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Research article

A bioinformatic analysis found low expression and clinical significance of ATF4 in breast cancer

Lujing Shao [a,1], Zhounan Zhu [a,1], Xinyan Jia [b,1], Yabin Ma [c,**], Chunyan Dong [a,*]

[a] Department of Oncology, East Hospital Affiliated to Tongji University, Tongji University School of Medicine, Tongji University, Shanghai, 200092, PR China
[b] Jinzhou Medical University, Jinzhou, Liaoning, 121000, PR China
[c] Department of Pharmacy, East Hospital Affiliated to Tongji University, Tongji University School of Medicine, Tongji University, Shanghai, 200092, PR China

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ABSTRACT

Background: Activating Transcription Factor 4 (ATF4) expression exhibits differential patterns across different types of tumors. Besides, the pathogenesis of breast cancer is complex, and the exact relationship between ATF4 and ATF4 remains uncertain.

Methods: The analysis of ATF4 expression was conducted by utilizing The Cancer Genome Atlas (TCGA) pan-cancer data, while the gene expression profile of breast cancer was checked by the comprehensive database-Gene Expression Omnibus database. In order to gain a more comprehensive understanding of the specific cell types that exhibit ATF4 expression within the microenvironment of breast cancer, we conducted a single-cell analysis of ATF4 using two distinct datasets of human breast cancer (GSE114717 and GSE11088, respectively). The spatial distribution of ATF4 within a tissue was demonstrated based on datasets obtained from the Human Protein Atlas (HPA) and SpatialDB. The clinical prognostic significance of ATF4 was assessed by analyzing clinical survival data obtained from TCGA, GSE4830, and GSE25055 datasets. We used the R package clusterProfiler to carry out an enrichment analysis of ATF4. We assessed how ATF4 impacts the growth and movement of breast cancer cell lines. We manipulated ATF4 levels using plasmid transfection techniques.

Results: The expression of ATF4 was found to be suboptimal and demonstrated a significant correlation with enhanced disease-specific survival ($p = 0.012$) and overall survival ($p = 0.032$) in breast cancer as well as other malignancies. We conducted an analysis to investigate the interaction between the infiltration level of immune cells and the expression of ATF4, using samples obtained from TCGA with known immune cell infiltration scores. Furthermore, a notable positive correlation exists between the elevated expression of ATF4 and immune-related genomes, specifically those associated with chemokine as well as immunity. Subsequent examination revealed a notable augmentation in the cytodifferentiation of T cells into regulatory T (Treg) cells within tissues exhibiting elevated levels of ATF4 expression. ATF4 exhibits notable upregulation in the MDA-MB-231 cell, thereby exerting a substantial impact on cell proliferation and migration

* Corresponding author. Department of Oncology, East Hospital Affiliated to Tongji University, Tongji University School of Medicine, Tongji University, Shanghai, 200092, PR China.
** Corresponding author.
E-mail addresses: slujing94@126.com (L. Shao), zhuzhounan@tongji.edu.cn (Z. Zhu), J17861170060@163.com (X. Jia), YabinMa23@126.com (Y. Ma), cy_dong@tongji.edu.cn (C. Dong).
[1] Lujing Shao, Zhounan Zhu and Xinyan Jia contributed equally to this article.

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upon its knockdown. Conversely, the overexpression of ATF4 in the MCF7 Luminal A breast cancer cell line can also modulate cellular function.
Conclusions: Our study suggests that ATF4 helps T cells differentiate into Treg cells in breast cancer. ATF4 can represent a clinically useful biomarker to predict the overall survival rate, especially in patients with different subtypes of breast cancer. Provide certain guidance value for the development of targeted drugs or inhibitors targeting ATF4.

1. Introduction

Breast cancer is the most common cancer in women worldwide. It is also the leading cause of cancer deaths in women [1]. In recent years, the incidence of female breast cancer has risen sharply. The burden of the disease is increasing, China leads the world in the number of female breast cancer cases and deaths. It accounts for 17.6 % and 15.6 % of the world's female breast cancer cases and deaths [2]. Currently, there is a lack of effective markers for early screening and diagnosing breast cancer. The advancement of high-throughput sequencing technology, has facilitated the identification of an increasing number of genes that serve as early tumor markers for breast cancer. However, to identify more key driver genes that can influence the immune microenvironment of breast cancer and breast cancer progression is still necessary. Recently, many researchers have found that ATF4 expression is upregulated in a variety of tumors and is involved in processes related to the regulation of tumor progression [3,4], this implies that ATF4 could be a potential target to treat breast cancer.

Activating transcription factor 4 (ATF4) is a member of the ATF/CREB family, also known as cyclic adenylic acid (cAMP)-response element binding protein 2 (CREB2) [5]. The ATF family is a group of transcription factors that contain a basic leucine zip region (bZIP), which is associated with protein interactions [5]. It was reported that ATF3 could enhance breast cancer-initiating cell features. ATF3 could also provide feedback on TGFβ [6]. Furthermore, ATF5 has the ability to activate PI3K/Akt pathway, which mediates the proliferation of tumor cells [7].

ATF4 plays an essential role in responses induced by stress signals such as hypoxia, amino acid deficiency, oxidative stress and endoplasmic reticulum stress (ERS) [8]. A study found that ATF4 increased the multiplication and movement of hepatoma carcinoma cells. It did this by increasing the amount of HKDC1 [9], and the expression of ATF4 was negative and weakly positive in normal cervical tissue cells and strongly positive in cervical cancerous tissue [10]. Upregulation of ATF4 expression inhibits the formation of extracellular matrix, resulting in reduced adhesion between laryngeal cancer cells, themselves and normal tissue cells, which in turn makes the tumor cells more invasive [11]. Recently, there are some reports on the research of ATF4 regulating breast cancer. For example, in an in vivo mouse breast cancer model, ATF4 enhances macrophage recruitment through enhanced M-CSF expression and enhances HIF-1α expression to promote angiogenesis, thereby promoting breast tumor growth [12]. In radiation resistant TNBC, activation of ATF4 can increase intracellular levels of reduced glutathione and clearance of reactive oxygen species after radiation, leading to radiation resistant [13]. In addition, HER2 can facilitate the proliferation and migration of breast cancer cells. It upregulates the expression of ATF4-ZEB1 [14].

