ELF5 inhibits migration and invasion of breast cancer cells via regulating CD24 expression

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Research

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Abstract

Objective: E74-like factor five (ELF5) is a basic transcriptional factor that plays a key role in breast tissue and glandular development. However, the molecular mechanism by which ELF5 mediates the biological functions of breast cancer cells has not been elucidated. We hypothesize that ELF5 regulate CD24 transcription though an E26 transforming sequence (ETS) cis-element in the CD24 promoter region.

Methods: Human breast cancer cell line MCF-7 was transfected with Myc-ELF5 plasmid in over-expression experiment. T47D cells were transfected with ELF5-shRNA plasmid in knockout experiment. The expression level of ELF5 `protein was analyzed by Western blot. MTT assays and Clonal formation assays were used to assess the proliferation properties of cells. Scratch wound-healing assays were performed to determine cell migration and invasive activities. CD24 protein expression was analysis by flow cytometry. Using the bioinformatics tool JASPAR, we identified high-scoring ETS-like sequences in the CD24 gene promoter. To confirm the ETS, Chromatin immunoprecipitation (ChIP) analysis was used. DNA fragments of a putative or mutated ETS-like sequence were synthesized and ligated into a pGL3 basic plasmid to construct the CD24 promoter luciferase reporter systems which was used to detect ELF5 regulation of CD24 transcriptional activity.

Results: In this study, we examined the effect of ELF5 on human breast cancer cell lines MCF-7 and T49D, and confirmed that ELF5 act as a suppressor of cell proliferation, migration and invasion. In further research, the interaction between ELF5 and CD24 was characterized in breast cancer cells. We found that CD24 was a target gene of ELF5 through ChIP-Sequence assays, and proved that ELF5 could bind to ETS cis-element on the proximal promoter of CD24 gene. Importantly, experiments verified that CD24 was upregulated after ELF5 overexpression in MCF-7 cells, while knockdown of CD24 expression caused MCF-7 cells to restore cell proliferation, migration and invasion activity in adaptive ELF5 expression.

Conclusions: This study not only found that ELF5 can inhibit cell proliferation, migration and invasion in breast cancer cells, but also found that ELF5 induced CD24 expression by binding to the ETS cis element in the CD24 gene promoter sequence. This study provided a molecular mechanism for ELF5 to inhibit breast cancer from a new perspective, and provided further theoretical support for the treatment and prevention of breast cancer.

1. Introduction

Breast tumor is one of the gynecological disease,and it ranks second among female cancer patients in terms of mortality rate [1]. Despite many clinical advances in breast cancer treatment, the number of cases is still increasing. According to statistics, about 279,000 new cases are detected in the annual incidence of breast cancer in women, and it is still growing at an annual rate of about 2% in China [2]. In clinical treatment, the metastasis and invasion of tumor cells is a key hazard for the treatment of malignant tumors, and it is also the goal that many researchers pay attention to and overcome [3]. Therefore, it is extremely important to elucidate specific molecular mechanisms related to breast cancer

cell proliferation and metastasis, in order to design appropriate therapeutic agents to improve the clinical treatment of patients.

E26 transforming sequence (ETS) transcriptional factors plays vital role in breast tissue and gland development, and its expression varies with the degree of tissue differentiation. The ETS transcriptional factor superfamily contains more than 30 members. The common feature of ETS member protein is that they possess a conserved 85 amino acid DNA-binding domain that can recognize and bind the 5'-GGAA/T-3' sequence in the promoters or enhancers of the target genes. The ETS transcription factor family not only regulates a series of normal physiological processes, such as maintaining the harmonious living activities of the organism, but also modulate complex pathophysiology during tumorigenesis [4–6]. As a member of the ETS superfamily, E74-like factor Five (ELF5) is an essential regulator in mammary gland development [7, 8], especially in the differentiation progress of breast gland cell subtype transformation. Experiments have shown that ELF5 could induce estrogen receptor (ER)-positive luminal subtypes of breast cells to ER-negative basal subtypes and inhibit differentiation of breast precursor cells into estrogen-sensitive phenotypes [9, 10]. A number of reports indicate that ELF5 play a crucial role in several human tumors such as prostate cancer, ovarian carcinoma and kidney cancer [11], but little is known about the effect of ELF5 on breast cancer cells.

Tumor cells invasion and metastasis are a series of dynamic processes involving multiple cell membrane proteins [12]. The function of cell membrane molecules, especially glycoprotein, are not limited to cell recognition, adhesion, migration, or regulating cell proliferation and differentiation [13].They can also serve as biomarkers for certain tumor diseases [14]. CD24 serves as one such candidate for labeling molecule. CD24 is a highly glycosylated mucin-like antigen on the surface of cell membranes which is either N-linked or O-linked glycosylation within its correspondingly amino acid residue. In addition to glycosylated CD24, which can activate different integrin cluster, it adheres to the extracellular matrix (such as laminin or fibronectin) [15]. As a ligand for P-selectin, CD24 can also facilitate the rolling activity of breast cancer cells on intravascular and then adheres to vascular endothelial cells. CD24 is frequently expressed in human breast cancer cells and is always associated with tumorigenesis and self-renewal through signal transducer [16]. Breast cancer cell with $CD44^{high}CD24^{low}$ phonotype have been proved to have stem cell properties and represented highly drug resistance [17, 18]. It is particularly worth mentioning that CD24 cross-linking may induced MCF-7 cell apoptosis and inhibit migration activity [19].

