression groups shown by violin chart/diagram. (B) Fractions of 22 tumor-infiltrating immune cells in the high and low ELK1 expression groups. (C) Lollipop chart showing the correlation between ELK1 expression and various immune cells (Spearman correlation analysis). (D) Correlation matrix illustrating the relationship between ELK1 and immune checkpoint-related genes. $^{*}P < 0.05$, $^{***}P < 0.01$. ELK1, ETS-like gene 1.

siELK1 group compared to the negative control group ($P = 0.004$) (Fig. 2B-C). Similarly, ELK1 silencing inhibited the proliferation rate on the third ($P = 0.034$) and fourth day ($P = 0.005$), as well as the formation of cell clones ($P = 0.015$) in MDA-MB-231 cells (Fig. 3B-C). Flow cytometry apoptosis assays demonstrated an increase in early apoptosis in cells transfected with siELK1 compared to the control groups (Figs. 2D, 3D).

To elucidate the molecular mechanisms underlying ELK1-mediated effects on proliferation and apoptosis, we assessed the expression of phosphatase and tensin homology (PTEN). Our analysis revealed upregulation of PTEN in both MCF-7 and MDA-MB-231 cells following siELK1 transfection (Figs. 2E, 3E). These results indicate that ELK1 silencing may inhibit cell proliferation and promote apoptosis in breast cancer cells via regulation of PTEN.

Comprehensive analysis of tumor microenvironment, TIICs and immune checkpoints in the ELK1 high and low groups from TCGA

We commonly assess the presence of immune and stromal cells within the tumor microenvironment using the ESTIMATE algorithm. According to ESTIMATE's principle, higher scores for immune and stromal components indicate a greater abundance of these cells within the tumor microenvironment, meanwhile elevated ESTIMATE scores correspond to fewer tumor cells[11]. Our study revealed a significant upregulation of ELK1 expression in samples with lower immune/stromal or ESTIMATE scores (more tumor cells). These results suggested that ELK1 was highly expressed in breast cancer cells (Fig. 4A).

The CIBERSORT algorithm was used to estimate the immune cell infiltration score for each sample, and data of 22 immune cell subtypes were obtained[12]. The differences in the