In this study, we assessed ATF4 expression in different tumor types. We analyzed three cohorts: the Cancer Genome Map (TCGA), Genotypic Tissue Expression (GTEx), and Gene Expression Omnibus (GEO). Additionally, we examined the connection between ATF4 expression and prognosis of breast cancer patients. Researchers observed that the expression of ATF4 in breast cancer tissues showed a lower level compared to that in adjacent and normal tissues. An inverse relationship was observed. The expression of ATF4 correlates with lower overall, disease-specific, and relapse-free survival rates in individuals with breast cancer. Furthermore, it is anticipated that ATF4 plays a role in the chemokine signaling pathway. Experts expected it to play a part in both the innate and adaptive immune systems. Given the significance of immune cell infiltration in relation to the overall survival of individuals with cancer, our study sought to investigate the relevance between ATF4 expression and levels of immune cell infiltration. Our findings indicate that tissues exhibiting elevated ATF4 expression are associated with heightened infiltration of Treg cells, while infiltration of Th cells is diminished. In order to conduct a more comprehensive investigation into the expression of ATF4 in various subtypes, we employed three negative breast cancer (TNBC) cell lines, namely MDA-MB-231, as well as MCF7, a the Luminal A breast cancer cell line. Results showed that the expression of ATF4 was elevated in MDA-MB-231 cells. Subsequently, the knockdown of ATF4 resulted in a notable deceleration of cell proliferation and migration in these cells. Conversely, the overexpression of ATF4 in MCF7 cells induced a significant acceleration of cell proliferation and migration in this cell line. The findings of our research indicate that ATF4 exhibits potential roles in various subtypes of breast cancer, thereby elucidating the underlying mechanisms by which ATF4 regulates Treg cells and the associated cascade of inflammatory factors within the microenvironment of breast cancer.

2. Methods

2.1. Data acquisition and analysis

We obtained clinical data and expression of ATF4 from the GTEx and TCGA pan-cancer datasets. This information was obtained from the UCSC Xena database, which is accessible in (https://xenabrowser.net/datapages/). The complete names of tumor abbreviations used in TCGA are provided in Supplementary Table 1. To assess ATF4 expression, we obtained tumor tissue samples data from the TCGA database. We obtained normal tissue samples by combining normal tissues from TCGA and GTEx databases. Analysis was

conducted by R v4.0.3 software.

2.2. Single-cell analysis of ATF4

Tumor Immune Single-cell Hub (TISCH) website (http://tisch.comp-genomics.org/documentation/) provided Single-Cell Analysis of ATF4 expression. The heatmap, scatter diagrams, and violin plots were used to quantify the expression of ATF4 in major lineage in all cancers.

2.3. Immunohistochemistry

We collected immunohistochemical staining data from human protein profiles (https://www.proteinatlas.org/)Determine the specific expression of ATF4 based on different magnification factors. In addition, we collected the tissues of two patients and tumor tissues of three negative breast cancer mice, and detected the expression of ATF4 in different breast cancer subtypes.

2.4. Prognosis anaylsis

Prognosis anaylsis was performed using BEST tool (https://rookieutopia.com/app_direct/BEST/) which provided multiple gene datasets in TCGA and GEO.

2.5. Correlation and enrichment analysis

PPI network analysis based on GeneMANIA (http://www.genemania.org) [15], which identifies associated genes with ATF4. The genes most positively associated with ATF4 were selected for enrichment analysis. Gene Ontology (GO) analysis was performed using EnrichGO function in the R package “clusterProfiler”. was utilized to carry out Gene Ontology (GO) analysis. The Enrich Kyoto Encyclopedia of Genes and Genomes (KEGG) function of the R package “clusterProfiler” was utilized to conduct KEGG analysis.

2.6. Immune cell infiltration

The immune cell infiltration scores of TCGA pan-cancer were previously obtained using CIBERSORT. We downloaded these scores. To estimate immune cell infiltration, we segregated samples from The Cancer Genome Atlas (TCGA) into two groups, high-expression ATF4 group and low-expression ATF4 group, on the basis of the mid-value expression level of ATF4.

2.7. Somatic mutation analysis

We used RNA-sequencing expression (level 3) profiles, genetic mutation for breast cancer patients from the TCGA dataset (https://portal.gdc.com). R package “maftools” were used to visualize the mutations [16,17].

2.8. Cell culture and plasmid transfection

The cells utilized in this study were obtained from the Cell Resource Center of Shanghai Academy of Biological Sciences, which is the affiliation of the Chinese Academy of Sciences. Prior to their use, all cell lines were subjected to rigorous testing to confirm the absence of mycoplasma contamination. The 4T1, EMT6, MDA-MB-231 and MCF7 cell lines were cultivated in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10 % fetal bovine serum (FBS) at a temperature of 37 °C in a humidified incubator with 5 % carbon dioxide (CO_2) concentration. The MCF10a cells were cultured in a specialized culture medium. The cell culture experiments were conducted in triplicate, with three replicates per group for each repetition. The MCF7 cells were transfected with the plasmid OE-ATF4, whereas the MDA-MB-231 cells were transfected with the plasmid SH-ATF4, resulting in the overexpression and knockdown of ATF4, respectively.

2.9. Western immunoblotting

MCF10a, MCF7 and MDA-MB-231 cells were lysed in RIPA lysis buffer. Samples were resolved onto SDS-polyacrylamide gels and imprinted on PVDF membranes. Primary antibodies against anti-ATF4 (#11815, cell signaling) and GADPH (#2118, cell signaling) were used. Images were conducted with a C-Digit chemiluminescent Western blot scanner (LI-COR, Lincoln, USA).

2.10. Quantitative real-time RT-PCR

We used TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) for total RNA extraction, on the basis of the instructions. The total RNA sample was subjected to reverse transcription, using a random primer and M-MLV Reverse Transcriptase (Takara). The SYBR Green I Master Mix reagent by ABI 7500 system (Applied Biosystem, Foster, CA, USA) was utilized to carry out quantitative real-time RT-PCR. Primers used as shown in Supplementary Table 2. Results were calculated by using $2-(\Delta\Delta Ct)$ method.

2.11. Cell proliferation and migration

The cell proliferation experiment was detected using the CCK8 reagent kit, and the specific operation was carried out according to the operating instructions. The proliferation ability was evaluated based on the OD values measured at different time points. Cell migration experiment, also known as scratch experiment, detects the proportion and rate of cell migration over scratches within a certain period of time.

2.12. Animal experiments

The study utilized female nude mice that were 5 weeks old, which were acquired from the Shanghai Model Organisms Center located in Shanghai, China. The mice were cared for and utilized in accordance with a protocol that received approval from the Ethics Committee of the East Hospital, which is affiliated with Tongji University in Shanghai, China.

Mice were subcutaneous injected cell suspension (approximately 5*10^4 cells) to induce breast cancer model. Totally 24 female mice (5-week old) were fall into four groups including 4T1-control (4T1), ATF4 knockdown in 4T1 (4T1-shATF4), EMT6-control (EMT6), and ATF4 knockdown in EMT6 (EMT6-shATF4) at random. Tumor tissues were collected for further flow cytometry.

