OTUD7B knockdown inhibits the proliferation and stemness of breast cancer cells by destabilizing FOXM1

HEBING WANG1*, SUMEI HAN2*, JIAN XIAO1, XINGHANG FU1, WENFENG CHEN2 and DEXIANG ZHUO3

Departments of 1Breast Surgery, 2Dermatology, and 3Laboratory Medicine, Sanming First Hospital Affiliated to Fujian Medical University, Sanming, Fujian 365000, P.R. China

Received November 4, 2022; Accepted October 16, 2023

DOI: 10.3892/ol.2024.14235

Abstract. Breast cancer is a leading cause of cancer-related death in women worldwide; therefore, there is an urgent need to develop novel therapies and drugs that prolong the survival and improve the quality of life of patients with breast cancer. In the present study, the effects and underlying mechanisms of OTU domain-containing 7B (OTUD7B) knockdown on breast cancer were investigated using MDA-MB-468, MDA-MB-453 and MCF7 cell lines. The results of Cell Counting Kit 8, colony formation and tumor sphere formation experiments showed that OTUD7B knockdown caused a significant decrease in the proliferation and sphere formation ability of MDA-MB-468, MDA-MB-453 and MCF7 cells in vitro. Moreover, western blotting results showed that CD44, EpCAM, SOX2 and Nanog protein levels were significantly decreased following OTUD7B knockdown. These findings indicated that OTUD7B knockdown reduced the proliferation and stemness of breast cancer cells. Co-immunoprecipitation assays demonstrated that OTUD7B interacted with forkhead box protein M1 (FOXM1) and reduced the polyubiquitylation of FOXM1 in breast cancer cells; accordingly, FOXM1 protein levels were significantly decreased by OTUD7B knockdown. Furthermore, the overexpression of FOXM1 reduced the inhibitory effects of OTUD7B knockdown on breast cancer cells. The findings of the present study provide new insights into the oncogenic role of OTUD7B in breast cancer and indicate that OTUD7B may serve as a therapeutic target for breast cancer.

Introduction

Breast cancer is the most common cancer type and a leading cause of cancer-related death in women worldwide. Breast cancer is clinically classified into four subtypes that are characterized by the expression of hormone receptors: Estrogen receptor (ER)-positive, progesterone receptor (PR)-positive, human epidermal receptor 2 (HER2)-positive, and triple-negative for ER, PR and HER2 (1,2). Breast cancer is treated by various methods, including surgery, chemotherapy, radiotherapy and endocrine therapy (3,4). For early-stage breast cancer, there is a good chance of patients achieving cancer-free status following multimodal therapy (3). By contrast, advanced breast cancer is typically regarded as incurable, with patients surviving for only 2-4 years (5). Thus, there is an urgent need to develop novel therapies and drugs that can prolong survival and improve the quality of life of patients with advanced breast cancer.

Deubiquitinases are components of the proteasome system that remove conjugated ubiquitin molecules from target proteins, and thus regulate their stability and bioactivity (6). Deubiquitinases are involved in several biological processes related to cancer development, including proliferation, cell cycle progression, apoptosis and metastasis (7). Increasing evidence has shown that deubiquitinases contribute to breast cancer growth, metastasis, immunosuppression, chemosensitivity and radiosensitivity (8). OTU domain-containing 7B (OTUD7B), a deubiquitinase is a member of the A20 subgroup of the ovarian tumor protein superfamily, which is often dysregulated in human cancer and may serve as a valuable prognostic predictor (9-14). In ERα-positive breast cancer, OTUD7B deubiquitylates ERα, leading to its stabilization; through this mechanism, OTUD7B promotes the proliferation of breast cancer cells (15). In ERα-negative breast cancer, silencing OTUD7B markedly inhibits the migration and

Correspondence to: Professor Dexiang Zhuo, Department of Laboratory Medicine, Sanming First Hospital Affiliated to Fujian Medical University, 29 Liedong Street, Sanyuan, Sanming, Fujian 365000, P.R. China
E-mail: wubishou@fjmu.edu.cn

Dr Wenfeng Chen, Department of Dermatology, Sanming First Hospital Affiliated to Fujian Medical University, 29 Liedong Street, Sanyuan, Sanming, Fujian 365000, P.R. China
E-mail: wenfengchen@fjmu.edu.cn

*Contributed equally

Abbreviations: OTUD7B, OTU domain-containing 7B; FOXM1, forkhead box protein M1; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal receptor 2; RT-qPCR, reverse transcription-quantitative PCR; HCC, hepatocellular carcinoma; Co-IP, co-immunoprecipitation; CHX, cycloheximide; MFI, mean fluorescence intensity; ns, not significant

Key words: breast cancer, deubiquitinase, OTUD7B, FOXM1, stemness
invasion of cancer cells in vitro, and significantly impairs lung metastasis in vivo (16). Furthermore, high OTUD7B expression is associated with poor paclitaxel response in patients with triple-negative breast cancer (17).