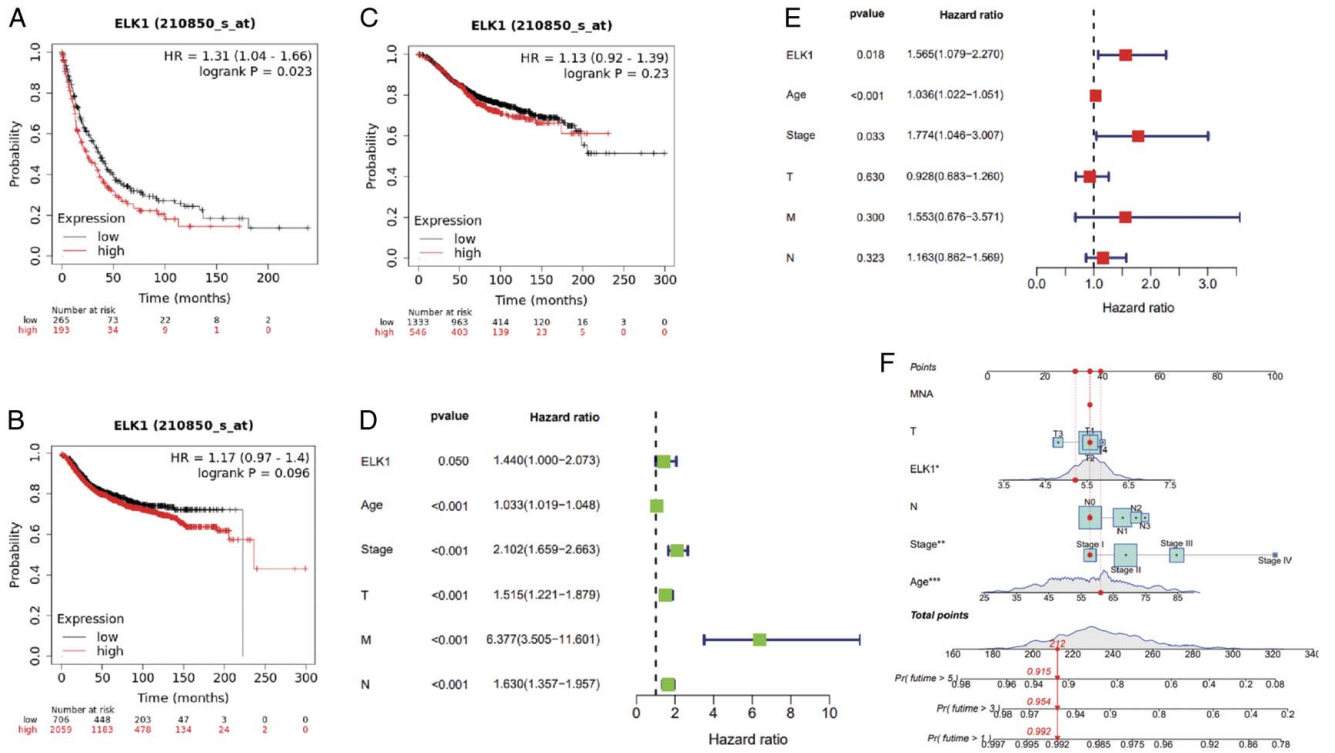

Figure 5. Prognostic significance of ELK1 in breast cancer. (A–C) Kaplan–Meier plots indicating the association between ELK1 expression and patients' post-progression survival (A), distant metastases-free survival (B), and overall survival (C). (D) The prognostic factors of breast cancer via univariate cox regression analysis. (E) The prognostic factors of breast cancer via multivariate cox regression analysis. (F) patient age, stage, and ELK1 expression were used to construct a nomogram to predict the 1-year, 3-year and 5-year survival rate of breast cancer patients. *$P < 0.05$, **$P < 0.01$, ***$P < 0.01$. ELK1, ETS-like gene 1.

fraction of each immune cell type between the high and low ELK1 groups were analyzed. Notably, the infiltration density of memory B cells, CD8 T cells, regulatory T cells (Tregs), gamma delta T cells, and activated NK cells were significantly increased in the low ELK1 expression group, and negatively regulated by ELK1. Conversely, the infiltration density of memory resting CD4 T cells and resting NK cells were significantly increased in the high ELK1 expression group, and positively regulated by ELK1 (Fig. 4B-C). In addition, expression of immune checkpoint genes (CD276, CD44) was positively related to ELK1 expression (Fig. 4D). These results imply that ELK1 plays a crucial role in regulating breast cancer immune microenvironment.

Evaluation of the independent prognostic value of ELK1 in breast cancer and construction of a predictive Alignment diagram

The Kaplan–Meier Plotter analysis revealed a significant association between elevated ELK1 expression and poor post-progression survival (PPS) among breast cancer patients (Fig. 5A). However, there was no obvious correlation with DMFS or OS (Fig. 5B, C).

The Cox survival analysis exhibited a meaningful survival difference between the high and low ELK1 expression groups. Univariate COX analysis exhibited that ELK1 expression, age, stage and TNM status had independent prognostic significances in breast cancer (Fig. 5D). Additionally, multivariate COX analysis indicated that ELK1 expression, age and stage independently influenced the prognosis of breast cancer patients (Fig. 5E). Alignment diagrams are mainly used for prognostic predictions in cancer patients[13]. In our research, ELK1 expression, patient age, and stage were used to construct an Alignment diagram to predict the 1-year, 3-year and 5-year survival rate of breast cancer patients, which could offer valuable insights for individualized clinical assessment and treatment strategies (Fig. 5F). These results display that ELK1 could be considered as an independent prognostic factor for breast cancer patients.