2.13. Statistical analysis

The data are presented as means ± standard error (SE) or median (interquartile range, IQR), unless otherwise specified. All figures were generated using GraphPad Prism version 6.0 (Graph-Pad Software, San Diego, CA). Statistical analysis was conducted using SPSS 23.0 (SPSS Inc, Chicago, IL, USA). Significant differences were assessed using either Student's *t*-test or one-way ANOVA, followed by the Mann-Whitney *U* test. Statistical significance was attributed to values of $P < 0.05$.

3. Results

3.1. Pan-cancer ATF4 expression analysis

Initially, we evaluated the expression of ATF4 in a comprehensive dataset encompassing multiple cancer types obtained from The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) project. The analysis demonstrated a decrease in ATF4 expression in 16 tumor types, namely ACC, BLCA, BRCA, CESC, COAD, ESCA, LAML, LUAD, LUSC, OV, PRAD, READ, SKCM, THCA, UCEC, and UCS. In contrast, its expression was high in CHOL, DLBC, GBM, HNSC, KIRC, LGG, LIHC, PAAD, TGCT and THYM (Fig. 1A). Then we evaluated the RNA expression of ATF4 in various organs and found that ATF4 was expressed in all tissues without tissue specificity (Fig. 1B). To explore the ATF4 expression in human breast cancer, we found that ATF4 was lower expressed in breast cancer

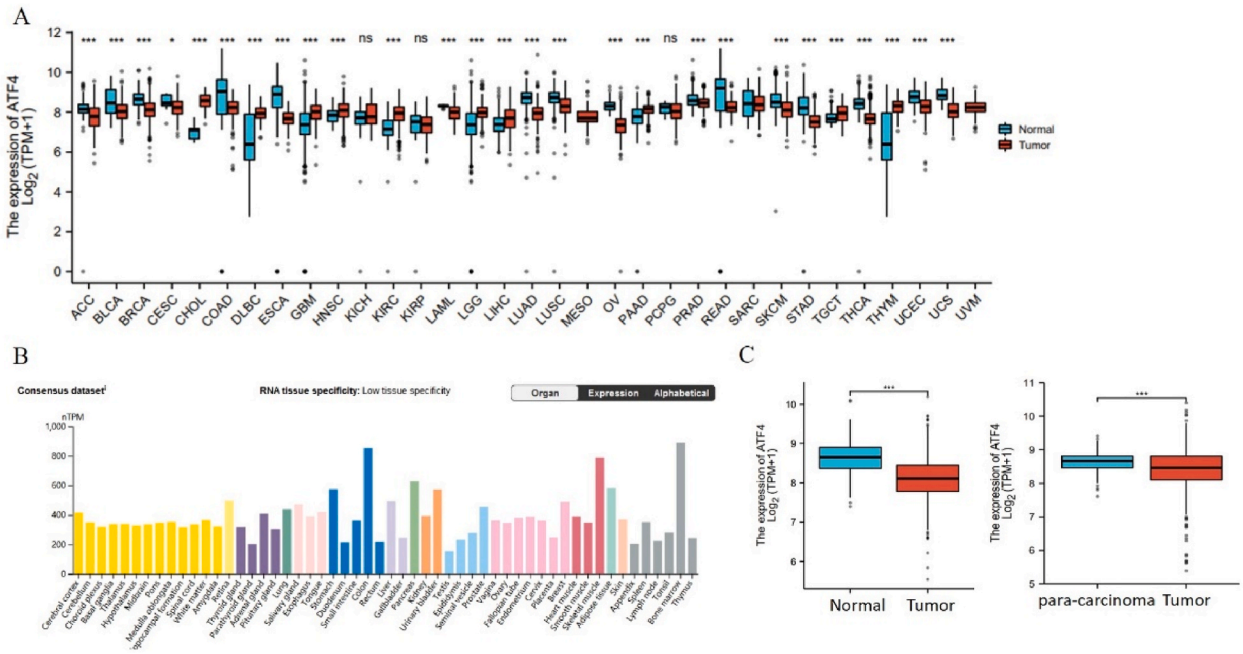

Fig. 1. Pan-cancer ATF4 expression analysis. (A) ATF4 expression in tumor and normal tissues in TCGA and GTEx pan-cancer data. (B) ATF4 expression in tumor cell lines. (C) Correlation between ATF4 expression and RNAss.

when compare to normal tissues and para-carcinoma (Fig. 1C).

3.2. ATF4 expression in human breast cancer

Using immunohistochemistry, we further validated ATF4 expression in breast cancer based on HPA datasets (Fig. 2A left column). Furthermore, two patients' breast cancer samples were obtained for additional elucidation. The findings verified that, in contrast to normal breast and para-carcinoma tissues, ATF4 was not highly expressed in breast cancer tissues (Fig. 2A middle column). The mouse triple negative breast cancer tissue was chosen for easy comparison due to the limited quantity of clinical triple negative breast cancer tissue samples. The findings demonstrated that mouse TNBC tumor tissue had comparatively high levels of ATF4 expression (Fig. 2A right column).

3.3. Relationship between the expression of ATF4 expression and the prognosis

In order to assess the usefulness of ATF4 in predicting the prognosis of patients with breast cancer, an analysis was conducted in BEST, which included several breast cancer datasets such as TCGA, GSE4830, and GSE25055, to examine the interrelationship between ATF4 expression and overall survival (OS), disease free survival (DSS), and recurrence-free survival (RFS). The results showed that lower expression of ATF4 was remarkable related to improved OS, DSS, and RFS in TCGA-BRCA ($p = 0.032$, 0.012 and 0.038 respectively) (Fig. 3A–C). When examining various subtypes of BRCA (PAM50), there were notable differences in ATF4 expression among Basal, Her2, LumA, LumB, and normal breast cancer ($p = 0.0042$ and $p < 0.001$, respectively). Consistent with the results from TCGA-BRCA, lower ATF4 expression was significantly correlated with improved OS and RFS ($p = 0.0044$ and $p < 0.001$, respectively) (Fig. 3D–G).

3.4. Correlation and enrichment analysis

In order to ascertain the potential role of ATF4, along with its associated pathways, a correlation analysis was conducted between ATF4 and various biological processes using TCGA dataset (Fig. 4A). Additionally, a correlation analysis was performed between ATF4 and other genes using Gene MANIA (Fig. 4B). The genes that exhibited the strongest positive association with ATF4 were chosen for enrichment analysis. We conducted additional analysis on the potential functional pathways using the cluster Profiler R package. The findings from the enrichment analysis of the "C2: Reactome gene sets" indicate that ATF4 is predominantly linked to RNA polymerase II transcription, cellular responses to external stimuli, transcriptional regulation by TP53, and regulation of TP53 activity (Fig. 4C).

