Original Article
The use of MFAP2 for diagnosis, prognosis and immunotherapy of triple-negative breast cancer

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Abstract: Objectives: Triple-negative breast cancer (TNBC) is characterized by significant heterogeneity, presenting a formidable challenge with a poor prognosis and a deficiency of efficacious treatment options. Methods: In this comprehensive study, we investigated the multifaceted role of Microfibril-associated glycoprotein 2 (MFAP2) in TNBC using a combination of bioinformatics analysis involving Gene Expression Omnibus (GEO), OncoDB, UALCAN, Human Protein Atlas (HPA), TIMER, STRING, DAVID, and GSCA databases and in vitro experiments, such as cell culture, MFAP2 gene knockdown, RT-qPCR, western Blot, colony formation, Cell counting kit-8, and wound healing assays. Results: Our findings demonstrated a significant up-regulation of MFAP2 mRNA in TNBC cell lines, emphasizing its potential as a diagnostic biomarker. Validation across multiple datasets further affirmed the elevated expression of MFAP2 in TNBC tissues, underscoring its prognostic relevance. Notably, our study revealed a correlation between MFAP2 expression and immune cell infiltration, suggesting its role in shaping the tumor microenvironment. STRING analysis unveiled interactions with proteins involved in elastic fibers and extracellular matrix constituents. Furthermore, KEGG pathway analysis highlighted enrichment in the TGF-beta signaling pathway, implicating MFAP2 in key cancer-related processes. Drug sensitivity analysis identified potential therapeutic targets, supporting MFAP2's utility in personalized treatment strategies. In vitro experiments corroborated the oncogenic impact of MFAP2, demonstrating its influence on TNBC cell proliferation and migration. Conclusion: These comprehensive findings position MFAP2 as a promising biomarker and therapeutic target in TNBC, offering valuable insight for future research and clinical application.

Keywords: MFAP2, TNBC, prognosis, treatment

Introduction

Breast cancer (BC) displays diversity and can be classified into two subtypes: triple-negative breast cancer (TNBC) and non-triple-negative breast cancer (NTNBC) [1, 2]. TNBC is distinguished from NTNBC based on notable clinicopathological characteristics, including an increased likelihood of recurrence, larger tumor size, lymph node metastases, and a more unfavorable prognosis [3, 4]. Approximately 15-20% of invasive BC cases are characterized as TNBCs. TNBC is identified by the absence of progesterone receptors, estrogen receptors, and HER2 expression [5, 6]. Compared to other types of breast cancer, TNBC presents with an earlier onset and a higher degree of malignancy [6]. TNBC is a subtype with a less favorable prognosis compared to other BC subtypes, primarily because of the lack of effective treatment options [7, 8]. Although there has been extensive research into the molecular changes linked to TNBC, the identification of reliable immune-related biomarkers for TNBC is imperative for diminishing mortality rates and advancing the development of novel targeted therapies.

Microfibril-associated glycoprotein 2 (MFAP2) comprises a 183-amino-acid protein with two domains [9]. It exists in two forms: extracellular MFAP2, a protein that binds to fibrin, collagen VI, tropoelastin, deproteinized, and biglycan14; and intracellular MFAP2, a functional protein that upregulates downstream genes associated with cell adhesion, movement, and matrix

remodeling. Over the past decade, abnormal expression of MFAP2 has been identified in various malignant tumors. In melanoma, MFAP2 is overexpressed, exerting its influence on EMT-related proteins and the Wnt/β-catenin pathway to enhance melanoma invasion and migration abilities [10]. MFAP2 up-regulation has been observed in gastric carcinoma, where it enhances cancer cell migration through the MFAP2/integrin α5β1/FAK/ERK pathway [11, 12]. Additionally, MFAP2 has been reported to activate the TGF-β/SMAD2/3 pathway in gastric carcinoma, expediting the transition of gastric carcinoma from an epithelial cell phenotype to a mesenchymal phenotype [13]. Earlier research has highlighted the high affinity of MFAP2 to TGF-β superfamily members in adipose tissue [14-16].

Nonetheless, investigations into the connection between MFAP2 and TNBC have been lacking. In this study, we utilized various TNBC cell lines and accessed multiple databases, including GEO, TCGA, UALCAN, GEPIA2, and TISIDB, to scrutinize MFAP2 expression in TNBC and its correlation with prognosis. Additionally, we delved into the association between MFAP2 expression and gene mutations, promoter methylation, and immune infiltration. Our findings indicated that abnormal MFAP2 expression was linked to changes in its promoter methylation, influenced immune infiltration in the tumor microenvironment, and served as a prognostic risk factor for TNBC. This research should establish a robust foundation for comprehending the role of MFAP2 in TNBC immunotherapy.

Materials and methods

TNBC cell lines

A total of five human normal breast epithelial cell lines (HBL-100, MCF-10A, MCF-12A, HMEC, and 184B5) and 15 TNBC cell lines (MDA-MB-231, MDA-MB-468, BCap37, Hs 578T, BT-549, HCC1937, HCC1395, HCC3153, SUM-229PE, SUM149PT, SUM1315M02, HCC70, BT-20, CAL148, MDA-MB-157) were acquired from the Cell Bank of the Chinese Scientific Academy. All cell lines were maintained following the supplier's instructions. Post-culturing, cells were incubated at 37°C with 5% CO_2 in a water-jacketed incubator from Thermo Scientific (Waltham, MA, USA). The cell culture medium was refreshed every two days, and experiments were initiated when cells exhibited logarithmic growth at 70-80% confluence.

Reverse transcriptase quantitative PCR (RT-qPCR)

Total RNA was isolated from normal breast epithelial cell lines (HBL-100, MCF-10A, MCF-12A, HMEC, and 184B5) and 15 TNBC cell lines (MDA-MB-231, MDA-MB-468, BCap37, Hs 578T, BT-549, HCC1937, HCC1395, HCC3153, SUM229PE, SUM149PT, SUM1315M02, HCC-70, BT-20, CAL148, MDA-MB-157) using TRIzol reagent (Mei5 Biotechnology, Co., Ltd., https://mei5bio.com/), following the manufacturer's instructions. Subsequently, the extracted RNA was converted into cDNA using the M5 Sprint qPCR RT kit with gDNA remover (Mei5 Biotechnology, Co., Ltd.), as per the manufacturer's guidelines. The extraction and reverse transcription processes were conducted in an enzyme-free environment. AceQ qPCR SYBR Green Master Mix (Vazyme Biotech Co., Ltd., https://www.vazyme.com/) was employed for quantifying the relative expression of MFAP2 (Sangon Biotech Co., Ltd.) in mRNA. The utilized primer sequences were as follows: For GAPDH, forward 5'-CAGGAGGCATTGCTGATGAT-3' and reverse 5'-GAAGGCTGGGGCTCATTT-3'; and for MFAP2, forward 5'-GTCCAACAGGAAGTCATCC-CAG-3' and reverse 5'-CCTGTGTATGGAGTAGA-GGCGG-3'.

Receiver operating characteristic (ROC) curve analysis

The diagnostic capability of MFAP2 in TNBC was evaluated through receiver operating characteristic (ROC) curve analysis. The area under the curve (AUC) was computed, where a greater AUC signifies superior diagnostic performance, and values approaching 1 indicate higher accuracy.