In the present study, we first identified ELF5 as a regulator of breast cancer cells activity. ELF5 significantly induces apoptosis and inhibits cell migration and invasion activity. Next, when exploring the regulatory relationship between ELF5 and CD24, we observed that ELF5 is a trans-acting factor that binds to the ETS cis-element of the CD24 gene promoter. Subsequently, experiments verified that CD24 was upregulated after ELF5 overexpression in human breast cancer cells, and that knockdown of CD24 expression caused MCF-7 cells to restore cell proliferation, migration and invasion activity in adaptive ELF5 expression. Therefore, it is inferred that ELF5 may affect the function of breast tumor cells by regulating the expression of CD24. This conclusion understands the molecular mechanism of ELF5

inhibition of breast cancer from a new perspective and helps to develop new breast cancer treatment methods.

2. Materials And Methods

2.1 Cell culture, and antibodies

HEK-293T, MCF-7, T47D, MDA-MB-231 and COS-7 cells were cultured as previously described [20]. Cells were grown at 37°C in a humidified 5% CO_2 atmosphere and then the cells were transfected with the appropriate plasmids according different experiments purpose using Lipofectamine 2000 (Invitrogen, Auckland, New Zealand). Protein expression was analyzed with Rabbit anti-ELF5 (Abcam, Cambridge, MA, USA), Mouse anti-CD24 antibody (Santa Cruz, Dallas, CA, USA), Rabbit anti-Myc and mouse anti-Flag antibodies (Sigma, Saint Louis, MO, USA).

2.2 Plasmid and luciferase reporter construction

Human ELF5 gene was cloned from a human cDNA library using the designed primer: 5'-GCTTGAAAACAAGTGGCATC-3' (forward) and 5'-TCTTCCTTTGTCCCCACATC-3' (reverse), and the amplified ELF5 DNA fragment was inserted into the pCMV6-AC-Myc-DDK expression vector at the EcoRI and XhoI sites. pGL3 vector and the CD24 luciferase reporter construction were performed as previously described [21], and primer for pGL3-CD24-promoter: 5'-GGGGTA-CCTAGGAATACCACCAGTTAGTT-3'(forward), 5'-GAAGATCTGCTAAGGCA-GGAGAATA-3' (reverse) .

2.3 RNA extraction, Real-time PCR and siRNA

Appropriate plasmids were transfected into MCF-7 cells and 24 hours. Total RNA was isolated from MCF-7 or T47D cells using RNAiso Reagent (Takara, Dalian, China). Total RNA (3 μg) was reverse transcribed using oligo (dT) primer and a Reverse Transcription System (Takara). The single-stranded cDNA was amplified by PCR using specific primers: CD24: 5'-GCTCCTACCCACGCAGATT-3' (forward) and 5'-GTGAGACCACGAAGAGACTGG-3' (reverse); ELF5: 5'- GCTTGAAAACAA-GTGGCATC-3' (forward) and 5'-TCTTCCTTTGTCCCCACATC-3' (reverse); and GAPDH: 5'- AGAACATCATCCCTGCCTCT-3' (forward) and 5'-CACCGGT-GCTCAGTGTAG-3' (reverse). The PCR products were analyzed by 1% agarose gel electrophoresis. Relative mRNA levels were determined using the ABI Prism 7500 sequence detection system with SYBR premix Ex Taq (Takara) as previously described[25]. Expression of target genes was determined according to the $2^{-\triangle\triangle CT}$ method using GAPDH as a reference gene. The following primer sequences were used: CD24-siRNA, 5'-UCUCUCUUCUGCAUCUUUATT-3' (forward) and 5'-UAAAGAUG-CAGAAGAGAGATT-3' (reverse).

2.4 Trans-well and scratch wound-healing assays

Scratch wound-healing assays were performed as previously described [24]. To test migration ability, a number of cells (1×10^5 per well) were placed and cultured at the upper chamber of 24-well trans-well plates with FBS-free medium. Cell invasion assays were performed in 24-well chambers with Matrigel

coated. Cells were transfected with Myc-ELF5 plasmid or control vector (Ctrl) and then plated in the upper chamber. After twenty four hours of incubation, the migrating and invading cells on the lower surface of the filter were fixed at first in 4% glutaraldehyde for 20 minutes, then stained in 0.1% crystal violet and 2% ethanol for 30 minutes. Cells for migratory was counted using Nikon TE2000-U microscope. The number of migrating and invading cells for each category of cells repeated three times.