Discussion

Early diagnosis plays a crucial role in the treatment of breast cancer patients, emphasizing the need to identify genes that can aid in diagnosis and prognosis. Several studies have highlighted the impact of ELK1 on tumor onset, progression, and metastasis. Knockdown of ELK1 has been shown to inhibit the malignant characteristics of bladder cancer cells[14], while ELK1 promotes the advancement and epithelial-mesenchymal transition of lung cancer by increasing B7-H3 expression[15].

In this study, we find that ELK1 expression was increased in breast cancer compared with para-cancer tissues, which corresponded to the results from TCGA data. Additionally, previous studies have shown that ELK1 promotes cell proliferation[16] and cell cycle progression in breast cancer[17]. Similarly, our research showed that interference of ELK1 decreased proliferation and increased apoptosis capacity of breast cancer cells, indicating ELK1 in breast cancer development. Moreover, transfection of

ELK1 siRNA led to upregulation of PTEN expression in breast cancer cells, aligning with previous findings[18,19]. Studies have also shown that ELK1 inhibition significantly induces PTEN expression, suppressing cell viability and clonal formation[8,20]. PTEN acts as a tumor suppressor by regulating various cellular processes including cell cycle, migration, and apoptosis, and is tightly related to the occurrence of various malignant tumors[21]. PTEN overexpression significantly declines the cell proliferation rate during the logarithmic growth phase and activates intrinsic apoptosis pathways[22]. These results suggest that ELK1 can affect the proliferation and apoptosis of various tumor cells by regulation of PTEN.

Several studies have experimentally elucidated that the tumor microenvironment significantly influences immunotherapy response and resistance to various cancers, and impacting their occurrence, development and prognosis[23]. Tumor-infiltrating lymphocytes (TILs) serve as valuable indicators of the tumor microenvironment and exhibit dynamic changes during tumor progression[24]. TILs have shown potential prognostic value in triple-negative and Her-2 overexpressed breast cancer[25,26]. In this study, we analyzed the breast cancer tumor microenvironment using the transcriptome data from the TCGA database by means of two algorithms, ESTIMATE and CIBERSORT. Our analysis revealed a correlation between the content of stromal and immune components in the tumor microenvironment and ELK1 expression levels. Thus, ELK1 appears to be closely associated with tumor immune cell infiltration and may contribute to the pathogenesis of breast cancer.

Given that ELK1 is highly expressed in breast cancer tissues, promotes proliferation, inhibits apoptosis of breast cancer cells, and is closely associated with tumor immune cell infiltration and poor post-progression survival (PPS), we assessed the independent prognostic significance of ELK1 in breast cancer using Cox survival analysis. Additionally, we constructed an Alignment diagram to predict the prognosis of cancer patients. Consistently, study has demonstrated that ELK1 is a strong, independent prognosticator of disease recurrence in prostate cancer, underscoring its unique role in prostate cancer progression[27]. Furthermore, ELK1 has been implicated in promoting the advancement of pancreatic cancer and conferring a poor prognosis[28].

In summary, this study demonstrates that ELK1 expression is highly expressed in breast cancer tissues and correlates with the growth of breast cancer cells. Moreover, TCGA data analysis indicates that ELK1 is related to tumor immune cell infiltration and may serve as an independent prognostic factor for breast cancer patients.

Ethical approval

Medical Ethics Committee of Shaoxing Hospital, Zhejiang University.

Consent

We have nothing to declare in this category.

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Author contribution

X.X.: visualization, writing the paper. Y.W.: data collection, data analysis. Q.W.: study design. S.L.: interpretation. G.M.: supervision, editing the paper.

Conflicts of interest disclosure

The authors declare no conflicts of interest.

Research registration unique identifying number (UIN)

Our study did not involve human subjects.

Guarantor

Xiuping Xu, Guofeng Mao.

Data availability statement

Any datasets analyzed during the current study are available upon reasonable request.

Provenance and peer review

Our paper was not invited.

Presentation

None.

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