Gene Expression Omnibus data collection and processing

Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) database is a widely utilized repository of tumor-related data [17]. We obtained the original datasets from the GEO databases. In our study, a total of two GEO datasets were acquired. To account for potential false-positive findings, an adjusted P-value

was computed for the GEO datasets. Differential expression of messenger RNAs (mRNAs) was determined by applying a threshold of Adjusted P < 0.05 and Log2 (Fold Change) > 1 or Log2 (Fold Change) < -1.

MFAP2 expression in pooled TCGA datasets

Two well recognized databases, including OncoDB (https://oncodb.org/index.html) and UALCAN (https://ualcan.path.uab.edu/analysis. html) are valuable resources in cancer research [18, 19]. OncoDB serves as a comprehensive oncogenomic database, providing a rich repository of cancer-related genetic alterations and clinical information. Researchers can explore and analyze data to better understand the molecular basis of cancer. UALCAN, on the other hand, focuses on facilitating accessible exploration of cancer transcriptome data from TCGA. It enables researchers to investigate gene expression, survival analysis, and molecular subtyping, enhancing insights into cancer biology and potential therapeutic targets. In the present study, OncoDB and UALCAN databases were utilized to analyze the expression of MFAP2 across the TCGA BC datasets.

MFAP2 expression in the Human Protein Atlas database

The Human Protein Atlas (HPA) database is a comprehensive resource providing detailed information on the expression and localization of proteins in various human tissues and cells, aiding in biomedical research. In the current study, MFAP2 protein expression in TNBC tissues samples was analyzed using HPA database.

Survival analysis of MFAP2

To validate the predictive efficacy of MFAP2, we employed the online tool UALCAN (http://ualcan.path.uab.edu/index.html) [18] to examine the association between MFAP2 expression and survival outcomes across various subtypes of breast cancer (BC) patients. Additionally, we conducted a thorough analysis of MFAP2 expression in diverse clinical variables of BC through the UALCAN platform.

Immune infiltration analysis

We examined the relationship between MFAP2 and immune infiltration using the "Gene" module of Tumor Immune Estimation Resource (TIMER, https://cistrome.shinyapps.io/timer/) [20, 21]. This online database provides comprehensive analysis of immune infiltrates in various cancer types, aiding researchers in understanding the tumor microenvironment.

Construction of a PPI network and Gene Enrichment analysis

The STRING online tool (https://string-db.org/) was employed to assess and integrate information pertaining to protein-protein interactions (PPIs), including their physical and functional associations [22, 23]. In order to further evaluate the interactions and functions of the MFAP2, we utilized the STRING platform to construct a PPI network.

DAVID (https://david.ncifcrf.gov/) is a bioinformatics tool widely used for functional analysis of large gene lists [24]. It aids in uncovering biological insight from diverse genomic data. In the present study, DAVID was used for the Gene Enrichment analysis of the MFAP2-associated genes.

Drug sensitivity analysis

GSCA (https://guolab.wchscu.cn/GSCA//) database is a resource that integrates genomic and drug sensitivity data, facilitating the exploration of associations between genetic features and drug responses in cancer. In the present study, GSCA database was used to perform a drug sensitivity analysis of MFAP2.

Immune checkpoint analysis

The GEPIA database (http://gepia.cancer-pku.cn/) [19, 25] was utilized to assess the expression of immune checkpoint genes in glioma and normal samples. Analysis focused on eight transcripts related to immune checkpoints: SIGLEC15, TIGIT, PD-L1, HAVCR2, PD-1, CTLA4, LAG3, and PDCD1LG2.

Cell transfection

MDA-MB-468 cells were plated onto 6-well plates at a density of 2×10^5 cells per well and cultured in complete medium for 24 hours. Following this, cells were transfected with MFAP2-siRNA using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's guidelines. Subsequently,

cells were incubated for 48 hours before being utilized for interference efficiency assessments or in vitro experiments. The siRNA sequence targeting MFAP2 was as follows: Sense: 5'-CCCACUAUAGCGACCAGAUTT-3'; Antisense: 5'-AUCUGGUCGCUAUAGUGGGTT-3'.

RT-qPCR and western blot analysis

We extracted proteins from control and MDA-MB-468 cell lines using lysis buffer containing protease inhibitors. For collection of the supernatant, the mixture was centrifuged for 20 minutes at 12000 rpm at 4°C. After boiling for 15 minutes at 100°C with 5× sodium dodecyl sulphate loading buffer, protein samples were obtained. In 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), proteins were separated and transferred to polyvinyl difluoride membranes (Millipore, USA). MFAP2 and ACTB primary antibodies were incubated at 4°C for 24 hours after membranes were blocked for 2 hours with 5% skim milk. An enhanced chemiluminescent kit (Millipore, USA) was used to visualize target bands after washing with TBST three times and incubating with specific antibodies for two hours. Finally, the quantification of protein bands was completed using Image J.

Colony-forming assay

Cells were distributed in 6-well plates at a density of 500 cells per well. After a 14-day incubation period, colony formation was assessed. 75% ethanol was introduced, and subsequently, crystal violet staining solution was applied to stain the cells. The resulting cell colonies were photographed and counted.

Cell counting kit-8 (CCK-8) assay

Cells were plated in 96-well plates at a concentration of $2.5×10^4$ cells/mL. Cell viability was evaluated through the CCK-8 assay at 24, 48, 72, 96, and 120 hours. A CCK-8 mixture was created by combining 10 µL of CCK-8 solution (Beyotime, Shanghai, China) with 90 µL of serum-free medium. This mixture was added to the wells and incubated for 2-3 hours. Subsequently, the absorbance of cells at a wavelength of 450 nm was measured using a microplate reader (BioTek, Epoch, USA).

Wound-healing assay

Cells were cultured in 6-well plates with complete medium. Upon reaching confluency, the monolayer was delicately disrupted by gently scraping with a sterile pipette tip. Subsequent removal of any resulting cell debris was done by rinsing with phosphate-buffered saline (PBS), and serum-free medium was introduced. Photographic documentation of cell morphology was conducted both at 0 hour and 24 hours after the monolayer disruption.

Statistical analysis

Statistical analyses were performed using R software. The Wilcoxon test compared two groups of continuous variables, while correlation analysis utilized the Spearman test. Prognostic analyses involved Kaplan-Meier curves and Log rank tests. A significance threshold of $P < 0.05$ applied to all statistical assessments.

Results

MFAP2 mRNA expression was up-regulated in TNBC cell lines

In our investigation, the RT-qPCR technique was employed to assess MFAP2 expression in 5 control cell lines and 15 TNBC cell lines. The results revealed a significant up-regulation of MFAP2 in TNBC cell lines compared to control cell lines (*p*-value < 0.05), as illustrated in **Figure 1A**. Additionally, ROC analysis indicated the potential of MFAP2 as a diagnostic biomarker for TNBC, as depicted in **Figure 1B**. Overall, these findings underscore the promising role of MFAP2 in TNBC detection.