2.5 Cell growth assays

MTT assays and Clonal formation assays were used to assess cells' proliferation. T47D or MCF-7 cells were transfected with appropriate plasmids, after 24 h transfected, then seeded in 96-well plates, with 2000 cells in each well and then subjected to MTT assay performed with a commercial kit (KeyGen) according to the manufacturer's protocol. The absorbance of the samples was test at 490 nm. For clonal formation assays, the transfected cells were immobilized with ethanol, stained with 0.1% crystal violet, and then following photographed.

2.6 Western blot assays and chromatin immunoprecipitation (ChIP) assays

Western blot assay and ChIP assays were conducted as previously described [22]. Amplifying the target DNA fragment was conducted with specific primers. For negative control, the primers (CD24-P-NC) were designed to amplify the sequence at -360 to -167 site of CD24 transcription start point. The following primer sequences were used for CD24-P-NC, 5'-GAATCTGTGCTTTGTGAACCAT-3' (forward) and 5'-AGGGTAGGGTGGAGACCTGTA-3' (reverse). For predicted target fragment primers (CD24-P-EBS) were designed to amplify the sequence at -100 to + 3 site of CD24 transcription start point. The following primer sequences were used for CD24-P-EBS, 5'-CCAGCCTGGGCAACAA-3' (forward), 5'-GCTGTCTTATCCTAGGAATAC-CACC-3' (reverse).

2.7 Statistical analysis

Data were examined as means ± SDs. Un-paired t-test was used as results from two groups were compared. Statistical analyses were carried out by one-way analysis of variance with Bonferroni's multiple-comparison correction for comparison among three or more groups. Statistical significance was considered at the $p < 0.05$ level.

3. Result

3.1 The Effect of ELF5 on breast cancer cells growth

To confirm the possible role of ELF5 in the progression of cancer cells, the endogenous expression of ELF5 protein was analysis in a panel of human cancer cell lines containing MCF-7, T47D, MDA-MB-231, HK293T and Hela cells. As shown in Fig. 1A, ELF5 was relative higher expressed in T47D and HK293T cell line, and comparatively at lower level expressed in MCF-7, MDA-MB- 231 and Hela cell lines.

Next, the low = expressing ELF5 breast cancer cell line MCF-7 cells were transfected with pCMV6-DDK-Myc-ELF5 plasmid. 24 hours after cell transfected, exogenous ELF5 `protein expression was analyzed by

Western-blot using Myc-tagged antibodies. In contrast, 24 hours after the knockout experiment with the ELF5-shRNA plasmid, the endogenous ELF5 protein was correspondingly reduced in the high- expressing ELF5 breast cancer cell line T47D cells (Fig. 1B).

Using MTT assays evaluate the effect of ELF5 in breast cancer cells growth; we then identified that exogenous transfection of ELF5 in MCF-7 cells inhibited cells growth activity. Conversely, knocked-down of ELF5 in T47D cells promote cells growth procession (Fig. 1C). Therefore, these data suggests that ELF5 may function as a breast tumor cells suppressor.

3.2 The ELF5 regulates cells cycle and migration activity

Given the established role of ELF5 in regulation of breast cancer cells growth, we next to determine the effect of ELF5 in cells cycle, cell migration and invasion activities. After 48 hours of ELF5 overexpression in MCF-7 cells, we observed a significant increase in the cell rates at G0/G1 phase from 41.88–52.62%, while the cell rates at S phase decreased from 48.03–38.68%, and the cell rates at G2/M decreased from 10.09–7.72%. (Fig. 2A). As a result, forced expression of ELF5 inhibited MCF-7 cells cycle process at G0/G1 phase. Meanwhile, 48 hours after knocked-down of ELF5 in T49D cells, the cells rates at G0/G1 phase decreased significantly from 50.41–42.45%, while cells rates at S phase increased from 16.21–20.05%, and cells rates at G2/M phase increased from 33.83–37.51% (Fig. 2B).

To explore the effect of ELF5 in motility on breast cancer cells, we overexpressed ELF5 in the breast cancer cell line MCF-7, and subsequently found that this operation indeed resulted in an inhibition of MCF-7 cells motility as wound healing assay indicated. Conversely, using the breast cancer cell line T47D, the motility ability was significantly increased upon ELF5 knocked-down assays (Fig. 2C).

Next, we want to examine whether ELF5 can affect the migration and invasion ability of breast cancer cells. Using Trans-well assays, we found that the migration and invasion ability were significantly decreased after MCF-7 cells deal with overexpression of ELF5 plasmid. In contrast, when knockdown of ELF5 in T47D cells using shRNA assay, the ability of migration and invasion was significantly elevated (Fig. 2D). Therefore, the results indicated that ELF5 could suppress the invasion and metastasis ability of breast cancer cells.

3.3 CD24 is transcriptional regulated by ELF5

When searching for the genes related to ELF5-mediated breast cancer cells effects, we tend to select cancer stem cell marker molecule, especially in breast cancer metastasis. The CD24 phonotype has been reported to express in breast cancer stem cells [23]. Studies have shown that the expression of ELF5 regulates the fate of breast stem/progenitor cells, which let us to explore the possibility of ELF5 regulating CD24 expression.