Functional enrichment and GO analysis showed that ATF4 was related to macromolecule biosynthetic process. ATF4 was also associated with cellular macromolecule biosynthetic process, positive regulation of macromolecule metabolic process, positive regulation of nitrogen compound metabolic process and positive regulation of macromolecule biosynthetic process (Fig. 4D). Furthermore, an examination of the KEGG pathway analysis revealed gene sets that were enriched and interacted with in pathways related to cancer, infection by the human T-cell leukemia virus, Hepatitis B, viral carcinogenesis, and infection with the Kaposi sarcoma-associated herpesvirus (Fig. 4E). These findings imply that ATF4 is connected to several pathways in breast cancer.

3.5. ATF4 expression was associated with T cell infiltration and differentiation

We evaluated the immune cell infiltration score of TCGA breast cancer. The analysis revealed high levels of infiltration for aDC, B

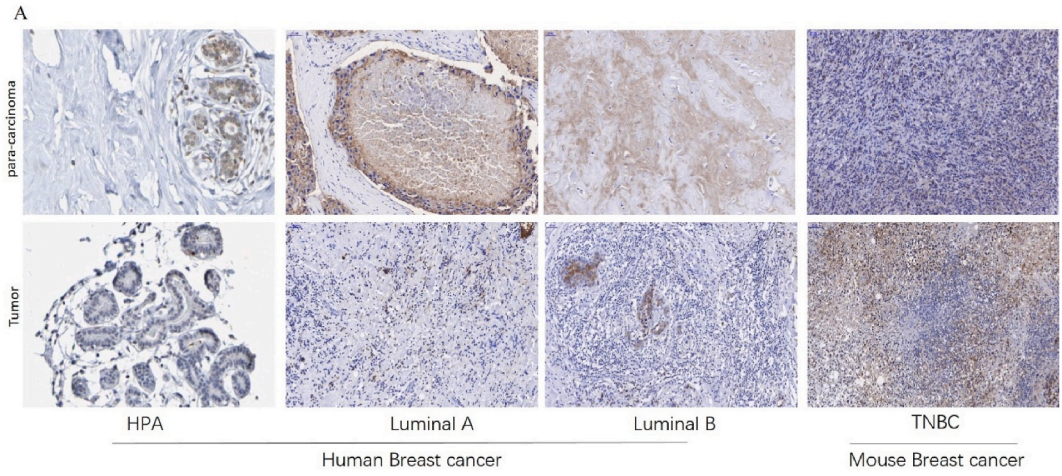

Fig. 2. Expression of ATF4 in single-cell level.(A) ATF4 expression in tumor and normal tissues in breast cancer. (B) ATF4 expression in tumor and para-carcinoma tissues in breast cancer. (C–E) ATF4 expression in single-cell level in human breast cancer.

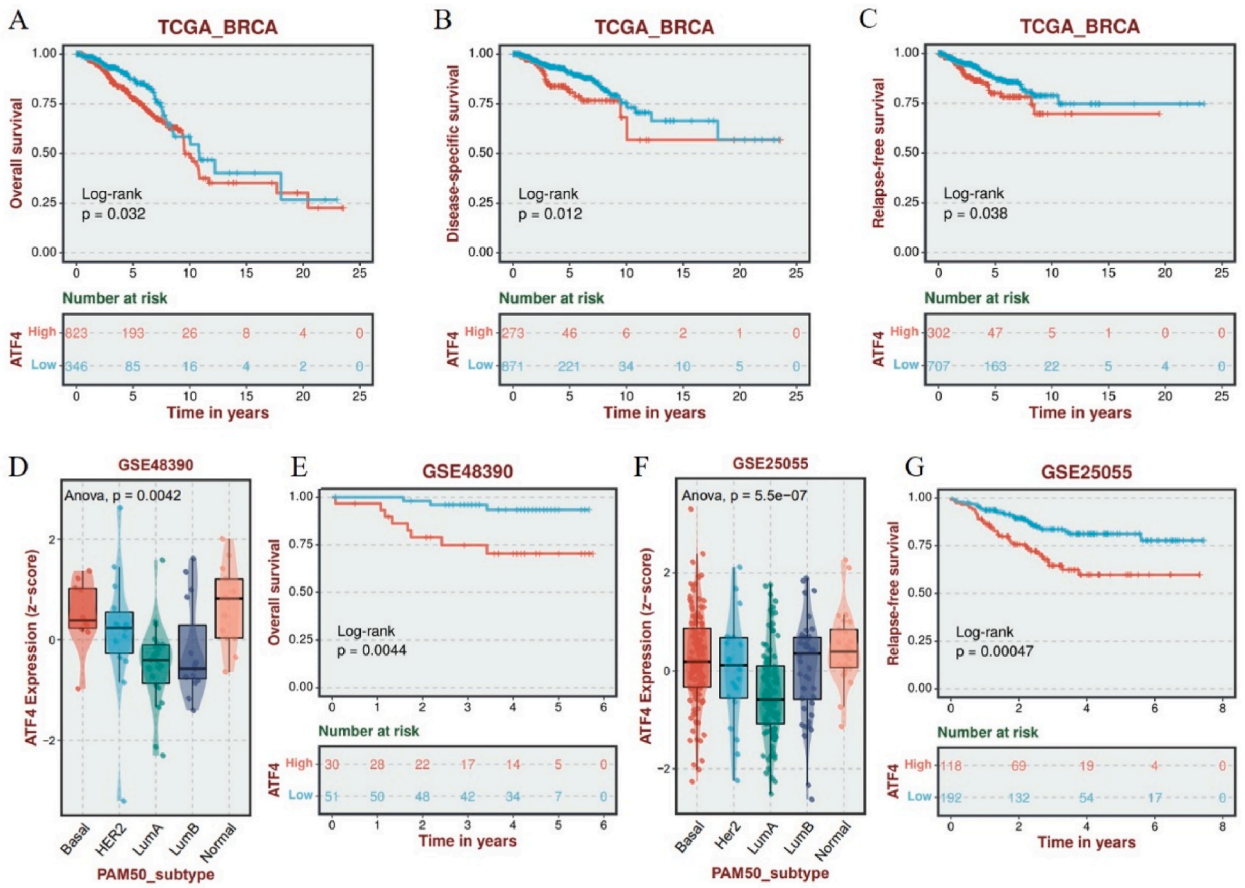

Fig. 3. Expression of ATF4 in breast tissues. (A) Representative images of Hematoxylin staining showing different pathological manifestations in normal and malignant breast tissues. (B) Representative images of immunohistochemistry showing ATF4 expression in benign and malignant breast cancer tissues. (C) Expression of ATF4 in spatial level.