Validation of MFAP2 expression in additional pooled datasets

To further validate MFAP2's expression and importance in TNBC, we conducted additional analyses of MFAP2 mRNA and protein expression levels using data from GEO, TCGA, and HPA web resources. The analysis of GEO datasets (GSE42568 and GSE33447) revealed a statistically significant increase (*p*-value < 0.05) in MFAP2 expression within TNBC samples compared to control samples (**Figure 1C**). Furthermore, ROC curves demonstrated a strong potential of elevated MFAP2 levels for diagnosing TNBC (**Figure 1D**). Additionally, analysis of the TCGA dataset using UALCAN and OncoDB databases, along with immunohistochemistry data from the HPA database, revealed an up-regulation of MFAP2 mRNA and protein expres-
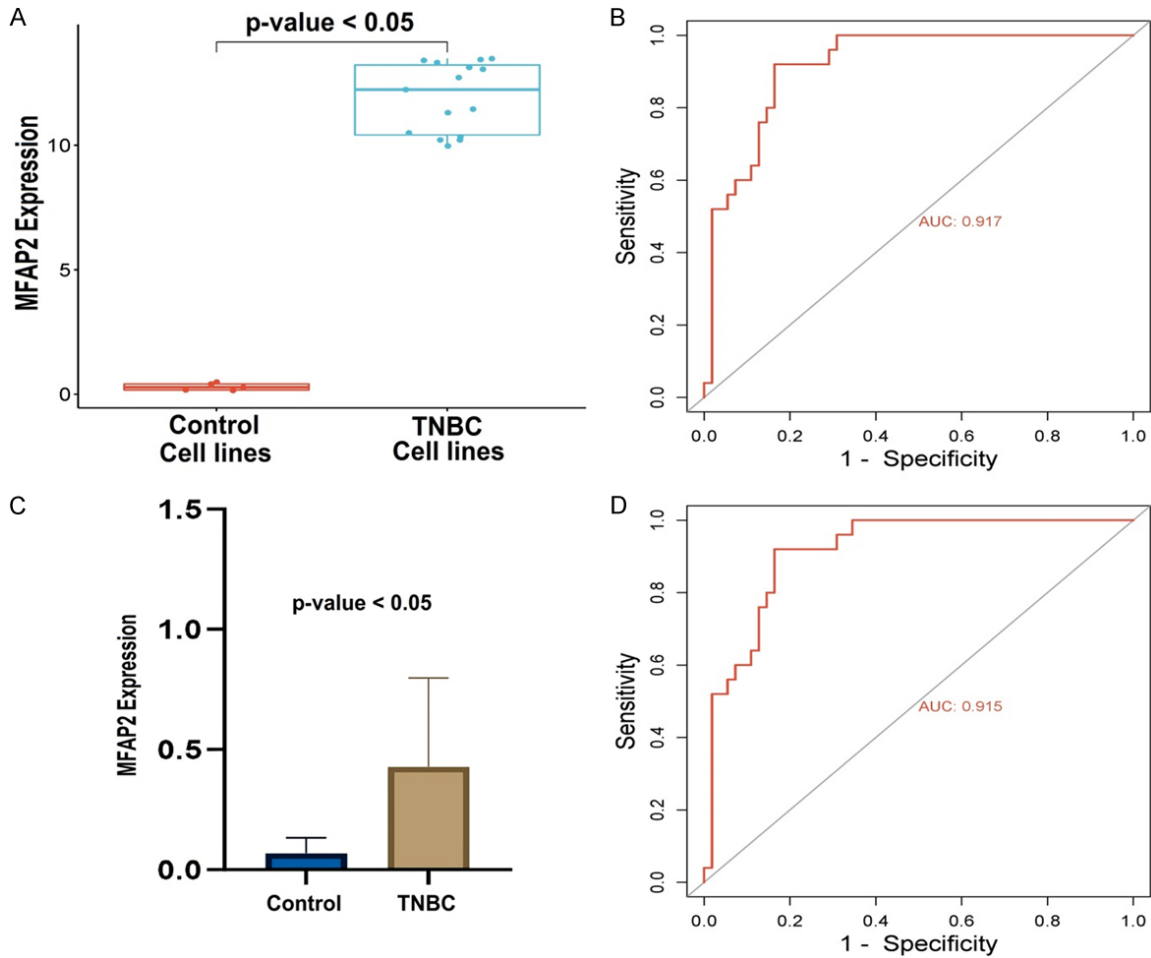
Figure 1. Document expression profile of MFAP2 mRNA in triple-negative breast cancer (TNBC) cell lines using RT-qPCR and datasets from the Gene Expression Omnibus (GEO). A. mRNA expression of MFAP2 in TNBC cell lines compared to normal control cell lines. B. ROC curve based on cell line expression data for MFAP2. C. mRNA expression of MFAP2 in TNBC and normal control tissue samples obtained from the GSE42568 and GSE33447 GEO datasets. D. ROC curve based on expression data from the GSE42568 and GSE33447 datasets for MFAP2. *P*-value < 0.05.

sion levels in TNBC tissues. This observation strongly indicates that MFAP2 plays a noteworthy role in the progression of TNBC, as illustrated in **Figure 2A-D**. Consequently, these results suggest that the expression of MFAP2 is a predictor of unfavorable outcome in TNBC patients.

Evaluation of the prognostic relevance of MFAP2 in BC

To validate the potential prognostic significance of MFAP2, we employed UALCAN to investigate the correlation between MFAP2 levels and overall survival in breast cancer (BC) patients. The survival curves unveiled a notable decrease in the overall survival rate for patients exhibiting elevated MFAP2 expression compared to