To estimate the relationship between ELF5 and CD24, we examined the genomic sequence upstream of the CD24 gene using an analysis tool (JASPAR). A putative ETS-binding sites (5’-ACCCGGAAAAG-3’) (EBS) was found at region of -367 to -357 within the CD24 gene promoter. Following COS-7 cells were transfected with either Myc-ELF5 or control vector. Twenty-four hours after transfection, ChIP assays

identified the correlation between ELF5 in the CD24 gene promoter and chromatin fragments corresponding to EBS (Fig. 3A).

To evaluated whether EBS within the CD24 gene promoter conferred ELF5-dependen transcriptional activity, DNA sequence involving wild type or indicated mutant EBS were inserted into the promoter region of a firefly luciferase reporter plasmids respectively, named pGL3-CD24-promoter plasmid and pGL3-CD24-promoter-Mut plasmid correspondingly (Fig. 3B). COS-7 cells were co-transfected with Myc-ELF5 or control vector together with the reporter plasmids. Twenty-four hours after transfection, reporter gene activity was measured, and the result showed that ELF5 enhanced CD24 transcriptional activity in these cells in a dose-dependent manner (Fig. 3C). Then, the ability of ELF5 to regulate the transcriptional activity of the CD24 gene was confirmed again using T47D cells. As shown in the Fig. 3D, Twenty-four hours after over-expression of ELF5 plasmid in T47D cells, the luciferase activation of the wild-type reporter gene was significantly increased as expected. However, ELF5-shRNA transfected T47D cells showed a decreased luciferase expression indeed. These experimental data therefore demonstrate that CD24 is a direct transcriptional target of ELF5.

3.4 ELF5 regulate CD24 expression in breast cancer cells

We first detected endogenous CD24 gene expression at the transcription level. In order to further study the effect of ELF5 on CD24 gene expression activity, 24 hours after transfection of MCF-7 cells with ELF5 plasmid, CD24 mRNA expression levels were increased (Fig. 4A). Next, analysis of protein expression by flow cytometry revealed that ectopic expression of ELF5 in MCF-7 cells significantly increased the expression of CD24 protein level (Fig. 4B). The above results prove that CD24 is regulated by ELF5 at the level of transcription and translation.

The above results demonstrated that ELF5 inhibits the proliferation and migration of breast cancer cells. Next, we want to ascertain whether ELF5 could modulate the growth, migration and invasion of breast cancer cells by regulating expression of CD24. After CD24-siRNA transfection, it was found that overexpression of ELF5 did not significantly affect the growth behavior of T47D cells (Fig. 4C). When co-transfection experiments performed in MCF-7 cells using CD24-siRNA and Myc-ELF5 plasmid, compared with the control, the cell migration and invasion activity were significantly improved (Fig. 4D). The results indicate that ELF5 modulate the growth, migration and invasion of breast cancer cells by regulating CD24 expression.

3.5 ELF5 regulates tamoxifen -resistant of MCF-7 cells

Chemo-resistance cells generally represented by malignant tumor cell that survive in chemotherapy treatment. We explored the effect of ELF5 on chemo-resistance in MCF-7 cells. Tamoxifen is a commonly used clinical treatment drug for breast tumors. In this experiment, the experimental group was treated with tamoxifen for MCF-7 cells and screened, while the control group was not treated with tamoxifen for cells. As shown in Fig. 4E, after ELF5 was overexpressed in the control cells, the number of early apoptotic cells increased from 2.6–21%, while the number of late apoptotic cells increased from 12.0–22.6%. However, after ELF5 was overexpressed in tamoxifen-treated cells, the number of early apoptotic cells decreased from 8.4–4.6%, and the number of late apoptotic cells decreased from 16.0–12.1% (Fig. 4F). This evidence suggests that CD24 is associated with chemo-resistance of tamoxifen in breast

cancer [24]. Therefore, these observations indicate that ELF5 modulate drug resistance in MCF-7 cells by regulating CD24 expression.

4. Discussion

In the present work, identifying ELF5 as a repressor is an essential step to elucidate that ELF5 regulates breast cancer cells in proliferation, invasion and metastasis activities. Our preliminary studies suggest that knockdown of ELF5 expression in T47D cells results in increase in cell proliferation. This result is also consistent with the conclusion of another group studies on ELF5, which found that 164 mitogenic genes were repressed by ectopic expression of ELF5 in breast cancer cells via ChIP-Sequence assays [8]. Indeed, the published data linking cyclin D2 repressed in mouse mammary gland as ELF5 localizing cis-regulatory regions of this gene [25]. Particularly, ELF5 inhibits mammary gland development and the epithelial-mesenchymal transition (EMT) in cancer cells by inhibited snail family transcriptional repressor 2 (Snail2) gene, which means that ELF5 plays a repressive role in metastasis activities [6]. However, the specific molecular mechanism of ELF5 regulating breast cancer cells need further study.