cell, CD8T cell, cytotoxic cell, DC, T cell, Th1 cell, NKCD56dim cell, and Treg. Conversely, the high-ATF4 expression group exhibited low levels of infiltration for Eosinophil, mast cell, T helper cell, Tcm, Tgd, and NK cell (Fig. 5A). Other immune cells, including macrophages, did not show significant differences in infiltration. Considering the functional aspects, indicating that high expression of ATF4 promotes the differentiation of T cells to Treg. The tumor's immunosuppressive state is related to this. These results suggest that breast cancer's immunosuppressive status is related to low-expressed ATF4. We further checked our results by the TIMER2 (http://timer.cistrome.org/) database [18–20]. We found that ATF4 expression was related to the T cells infiltration level, including T cell and Treg (Fig. 5B–C). For T cell differentiation, the expression of ATF4 was positively correlated with T cells, Th1, Th2 and Treg (Supplementary Figs. 1A–S1D) and negatively correlated with Tcm and T helper cell (Supplementary Figs. 1E–F). Furthermore, there was a significant correlation observed between ATF4 expression and various Treg markers in both pan-cancer and breast cancer. These markers include CD4, FOXP3, IL2RA, IL7R, TGFB1, and CTLA4, as depicted in Fig. 5D–E and Supplementary Figs. S2A–F. The findings of this study suggest that decreased ATF4 expression is linked to the infiltration of T cells and their differentiation into T helper cells or Treg cells within the immunosuppressive microenvironment of breast cancer. This regulatory mechanism plays a role in regulating the immune response.

3.6. Single-cell analysis of ATF4 in breast cancer

To further identify the cell types that express ATF4 in breast cancer microenvironment, we carried out the single-cell analysis of ATF4 in 2 single-cell datasets of human breast cancer (GSE114717 and GSE11088, respectively). The TISCH web tool was utilized to generate a heatmap illustrating the relative expression levels of ATF4 across various cell types (Fig. 6A). The findings of this study revealed that ATF4 exhibited predominant expression in immune cells, particularly T proliferative cells, T regulatory cells, and CD8 T effector cells (Fig. 6B–C).

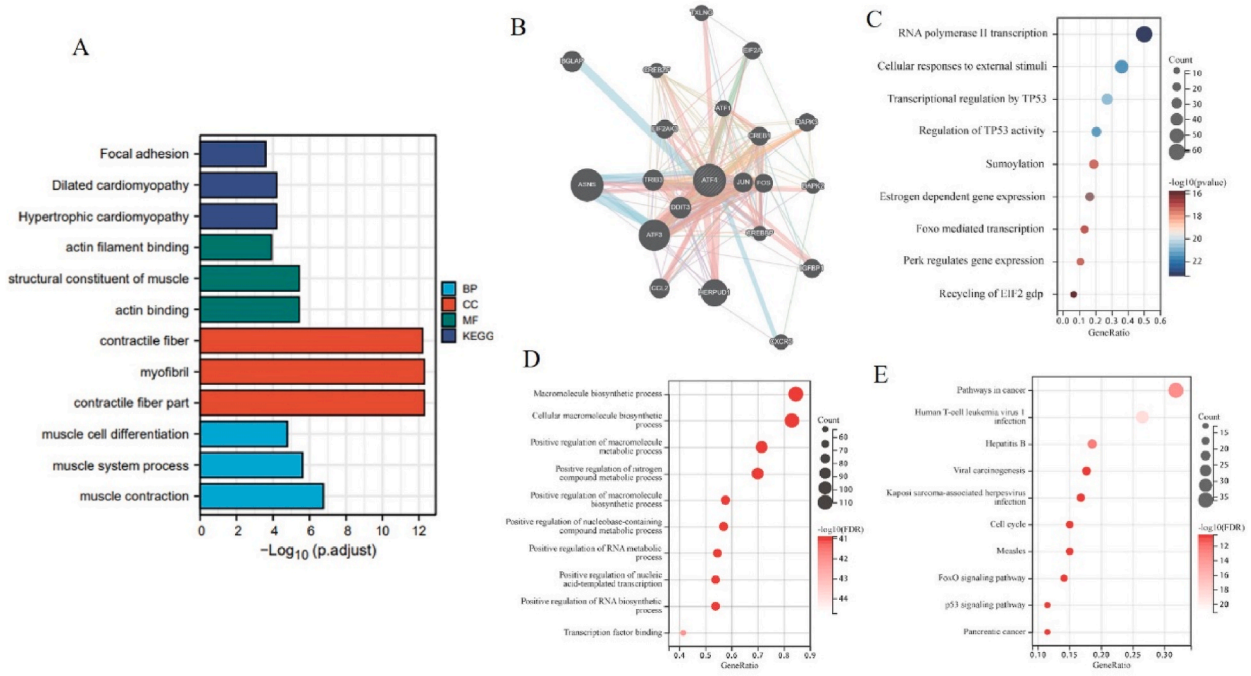

Fig. 4. Association between ATF4 expression and breast cancer prognosis. (A) Kaplan–Meier analysis of OS, DSS and RFS in TCGA-BRCA. Results with logrank p < 0.05 are shown. (B) ATF4 expression in different subtypes of breast cancer and OS in GSE48390. (C) ATF4 expression in different subtypes of breast cancer and OS in GSE25055.