those with lower expression levels (*p*-value = 0.018), as depicted in **Figure 3A**. Moreover, by conducting supplementary survival analyses, it was observed that MFAP2, coupled with cancer type (*p*-value = 0.012) or menopause status (*p*-value = 0.00036), could exert a significant influence on the survival outcomes of breast cancer (BC) patients, as illustrated in **Figure 3A**. An exploration of MFAP2 expression levels across various clinical variables of BC was carried out using the UALCAN online tool. The analysis revealed that the mRNA expression level of MFAP2 in BC tissues varied across different clinical variables such as Race, Gender, and Age, with higher levels observed compared to normal tissues (**Figure 3B**).
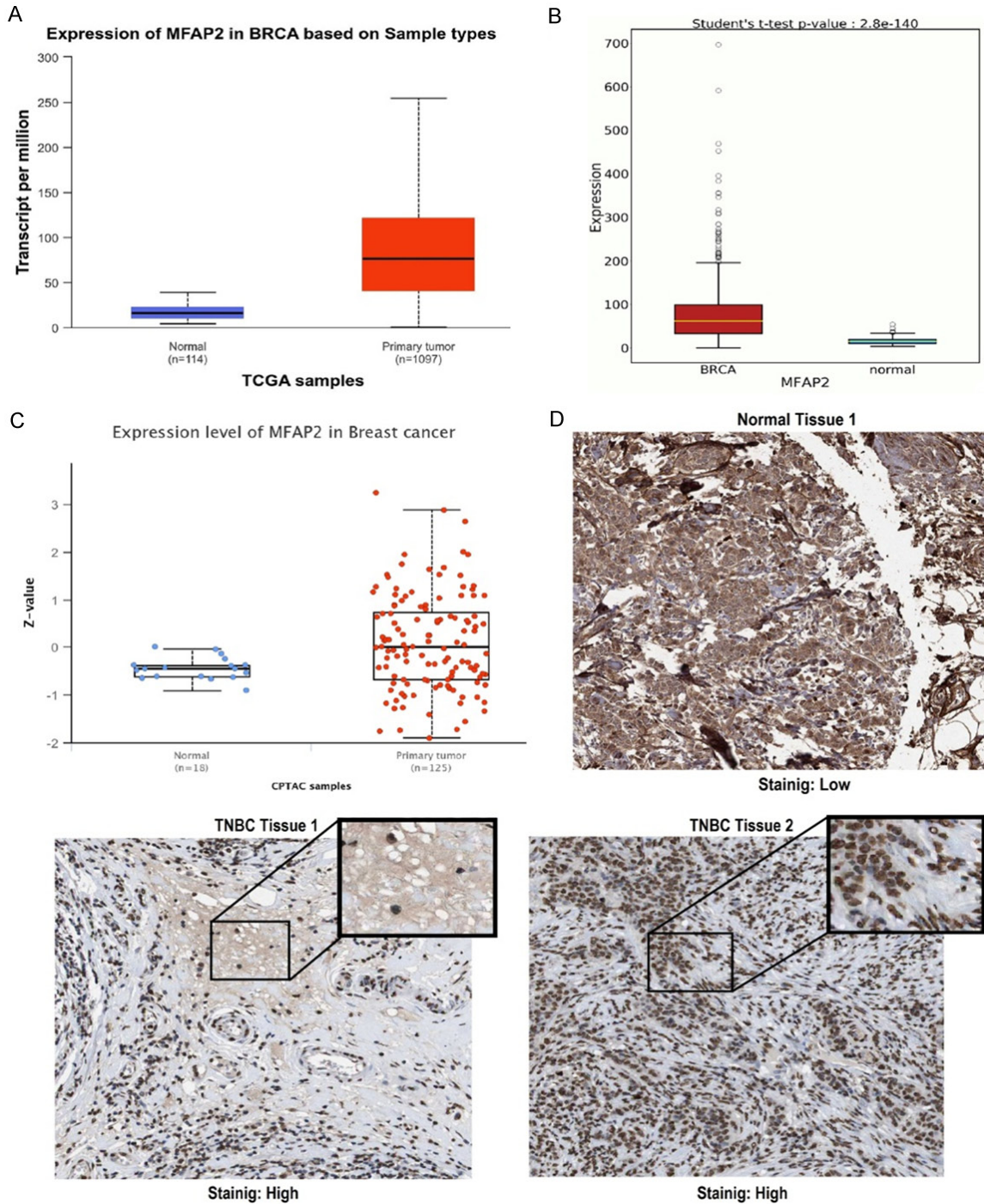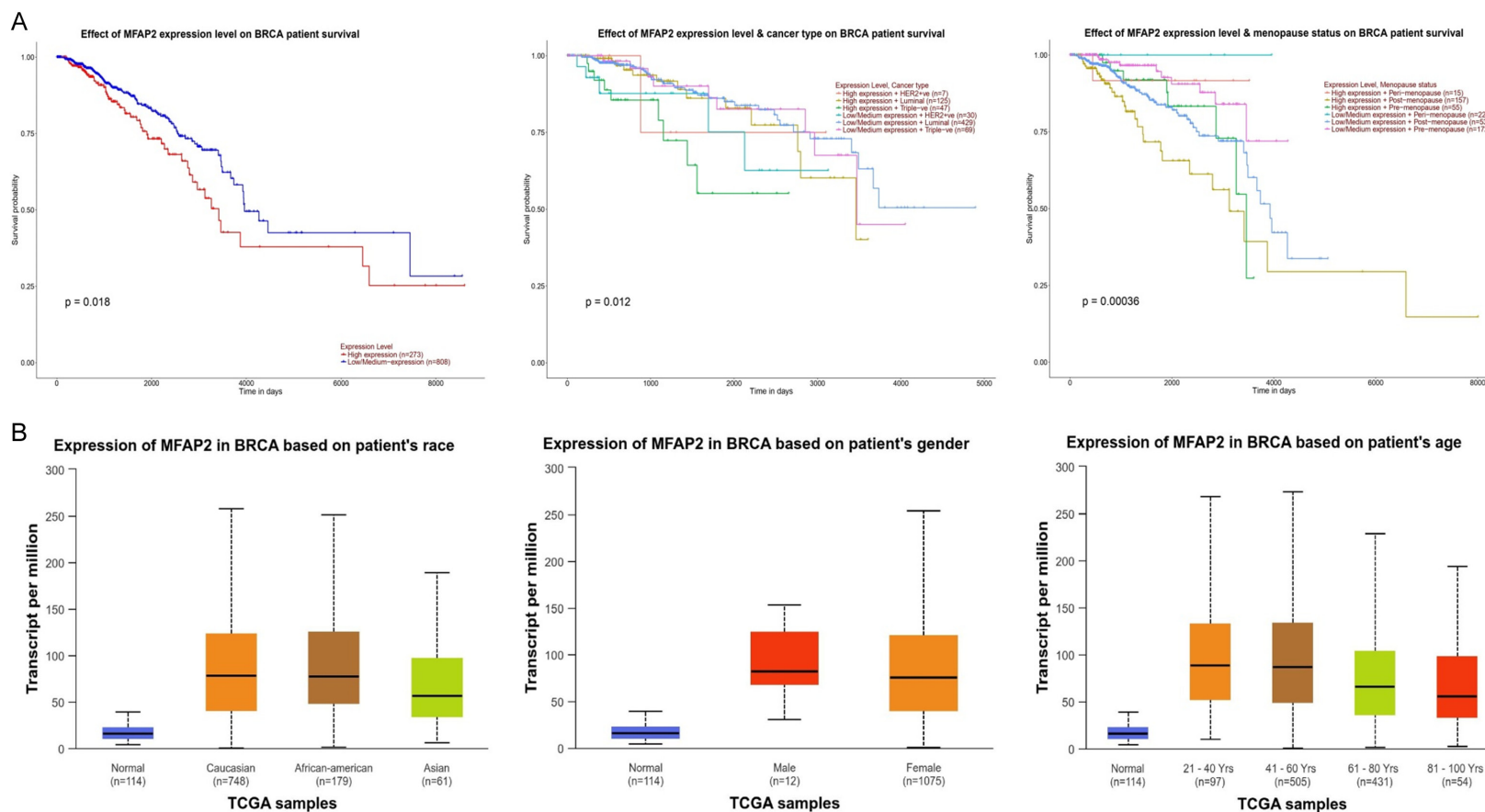
Figure 2. Validation of MFAP2 expression using the Cancer Genome Atlas (TCGA) and Human Protein Atlas (HPA) databases. A. Examination of MFAP2 mRNA expression across TCGA breast cancer (BC) samples using UALCAN. B. Assessment of MFAP2 mRNA expression across TCGA BC samples through OncoDB. C. Evaluation of MFAP2 protein expression across TCGA BC samples using UALCAN. D. Analysis of MFAP2 protein expression in triple-negative breast cancer (TNBC) and control tissue samples via the HPA. *P*-value < 0.05.