Studies confirm that both CD24 and ELF5 genes are associated with the undifferentiated state of breast cancer stem cell-like phenotypes [7, 16, 26], and are associated with cell proliferation, migration and invasion activity [27, 28]. The ELF5 gene regulate many epithelium-specific genes involving in normal and pathological tissues, especially in the later stages of inducing cells terminal differentiation [29]. CD24 is a molecular marker frequently present in breast cancer stem cells or carcinomas [27, 30]. As a highly glycosylated molecule, CD24 is localized in lipid rafts on the cell membrane via a glycosyl-phosphatidylinosoitpl (GPI) anchor. This lipid rafts microenvironment provided an important platforms for the biological functions of CD24. For example, CD24 can interact with sialic acid binding receptors or with different adhesion molecules to transmit different signaling pathways [19]·[27, 31]. In terms of cellular function, CD24 cross-linking could induce MCF-7 breast cancer cell apoptosis and inhibit cells migration [19, 32]. Our experimental data show that overexpression of ELF5 level indeed suppression of breast cancer cells migration and invasion, while the depletion of CD24 via knockdown led to restoration of cells proliferation, migration and invasion activity caused by over-expression ELF5 in MCF-7 cells.

The critical barrier in the treatment of breast cancer is drug resistance, which led to anti-estrogen chemotherapy resistance and tumors recurrence in more than 30% of cases. The molecular mechanism of chemotherapy resistance has not been fully understand, and it has become a focus of current research. Studies have found that ELF5 may determine breast cancer cell subtype by reducing the estrogen sensitivity of luminal subtype and promoting the basal characteristics of basal subtype cells, which helps cells acquire antiestrogen resistance [33]. CD24 is also associated with increase chemotherapy resistance. Merging evidence suggests that CD24 has been linked to drug resistance to tamoxifen, docetaxel and doxorubicin in breast cancer via in vitro and in vivo assays [34, 35]. Therefore, here we found that enforce ELF5 expression contributes to the formation of tamoxifen resistance in MCF-7 cells, and that ELF5 may regulate drug resistance of MCF-7 cells via CD24 expression.

5. Conclusions

In our study, we identified the role of ELF5 in promoting CD24 transcription, which may provide a novel mechanism for human breast cancer invasion and metastasis. First, we found that ELF5 not only inhibit the invasion and metastasis of breast cancer cells, but also repress the proliferation of breast cancer cells. Second, ELF5 could bind to CD24 gene promoter, which regulate the transcriptional level of CD24. Finally, we demonstrated that ELF5 could suppress the invasiveness of breast cancer cells via regulating CD24 activity. In addition, ELF5 regulated drug resistance in MCF-7 cells. This study may provide further theoretical supporting for the drug development targeting ELF5 or CD24 genes for breast cancer therapy.

Abbreviations

CHiP: Chromatin immunoprecipitation; ELF5: E74-like factor five; EMT: Epithelial-mesenchymal transition; ER: Estrogen receptor; ETS: E26 transforming; GPI: Glycosyl-phosphatidylinosoitpl; Snail2: Sequence snail family transcriptional repressor 2

Declarations

Acknowledgements

Not applicable

Authors' contributions

Conceived and designed the experiments: Xinjian Qu. Performed the experiments: Xinjian Qu and Qianqian Li. Contributed regent and analysis tools: Qianqian Li and Simei Tu. Wrote the paper: Xinjian Qu.

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Availability of data and materials

The datasets used and/or analyzed during this study are available from the corresponding author on reasonable request (quxinjian@dlut.edu.cn).

Ethics approval and consent to participate

There were no human tissues or animal experiments in this study.