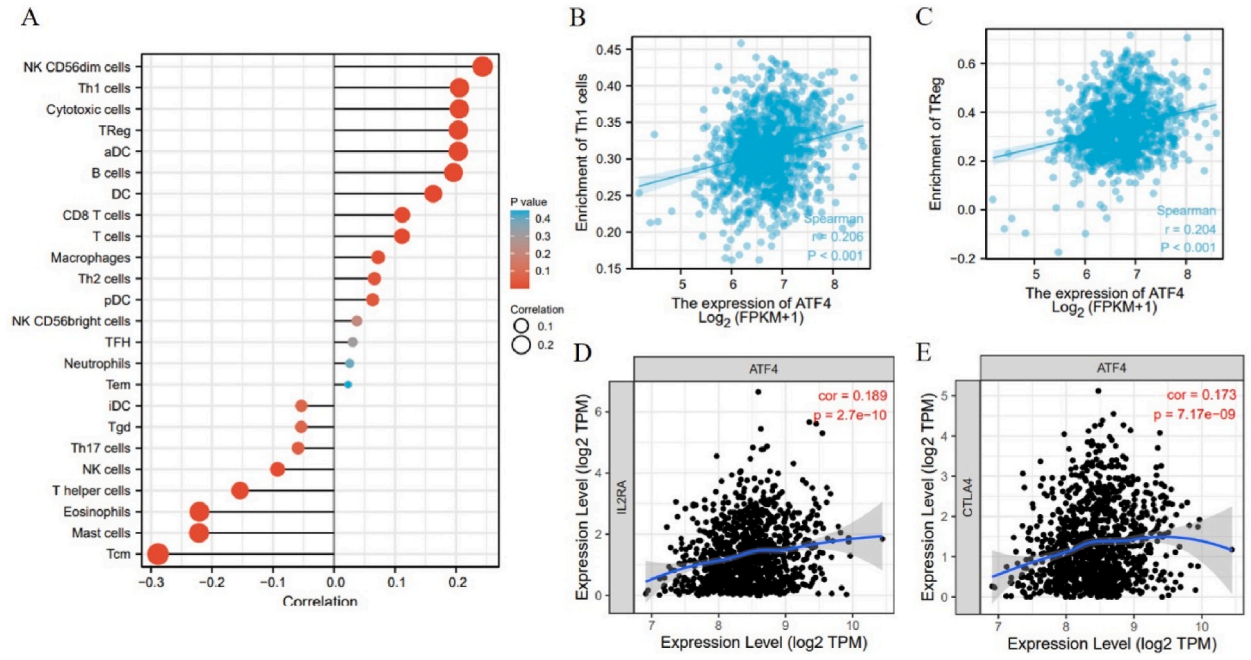

Fig. 5. Function and pathway enrichment analysis of ATF4 in breast cancer. (A) correlation analysis between ATF4 and other genes using GeneMANIA. (B)Significant Gene Ontology terms of thee hub genes most positively associated with ATF4 in "C2:Reactome gene sets". (C)Significant Gene Ontology terms of thee hub genes most positively associated with ATF4, including biological processes (BP), molecular function (MF), and cell component (CC). (D) Significant KEGG pathways of the hub genes most positively associated with ATF4.

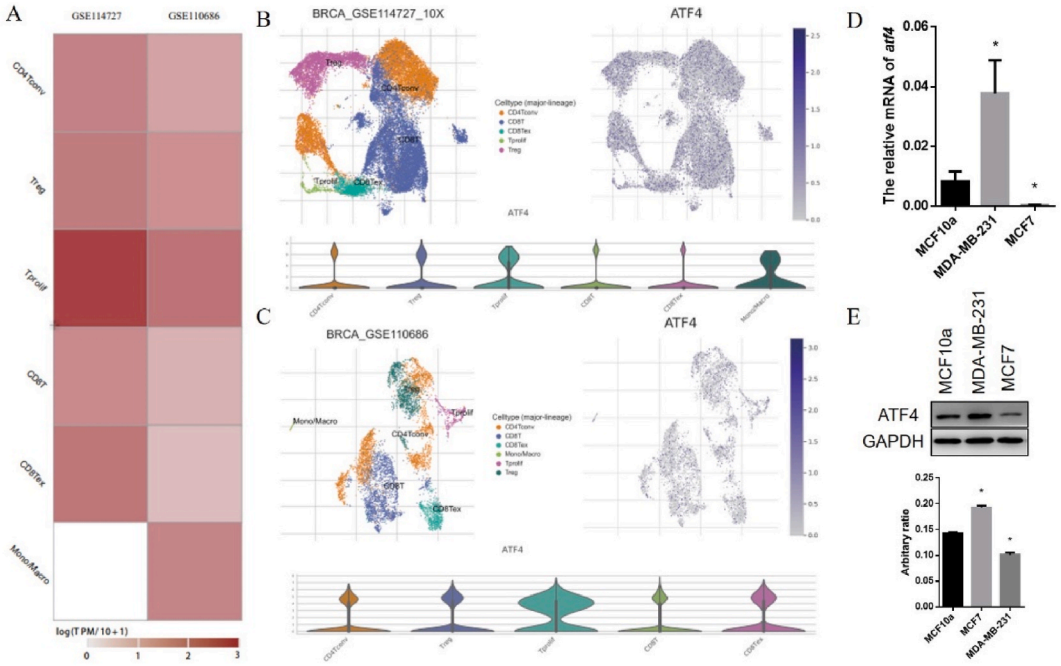

Fig. 6. Correlation between immune cell infiltration and ATF4 in breast cancer. (A) Correlation between immune cell infiltration and ATF4 expression, right represents positive correlation; the left represents negative correlation. (B) Correlation between ATF4 expression and T cell and Treg infiltration. (C) Correlation between ATF4 expression and Th1, Th2, Treg infiltration. (D) Correlation between ATF4 expression and IL7R and IL2A in breast cancer.

3.7. Somatic mutation analysis of ATF4 in triple negative breast cancer

Considering the high incidence rate and mortality of TNBC, and the high expression of ATF4 in TNBC, it is proposed that ATF4 may promote tumor progression and metastasis by regulating Treg cells in TNBC. We next mainly focused on the ATF4 expression in TNBC. By comparing the non-tumorigenic epithelial cell MCF10a, human breast cancer cell MCF7 and triple negative breast cancer cell MDA-MB-231, it was found that the expression of ATF4 in MDA-MB-231 was notably higher than that in the other two cell lines, both at mRNA and protein levels (Fig. 6D–E).

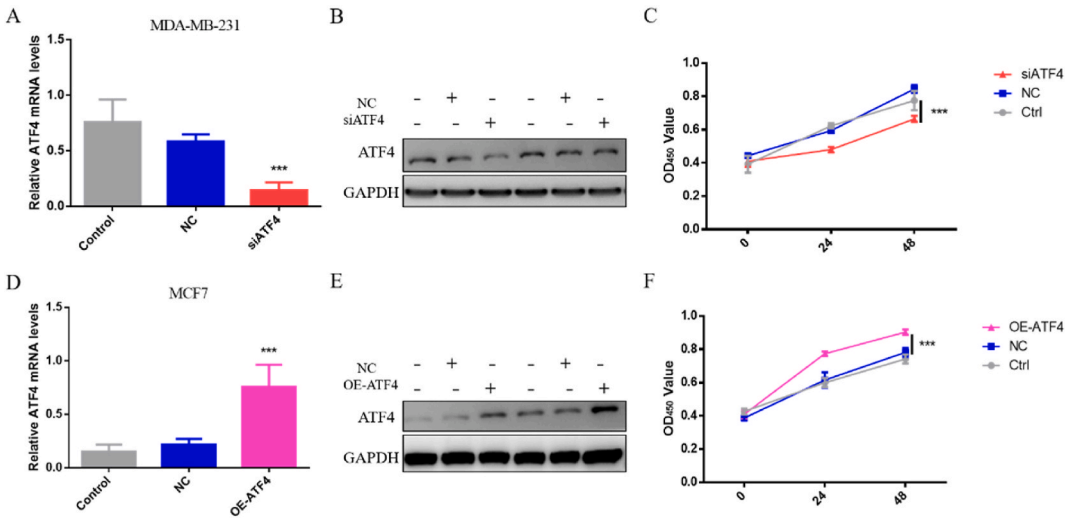

Fig. 7. Somatic mutation analysis of ATF4. (A) Somatic mutation rate of ATF4 in breast cancer. (B) Top ten genes and ATF4 mutation in different groups. (C) The mRNA levels of ATF4 in MCF10a, MCF7, MDA-MB-231 cells. (D–E) The protein levels of ATF4 (left) and the quantity of the protein level (right). * indicates the significant difference compared with MCF10a, $p < 0.05$.