Correlation between MFAP2 and immune infiltration

The TIMER network analysis tool was employed to investigate the association between MFAP2

transcription and immune infiltration in TNBC. We explored the relationship between MFAP2 expression and five immune cell types (B cells, CD4+ T cells, CD8+ T cells, neutrophils, and macrophages). A modest yet statistically signifi-

Figure 3. Prognostic significance and examination of MFAP2 expression across diverse clinical variables in breast cancer (BC). A. Using Kaplan-Meier curves from UALCAN, we analyzed the association between MFAP2 expression and overall survival (OS) in BC patients with varying clinical variables. B. Assessment of MFAP2 expression levels across different clinical variables in BC patients. *P*-value < 0.05.

cant correlation was observed between MFAP2 expression and tumor purity (r = -0.234, *p*-value < 0.05). Notably, B cell abundance exhibited a negative correlation with MFAP2 expression, while other immune infiltrates showed a positive correlation, as illustrated in **Figure 4A**.

Based on these findings, we conducted an analysis of differentially expressed immune checkpoint genes, including SIGLEC15, TIGIT, PD-L1, HAVCR2, PD-1, CTLA4, LAG3, and PDCD1LG2, in TNBC tissues and normal tissues. The results demonstrated an up-regulation in the expression of CTLA4, HAVCR2, LAG3, and PD-1 in TNBC tissues (**Figure 4B**), while the expression of SIGLEC15, TIGIT, PD-L1, and PDCD1LG2 was down-regulated in TNBC tissues (**Figure 4B**). These findings suggest that MFAP2 may represent potential targets for immunotherapy.

STRING and Gene Enrichment analyses

STRING analysis via the STRING database showed that MAFP2 protein interact with 10 other proteins including LOX1, DCN, ELN, FBN1, FBN2, LOX, MFAP1, MFAP3, MFAP5, and BGN to perform its task (**Figure 5A**). The Gene Ontology (GO) categories of the MFAP2-associated proteins were examined to ascertain the functional categories in which these genes were implicated.

The findings of the GO analysis demonstrated that, in terms of Cellular Component (CC), the MFAP2-associated proteins exhibited significant enrichment in "Elastic fiber, microfibril, beta-catenin destruction complex, Wnt signalosome, and lysosomal lumen, etc., terms" (**Figure 5B**). Furthermore, in the Molecular Functions (MF) analysis, the MFAP2-assoaciated proteins were predominantly associated with the "Protein-lysine 6-oxidase activity, oxidoreductase activity acting on the CH-NH2 group of donor oxygen, and extracellular matrix constituent conferring elasticity, etc., terms" (**Figure 5C**). Furthermore, the Biological Processes (BP) of MFAP2-assoaciated proteins include "Sequencing of TGFbeta in extracellular matrix, peptidyl-lysine oxidation, and peptide cross-linking via chondroitin 4-sulfate glycosaminoglycan, etc., terms" (**Figure 5D**). To gain further insight into the MFAP2-associated dysregulated pathways, a KEGG pathway analysis

was conducted. The findings revealed that the MFAP2, along with other interacting proteins, was predominantly enriched in the TGF-beta signaling pathways (**Figure 5E**).

*Drug sensitivity analysis of MFAP2*

To substantiate the ability of MFAP2 to inform personalized treatment for TNBC patients, we carried out a drug sensitivity analysis utilizing TNBC data sourced from the GSCA website. A bubble plot analysis, utilizing GDSC and CTRP IC_{50} drug data from the GSCA database, was conducted to explore the correlation between MFAP2 in breast cancer and the responsiveness of these tumors to small molecule drugs (**Figure 6**). We found that the expression of MFAP2 had negative correlation with 07 drugs, including TW 37, Olaparib, Elesclomol, Docetaxel, Cisplatin, Bleomycin (50 um), and AG-014699 (**Figure 6**) while positive correlation with 23 drugs, including AT-7519, BHG712, BIX02189, CAL-101, GSK690693, I-BET-762, KIN001-102, KIN001-236, KIN001-244, KIN-001-260, Methotrexate, NG-25, PHA-793887, Phenformin, PIK-9, 2QL-XI-92, STF-62247, TAK-715, THZ-2-49, TL-1-85, TPCA-1, Tubastatin A, ZSTK474 (**Figure 6**).

MFAP2 promoted the proliferation and migration of TNBC cells

Initially, we observed a significant up-regulation of MFAP2 in TNBC cell lines when compared to the control cell lines. To delve deeper into the functional implications of MFAP2 in TNBC, we employed siRNA-mediated knockdown of MFAP2 in MDA-MB-468 cells. The effectiveness of the knockdown was subsequently verified through RT-qPCR and western blot. These analyses confirmed that the expression of MFAP2 was markedly lower in the transfected MDA-MB-468 cells (si-MFAP2-MDA-MB-468) compared to the control MDA-MB-468 cells (Ctrl-MFAP2-MDA-MB-468) (**Figure 7A**, **7B** and Supplementary Figure 1). Furthermore, a colony-forming assay was conducted, revealing a notable decrease in the clonogenic potential of si-MFAP2-MDA-MB-468 cells upon the reduction of MFAP2 expression (**Figure 7C**, **7D**). CCK-8 assays indicated a significant reduction in the proliferative capacity of si-MFAP2-MDA-MB-468 cells following MFAP2 knockdown (**Figure 7E**). Additionally, we explored the impact
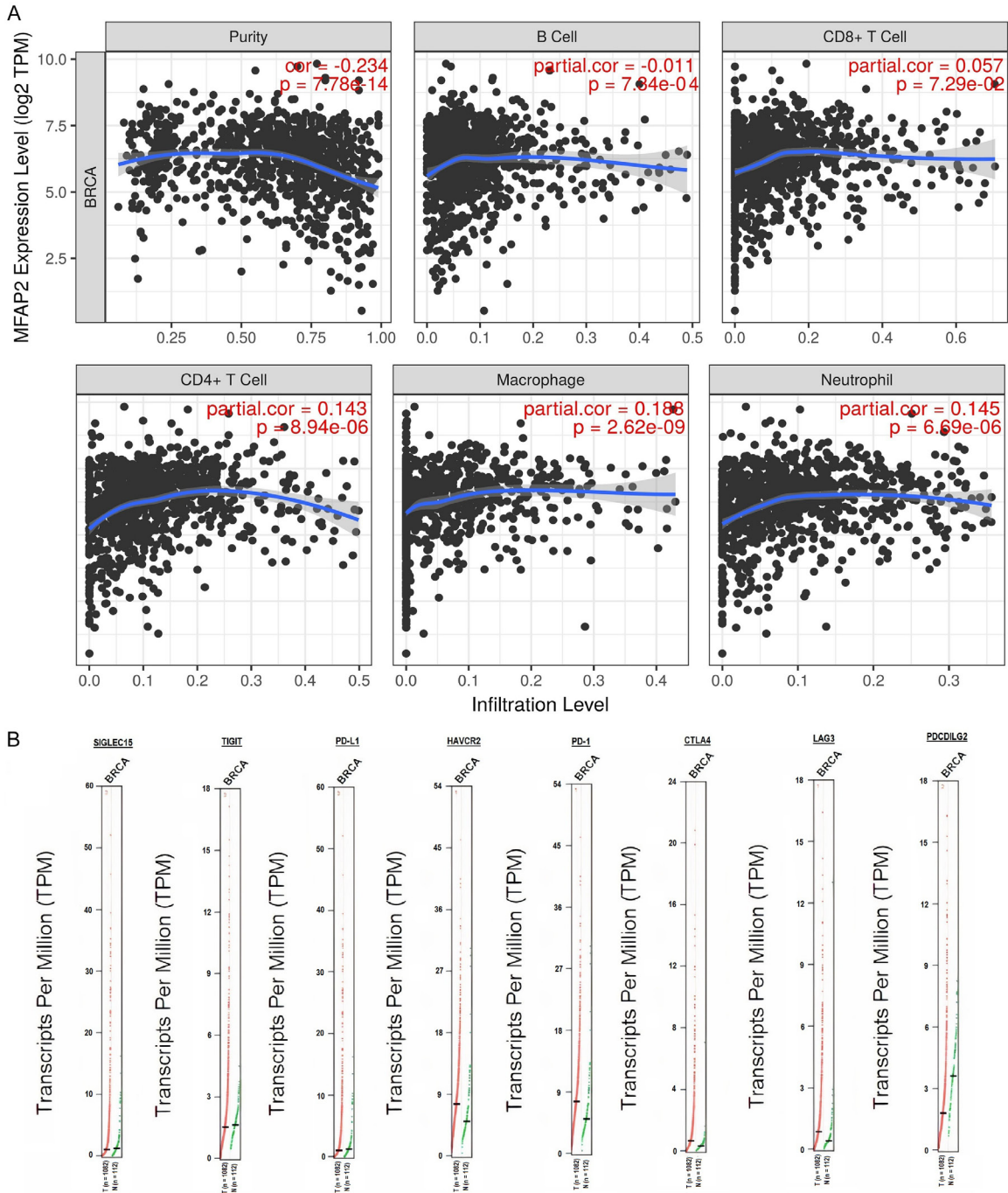
Figure 4. Analysis of immune infiltration in relation to MFAP2. A. Correlation between MFAP2 expression and immune cell abundance. B. Expression levels of immune checkpoints in breast cancer (BC) and normal tissues. *P*-value < 0.05.