Consent for publication

Not applocable

Competing interests

The authors declare that they have no competing interests

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Figures

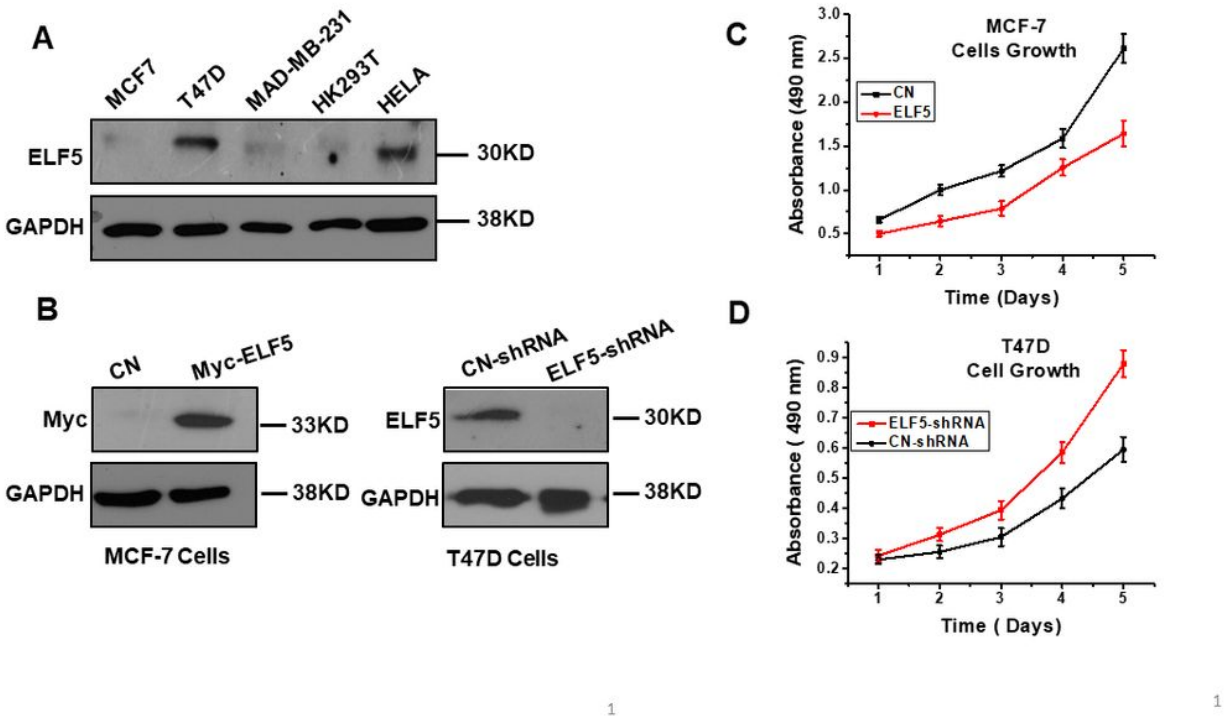

Figure 1

The Effect of ELF5 on breast cancer cells growth. (A) Western blot analysis the endogenous ELF5 protein expression level in several breast cancer cells. (B) Western blot analysis ELF5 overexpression in MCF-7 cells (left) and ELF5 knockdown in T47D cells (right). (C) MTT assays assess the effects of ELF5 overexpression on the growth in MCF-7 cells (up) and ELF5 knockdown in T47D cells (down). Bars represent means ± s.d. (n=3).