3.8. Knockdown or overexpression of ATF4 can significantly change the proliferation and migration of breast cancer cells

To further verify the effect of ATF4 on breast cancer, we selected the cell line MCF7, which represents LuminalA, and the cell line MDA-MB-231, which represents TNBC. MCF7 overexpressed ATF4 and MDA-MB-231 knocked down ATF4 through plasmid transfection, respectively (Fig. 7A–B, 7D-7E). Subsequently, the effect of ATF4 expression on the proliferation of MCF7 and MDA-MB-231 cells was detected. The results showed that after overexpression of ATF4 in MCF7, the proliferation rate increased. While after knocking down ATF4, the proliferation rate of MDA-MB-231 cells was obviously lower than that of the control group (Fig. 7C and F). In order to further explore the effect of ATF4 on the migration of TNBC, we used mouse triple negative breast cancer cell lines 4T1 and EMT6 to construct a stable viral strain that stably knocks down ATF4. The lower the expression of ATF4, the slower the migration rate (Fig. 8).

3.9. ATF4 regulates the progression of TNBC by regulating of treg cells

Using the above virus stable transfection strains, mice were subcutaneously tumorigenic and divided into four groups: 4T1 group, 4T1-shATF4 group, EMT6 group, and EMT6-shATF4 group. Retain tumor tissue for flow cytometry detection to analyze the composition and proportion of immune cells in the tumor tissue. Through comparative analysis, it was found that the results were close to the public database, that is, in the tumor tissue of TNBC, the expression of ATF4 was positively related to CD4, CD8, and Treg cells, but there was only a statistically significant difference with Treg cells.; There was a negative correlation with neutrophils, macrophages, and NK cells, and there was no statistical difference (Fig. 9A–H).

4. Discussion

The role of ATF4 in the pathogenesis of numerous diseases remains incompletely understood, particularly in relation to its involvement in different types of tumors, where research efforts have been limited. Hence, there is an imperative need to elucidate the precise function of ATF4 in the prognosis, advancement, and therapeutic approaches of cancer. Prior research has documented divergent functions of ATF4 in various types of neoplasms. For instance, in pancreatic ductal adenocarcinoma, the perk/eIF2/ATF4 signaling pathway is activated by knockout of pum1 gene to promote apoptosis and inhibit the growth, invasion and metastasis of tumor cells [21]. Overexpression of ATF4 can inhibit tumor proliferation in prostate cancer [22]. On the other hand, ATF4 promotes tumor growth of endometrial carcinoma by inducing CCL2 expression and macrophage recruitment [23].

Based on the findings of our study, the expression levels of ATF4 and its prognostic significance in pan-cancer were assessed using data from TCGA and GTEx obtained from UCSC Xena. Our analysis revealed that ATF4 exhibited significantly higher expression levels in CHOL, DLBC, GBM, HNSC, KIRC, LGG, LIHC, PAAD, TGCT and THYM, compared to normal tissues. Conversely, lower expression levels of ATF4 were observed in ACC, BLCA, BRCA, CESC, COAD, ESCA, LAML, LUAD, LUSC, OV, PRAD, READ, SKCM, THCA, UCEC and UCS. ATF4 expression levels vary across tumor types. This may indicate different functions and mechanisms.

Tumor microenvironment (TME) is composed of host stromal cells and tumor cells, including immune system components (such as macrophages and lymphocytes), vascular endothelial cells, fibroblasts, muscle fibroblasts, mesenchymal stem cells, adipocytes and extracellular matrix (ECM) [24]. More and more evidence supports clinic-pathological significance of tumor microenvironment immune cells to predict the survival status and curative effect of tumor patients [25]. Specifically, Regulatory T (Treg) cells expressing Foxp3 are essential to prevent autoimmunity and inhibit effective tumor immunity. Treg cells infiltrate into tumor tissue, which is usually related to the poor prognosis of cancer patients. Removal of Treg cells can enhance the anti-tumor immune response, but it may also trigger autoimmunity [26]. GO results showed that ATF4 was primarily associated with RNA polymerase II transcription, cellular responses to external stimuli, transcriptional regulation by TP53 and regulation of TP53 activity according to “C2: Reactome gene sets”. In addition, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis indicated an enrichment and crosstalk of the hub genes in cancer, human T-cell leukemia virus 1 infection and Hepatitis B. Furthermore, the KEGG pathway analysis revealed that the hub genes exhibited enrichment and crosstalk in various biological processes, including cancer, human T-cell leukemia virus 1 infection, and Hepatitis B. Through the examination of the association between ATF4 and the infiltration of immune cells, it was observed that the group exhibiting high expression of ATF4 displayed a notably elevated level of Treg infiltration. Conversely, within this group, a decreased level of T helper cell infiltration was observed in the context of breast cancer. Furthermore, the observed correlation between ATF4 and the expression of immune-suppressive genes suggests that ATF4 plays a crucial role in the regulation of tumor immunology. In conclusion, it is highly probable that ATF4 plays a significant role in the infiltration of immune cells and holds potential as a valuable prognostic biomarker for triple-negative breast cancer.

However, in this study, several limitations have been identified. A systematic bias in our study was inevitable since we used open databases to collect and analyze sequencing data, although we performed some wet experiments to verify the ATF4 expression in breast cancer, more experiments like the regulation of ATF4 to T-cell differentiation need to be conducted. In order to investigate mechanisms and apply clinically, well-designed experiments are required.

5. Conclusions

The findings of our study indicate that ATF4 plays a role in the differentiation of T cells into Treg cells in the context of triple-negative breast cancer. The ATF4 protein has the potential to serve as a valuable biomarker in the clinical setting, as it can be used to predict the overall survival outcomes of patients with triple-negative breast cancer.