of MFAP2 on the migratory ability of MDA-MB-468 cells, observing a clear increase in cell migration in si-MFAP2-MDA-MB-468 cells following MFAP2 knockdown (**Figure 7F**). Collectively, these findings underscore the crucial role of MFAP2 in TNBC.

Discussion

MFAP2 plays a vital role as a constituent of extracellular elastic microfibers, interacting with and influencing fibrin. Additionally, it serves as the foundational protein for the majority of
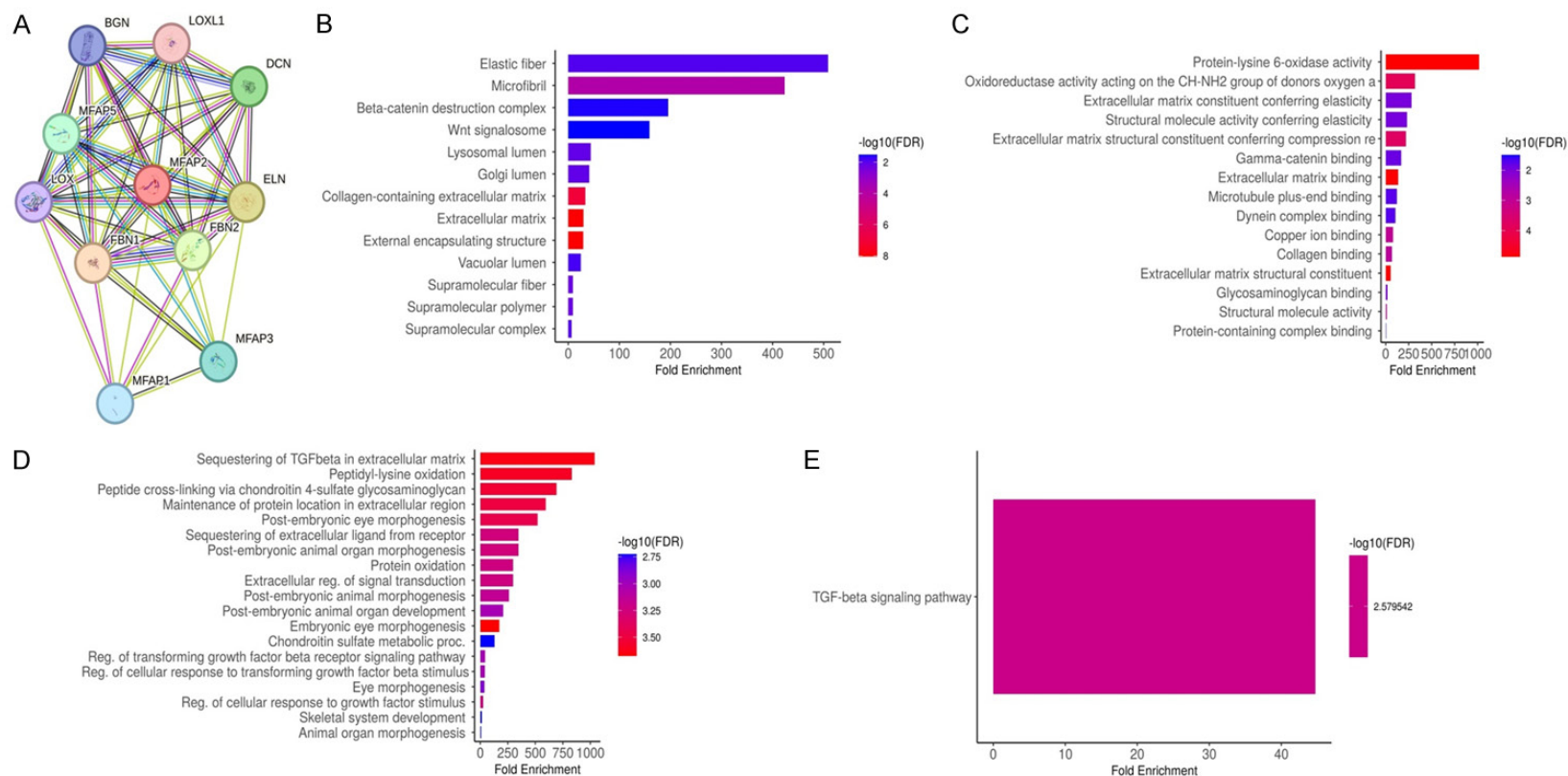
Figure 5. Constructing the Protein-Protein Interaction (PPI) network and conducting Gene Enrichment analysis for MFAP2. A. Construction of a PPI network for MFAP2. B. Bar graph representing Gene Ontology (GO) enrichment analysis in terms of Cellular Component (CC). C. Bar graph illustrating GO enrichment analysis in Molecular Function (MF). D. Bar graph depicting GO enrichment analysis in Biological Processes (BP). E. Bar graph representing the results of KEGG pathway analysis. *P*-value < 0.05.
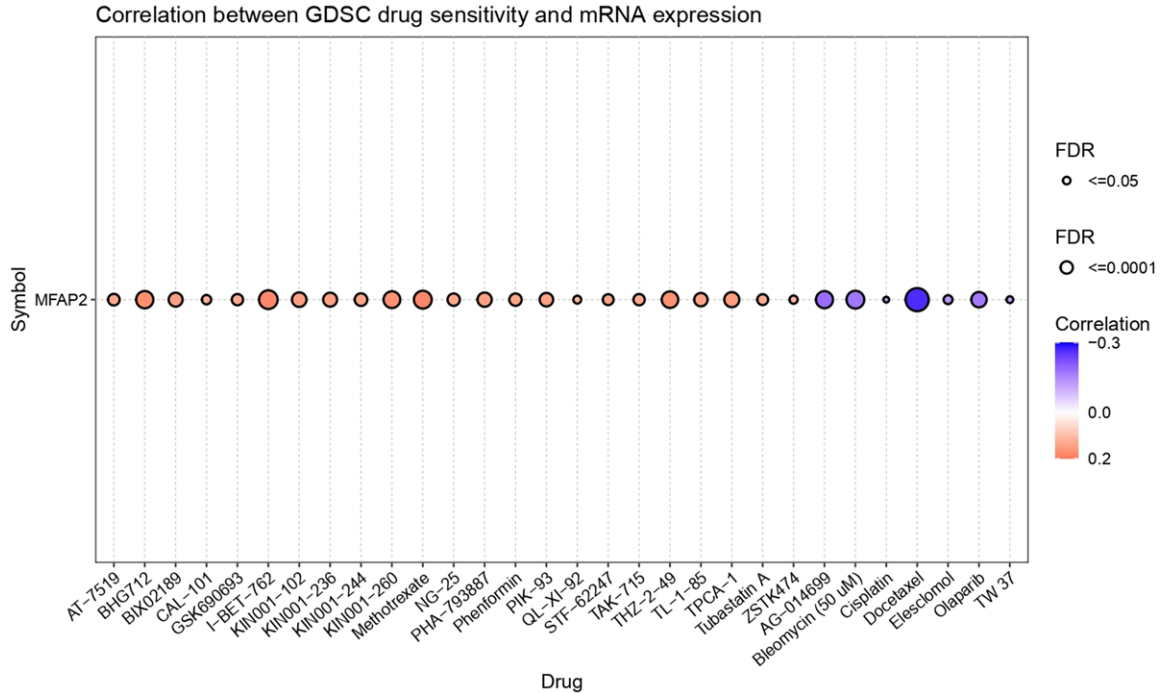
Figure 6. Examining the correlation between MFAP2 expression and drug sensitivity in breast cancer (BC) through the use of genomics of drug sensitivity in cancer and cancer therapeutics response portal IC$_{50}$ data within the gene set cancer analysis (GSCA) web server. *P*-value < 0.05.