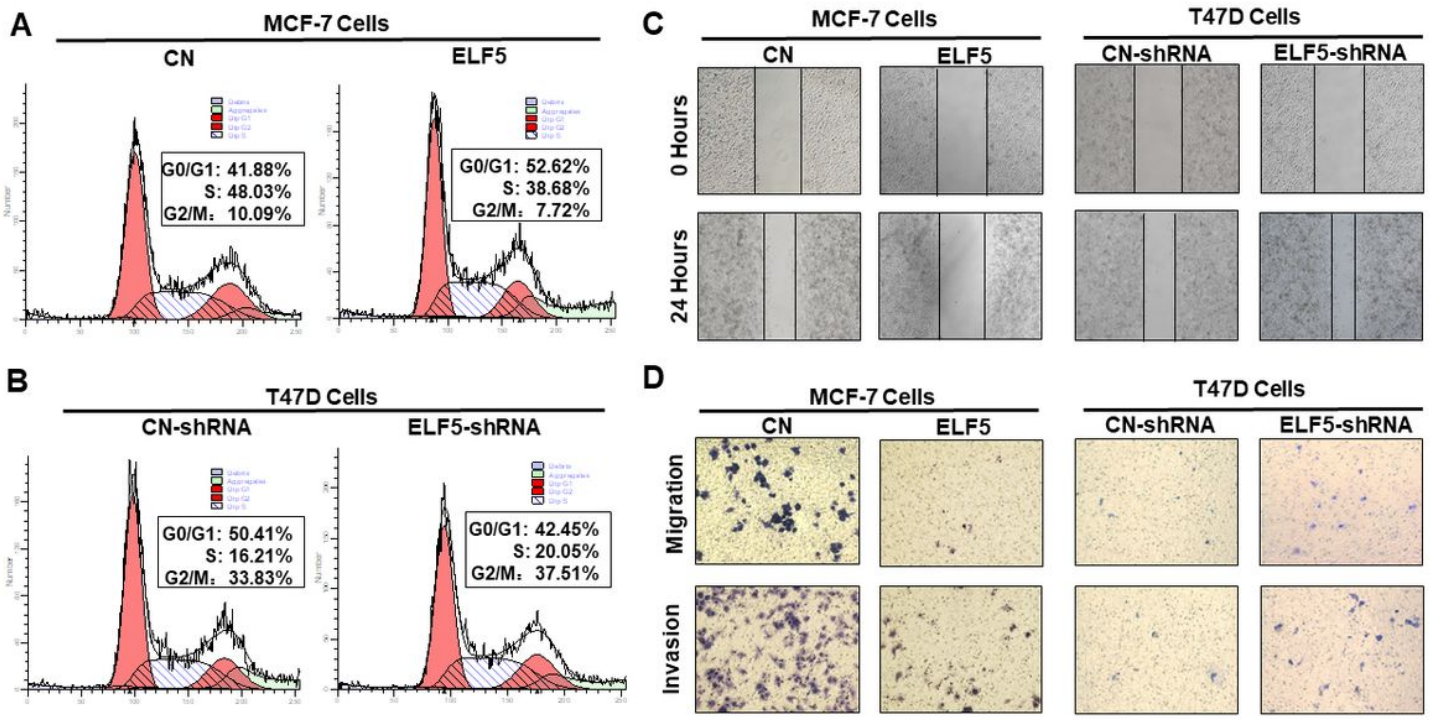

Figure 2

The effect of ELF5 on breast cancer cells cycle and motility phenotype. (A) The cell cycle was analyzed by FACS after 48h overexpression ELF5 in MCF-7 cells. (B) The cell cycle was analyzed by FACS after 48h knockdown ELF5 in T47D cells. (C) Scratch wound-healing assay performed to assess the effect of ELF5 overexpression on the motility of MCF-7 cells (left) and ELF5 knockdown on the motility of T47D cells (right). (D) Trans-well assay performed to evaluate the effects of ELF5 overexpression on the cellular migration and invasion of MCF-7 cells (left) and knockdown ELF5 on the cellular migration and invasion of T47D cells (right).