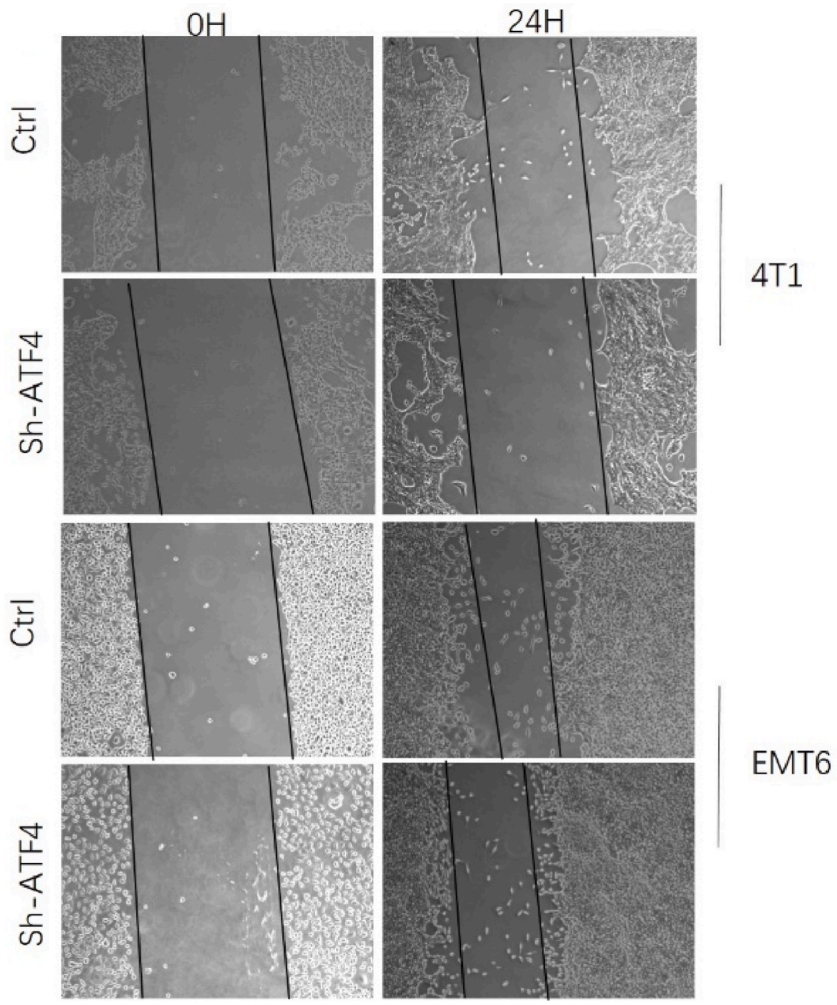

Fig. 8. Changes in cell migration rate after knocking down ATF4. Comparison of cell migration rates of mouse triple negative breast cancer cell lines 4T1 and EMT6 within 24 h before and after ATF4 is knocked down by lentivirus.

Ethics approval and consent to participate

Considering that the data are publicly available, no ethics approval is required for this study.

Consent for publication

All authors reviewed and approved the manuscript.

Data availability statement

Data associated with my study has not been deposited into a publicly available repository. The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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CRediT authorship contribution statement

Lujing Shao: Writing – original draft, Data curation. **Zhounan Zhu:** Software, Formal analysis. **Xinyan Jia:** Writing – review & editing, Validation. **Yabin Ma:** Validation, Supervision. **Chunyan Dong:** Funding acquisition, Conceptualization.

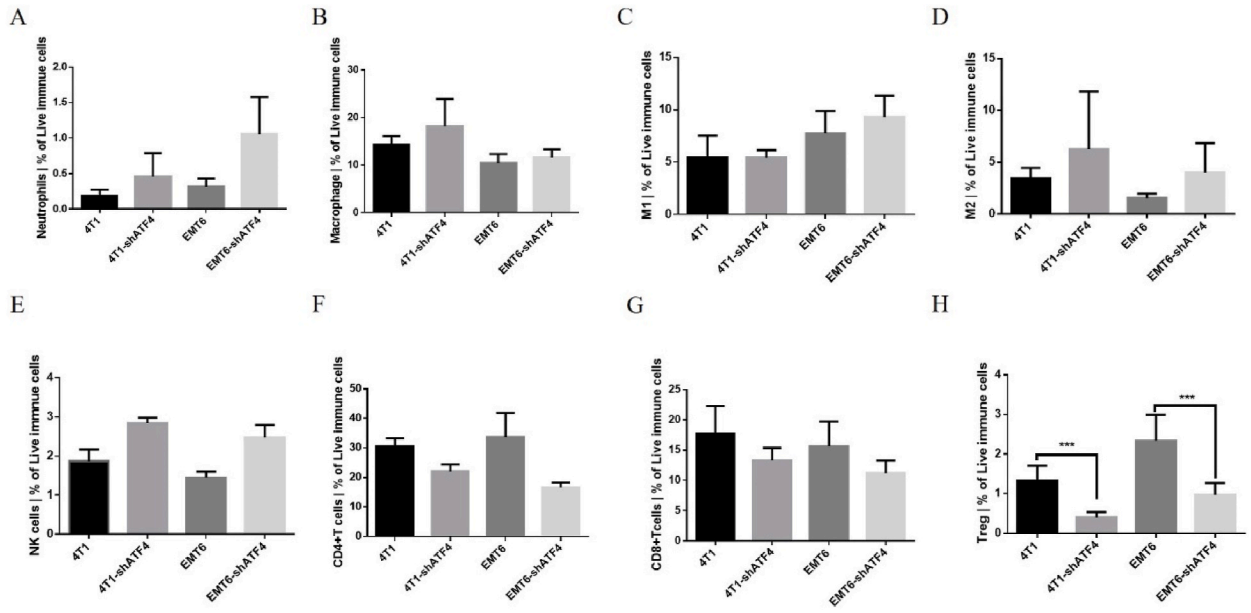

Fig. 9. Correlation between Tregs and ATF4 in TNBC. (A–H) Retain tumor tissue for flow cytometry detection to analyze the composition and proportion of immune cells in the tumor tissue. Using the above virus stable transfection strains, mice were subcutaneously tumorigenic and divided into four groups: 4T1 group, 4T1-shATF4 group, EMT6 group, and EMT6-shATF4 group. Pearson $p < 0.05$.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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List of abbreviations

ATF4	Activating transcription factor
TCGA	The Cancer Genome Atlas
GEO	Gene Expression Omnibus
GTEx	Genotype-Tissue Expression
GSCA	Gene Set Cancer Analysis
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
BRCA	Breast invasive carcinoma
CHOL	Cholangiocarcinoma
COAD	Colon adenocarcinoma
ESCA	esophageal carcinoma
GBM	glioblastoma multiforme
LAML	acute myeloid leukemia
LGG	lower grade glioma
LIHC	liver hepatocellular carcinoma
LUAD	lung adenocarcinoma
OV	ovarian cancer
PAAD	pancreatic adenocarcinoma
STAD	stomach adenocarcinoma
TGCT	testicular germ cell tumor
UCS	uterine carcinosarcoma

HNSC head and neck squamous cell carcinoma
SKCM skin cutaneous melanoma
THCA thyroid carcinoma

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24669.

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