vertebrate microfibrils [26-30]. Notably, MFAP2 exhibits a distinctive capability to interact with TGF-β family growth factors, Notch, Notch ligands, and various elastins [31]. Mutations in the MFAP2 gene have been associated with conditions such as thrombosis, thoracic aneurysms, metabolic disorders, and osteopenia in humans [32]. Research indicates that MFAP2 is prominently expressed in gastric cancer tissues, with its elevated expression significantly correlated with both overall and disease-free survival among patients with gastric cancer [9]. Moreover, MFAP2 may participate in activating the TGF-β/SMAD2/3 signaling pathway, contributing to the promotion of proliferation, migration, invasion, and epithelial-mesenchymal transition in gastric cancer cells [13]. An earlier study identified MFAP2 as a novel target of microRNA-29, proposing that the miR-29/MFAP2/integrin α5β1/FAK/ERK1/2 pathway could play a crucial role in the progression of gastric cancer [12]. Furthermore, investigations into hepatic carcinoma have highlighted the significance of MFAP2, with its overexpression in hepatic carcinoma being linked to cancer staging, poor OS, and disease-specific survival [11, 31]. In vitro experiments involving

MFAP2 knockdown have demonstrated that the down-regulation of MFAP2 inhibits the proliferation and migration of liver cancer cells [32].

In this study, we observed an elevation (*p* < 0.05) in both mRNA and protein expression levels of MFAP2 in TNBC cell lines and tissue samples when compared to control samples. Additionally, we identified a correlation (*p* < 0.05) between the expression of MFAP2 and various clinical variables, as well as the prognosis of TNBC patients. These findings strongly imply that MFAP2 functions as an oncogene in TNBC. Recent studies have uncovered a correlation between tumor immune infiltration and cancer growth. Data mining from public sources has indicated an association between MFAP2 transcription and immune infiltration in TNBC. Within tumor-infiltrating lymphocytes, two distinct subpopulations of CD4+ cells, namely Th1 and Th2 helper T cells, have been identified. Th2 cells are known for releasing IL-4 and IL-10, which can impede the host immune system, creating a conducive environment for tumor proliferation [33-36]. Additionally, macrophages exhibit two functional phenotypes influ-
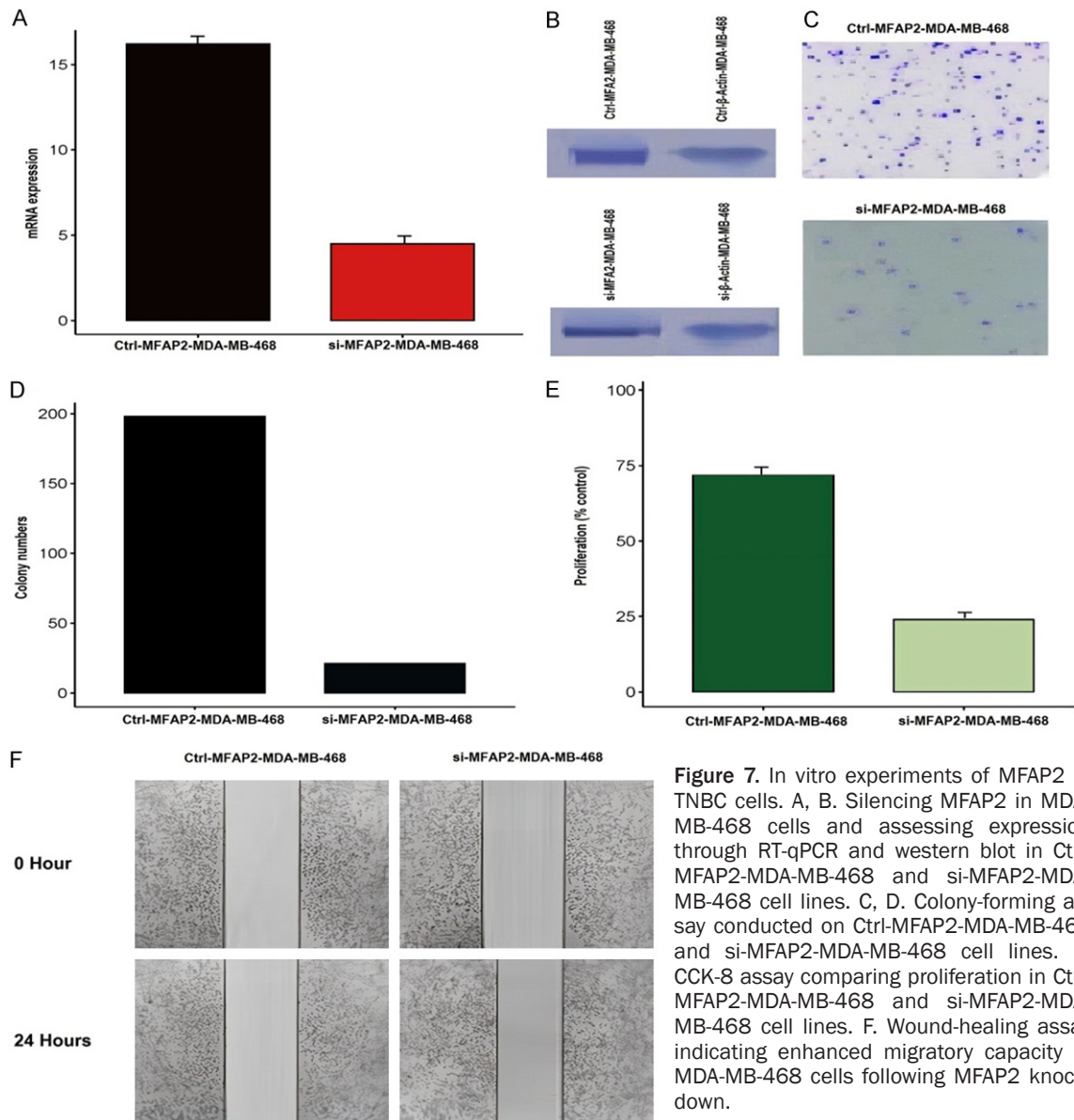
Figure 7. In vitro experiments of MFAP2 in TNBC cells. A, B. Silencing MFAP2 in MDA-MB-468 cells and assessing expression through RT-qPCR and western blot in Ctrl-MFAP2-MDA-MB-468 and si-MFAP2-MDA-MB-468 cell lines. C, D. Colony-forming assay conducted on Ctrl-MFAP2-MDA-MB-468 and si-MFAP2-MDA-MB-468 cell lines. E. CCK-8 assay comparing proliferation in Ctrl-MFAP2-MDA-MB-468 and si-MFAP2-MDA-MB-468 cell lines. F. Wound-healing assay indicating enhanced migratory capacity in MDA-MB-468 cells following MFAP2 knockdown.