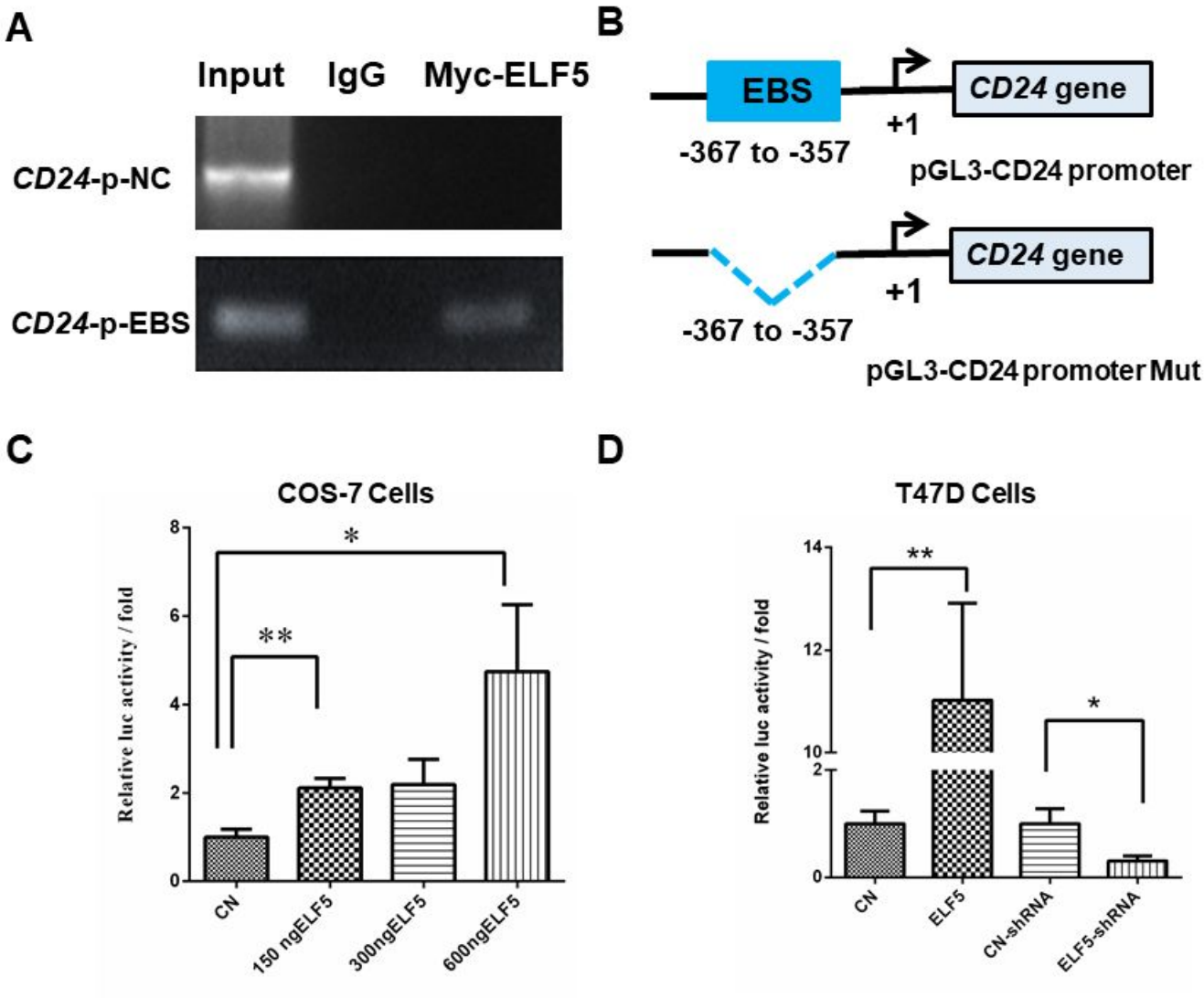

Figure 3

CD24 is transcriptional regulated by ELF5. (A) ChIP assays verified the association of ELF4 with the chromatin fragments corresponding to EBS within CD24 gene promoter. (B) Schematic illustrated the constructed CD24-WT-Luc plasmid (up) and CD24-Mut-Luc plasmid (down). (C) Firefly luciferase reporter assays showed ELF5 enhanced the transcriptional activity of CD24 in a dose-dependent manner in COS-7 cells. (D) Firefly luciferase reporter assays verified ELF5 regulate the transcriptional activity of CD24 gene in T47D cells.

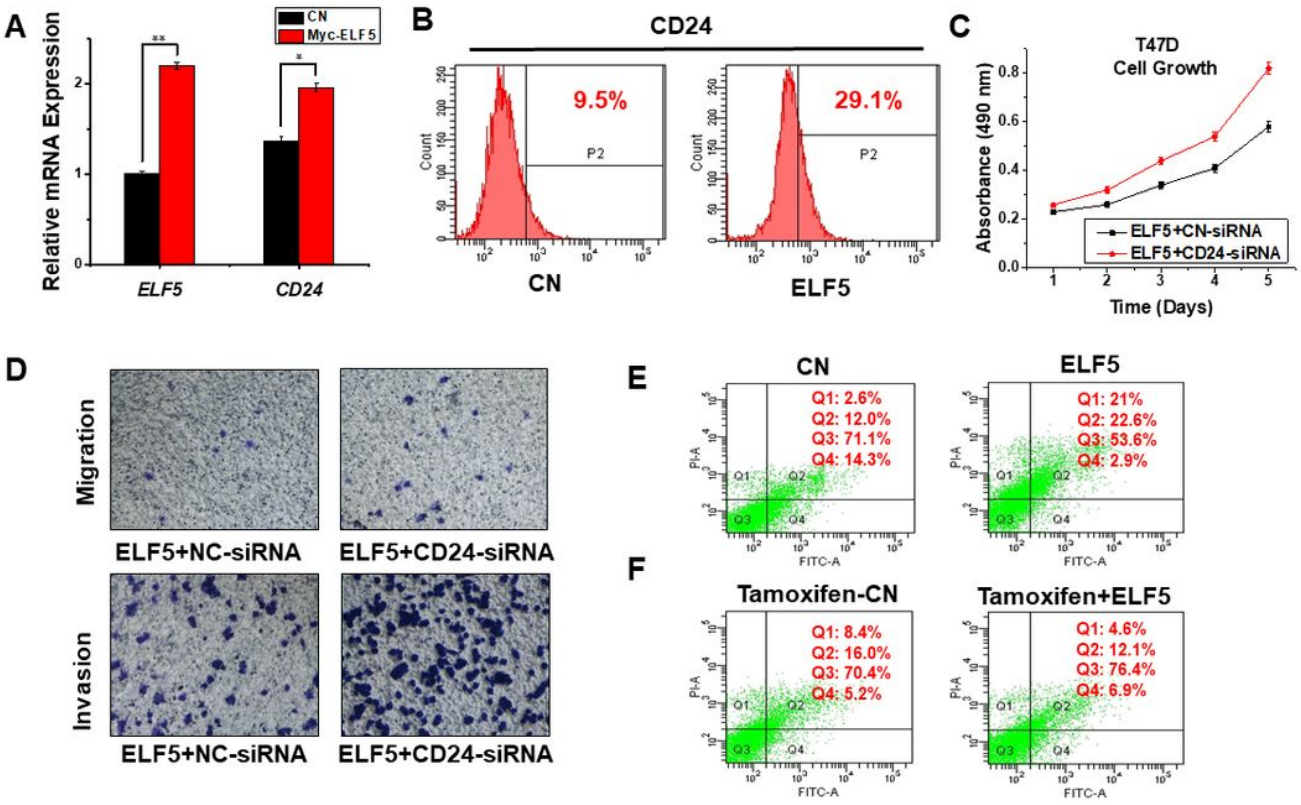

Figure 4

ELF5 regulates CD24 expression and regulates chemo-resistance of MCF-7 cells. (A) ELF5 regulate CD24 mRNA levels in breast cancer cells. (B) The CD24 protein expression levels were evaluated using flow cytometry analysis after over-expression Myc-ELF5 plasmid in MCF-7 cells. (C) The proliferation effect of knockdown CD24 in adapted ELF5 expression for T47D cells. (D) The migration and invasion effect of knockdown CD24 in adapted ELF5 expression for MCF-7 cells. (E) Flow cytometry analysis the effect of ELF5 in apoptotic character for MCF-7 cells without tamoxifen treated. (F) Flow cytometry analysis the effects of ELF5 in chemo-resistance character for MCF-7 cells with tamoxifen treated.