enced by signals in the tumor microenvironment: classically activated macrophages (M1) and alternatively activated macrophages (M2). M2 macrophages possess anti-inflammatory and carcinogenic properties, in contrast to the anti-tumor properties of M1 macrophages [37]. Our study revealed a significant correlation (p-value < 0.05) between MFAP2 expression and various immune cell types, including CD8+ T cells, CD4+ T cells, macrophages, and neutrophils. A prior investigation has also highlighted the positive association between MFAP2 over-expression and markers of Th2 cells and M2 macrophages in glioma [38], suggesting a pivotal role for MFAP2 in modulating tumor immu-

nity. Consequently, it is plausible that MFAP2 may exert similar effects on the immune infiltration of TNBC, positioning it as atarget for future immunotherapeutic interventions.

The up-regulation of MFAP2, particularly in conjunction with the TGF-beta signaling pathway as revealed by KEGG pathway analysis, implies a significant role in cancer development. This association suggests contributions of MFAP2 to uncontrolled cell growth, enhanced invasion and metastasis through epithelial-mesenchymal transition, modulation of the immune microenvironment, extracellular matrix remodeling, angiogenesis, and resistance to thera-

pies [39-42]. Further investigation is crucial to elucidate the specific mechanisms through which MFAP2 and TGF-beta signaling orchestrate these cancer-related pathways.

Furthermore, the functional role of MFAP2 in TNBC has not been previously confirmed through in vitro experiments. In our investigation, we extensively validated the oncogenic influence of MFAP2 using in vitro experiments. As expected, our experimental results aligned with the bioinformatic analysis, affirming that MFAP2 indeed plays a role in promoting cell proliferation and metastasis in TNBC. Finally, through drug sensitivity analysis, we revealed that the expression of MFAP2 had negative correlation with 07 drugs, including TW 37, Olaparib, Elesclomol, Docetaxel, Cisplatin, Bleomycin (50 um), and AG-014699 (**Figure 6**) while positive correlation with 23 drugs, including AT-7519, BHG712, BIX02189, CAL-101, GSK690693, I-BET-762, KIN001-102, KIN001-236, KIN001-244, KIN001-260, Methotrexate, NG-25, PHA-793887, Phenformin, PIK-9, 2QL-XI-92, STF-62247, TAK-715, THZ-2-49, TL-1-85, TPCA-1, Tubastatin A, ZSTK474. These findings signify MFAP2's potential as a predictive marker for drug response in TNBC, paving the way for tailored therapeutic approaches.

Conclusion

MFAP2 is pivotal in TNBC. We demonstrated a significant up-regulation of MFAP2 in TNBC cell lines and tissue samples, establishing its potential as a diagnostic and prognostic marker. The correlation between MFAP2 expression and immune cell infiltration emphasizes its involvement in the tumor microenvironment. Additionally, our in-depth analysis revealed enrichment in the TGF-beta signaling pathway, suggesting its influence on key processes in cancer development. Notably, drug sensitivity analysis highlighted MFAP2 as a potential target for personalized treatment strategies in TNBC. In vitro experiments provided functional validation, confirming the oncogenic impact of MFAP2. These comprehensive findings underscore MFAP2's multifaceted role and its promising implications for future therapeutic use in TNBC.

Disclosure of conflict of interest

None.

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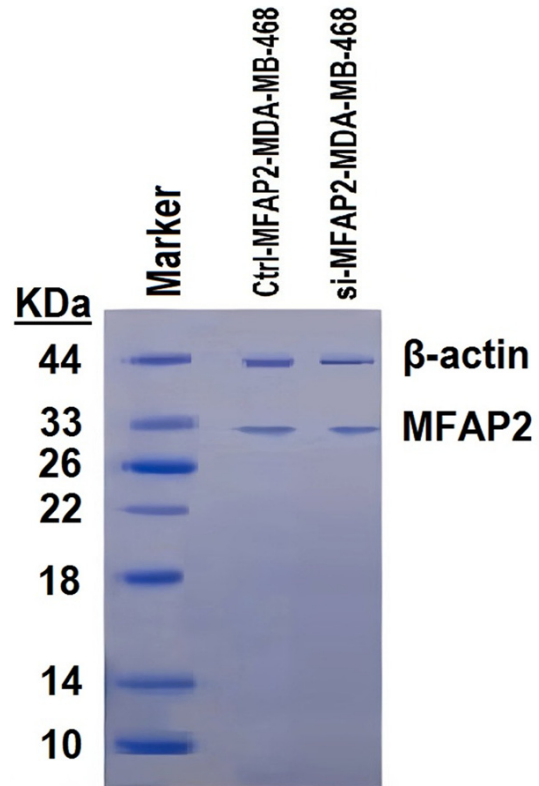
Supplementary Figure 1. Uncropped western blot images of MFAP2 and β-actin protein in control (Ctrl-MFAP2-MDA-MB-468) and transfected TNBC cell lines (si-MFAP2-MDA-MB-468).