In the present study, co-immunoprecipitation (co-IP) assay was used to investigate whether OTUD7B interacts with forkhead box protein M1 (FOXM1) and reduces FOXM1 ubiquitination to stabilize the FOXM1 protein. FOXM1 is a proliferation-specific transcription factor that is involved in the initiation, progression, metastasis and drug resistance of breast cancer (18). Furthermore, the present study investigated whether OTUD7B inhibits the proliferation and stemness of breast cancer cells by regulating the protein stability of FOXM1.

Materials and methods

Cell culture and tumor tissues. MCF-10A, MDA-MB-436, MDA-MB-231, MDA-MB-468, MDA-MB-453 and MCF-7 cells were obtained from Xiamen Immocell Biotechnology Co., Ltd., and were cultured in Dulbecco's modified Eagle's medium ( Gibco; Thermo Fisher Scientific, Inc.) containing 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.). Cells were cultured at 37°C and 5% CO₂ in an incubator.

A total of 10 pairs of breast cancer tissue samples and adjacent normal tissue samples (1 cm away from the edge of the tumor and free of tumor cells) were obtained from Sanming First Hospital Affiliated to Fujian Medical University (Sanming, China). The age range of the 10 female patients was 43-62 years (mean age, 53±6.5 years). All of the patients did not receive any other treatment, such as chemotherapy, target therapy or other preoperative treatment, except for surgical operation.

Plasmid construction. Plasmid encoding His-ubiquitin (His-Ub) was obtained from Xiamen Anti-HeLa Biological Technology Trade Co., Ltd. The plasmids encoding hemagglutinin (HA)-OTUD7B and Flag-FOXM1 were constructed by subcloning OTUD7B and FOXM1 cDNA into pCDNA3.1-HA and pCDNA3.1-Flag vectors (Xiamen Anti-HeLa Biological Technology Trade Co., Ltd.), respectively. FOXM1 cDNA was also inserted into plv-EF1a-MCS-c-Flag-bsd vector (Xiamen Anti-HeLa Biological Technology Trade Co., Ltd.), and the resulting plasmid was named plv-Flag-FOXM1, which was used to produce stable OTUD7B-knockdown and FOXM1-overexpression cells. Plasmids encoding a short hairpin (sh)RNA targeting OTUD7B (shOTUD7B) and a negative control shRNA (shNC) were constructed using the pLKO.1 vector (Xiamen Anti-HeLa Biological Technology Trade Co., Ltd.). The sequences for plasmid construction are listed in Table I.

To determine the knockdown or overexpression efficiency of plasmids in different breast cancer cells, MDA-MB-468, MDA-MB-453 and MCF7 cells were cultured in DMEM supplemented with 2% B27 and 5 µg/ml puromycin (Gibco; Thermo Fisher Scientific, Inc.) at 37°C. The optical density was determined using an absorbance reader (SpectraMax®, Molecular Devices, LLC) at 450 nm.

Colonies formation assay. Clusters of cells containing >50 cells were considered colonies. The same cells used in the cell proliferation assay were seeded in 6-well plates at a density of 1x10⁵ cells/well. After 10 days of culture, colonies were fixed with 4% paraformaldehyde overnight at 4°C. The fixed colonies were then stained with 0.1% crystal violet for 10 min at 26°C. A light microscope was used to count the colony numbers.

Sphere formation assay. The same cells used in the cell proliferation assay were seeded in 6-well plates (1x10⁴ cells/well) containing serum-free DMEM supplemented with 2% B27
NeuroMix (Absin Bioscience, Inc.), 20 ng/ml epithelial growth factor (Sigma-Aldrich; Merck KGaA), 20 ng/ml basic fibroblast growth factor (Sigma-Aldrich; Merck KGaA) and 4 µg/ml insulin. After 7 days of culture at 37˚C, the sphere diameters were measured under a light microscope.

Flow cytometric analysis. The same cells used in the cell proliferation assay were plated in 6-well plates (2x10^6 cells/well). After 24 h, the cells were harvested and incubated with FITC-conjugated antibodies for CD44 (cat. no. 12211-MM02-F; 1:200; Sino Biological, Inc.), CD24 (cat. no. 655154; 1:200; BD Biosciences) or EpCAM (cat. no. 10694-MM06-F; 1:200; Sino Biological, Inc.) at 26˚C for 30 min. The fluorescence intensity was determined using a NovoCyte flow cytometer (ACEA Biosciences; Agilent Technologies, Inc.) and NovoExpress software (1.4.1; ACEA Biosciences; Agilent Technologies, Inc.).

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was isolated from MCF-10A, MDA-MB-436, MDA-MB-231, MDA-MB-468, MDA-MB-453 and MCF-7 cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. RNA was reverse transcribed to cDNA using a reverse transcriptase cDNA synthesis kit (cat. no. RR036Q; Takara Bio, Inc.) according to the manufacturer's protocol. qPCR was conducted on the obtained cDNA with a Roche Lightcycler 480 system (Roche Diagnostics) and TB Green® premix (cat. no. RR820Q; Takara Biotechnology Co., Ltd.). The qPCR program included preheating (95˚C for 30 sec), 40 cycles of heating (95˚C for 5 sec), cooling and extension (60˚C for 30 sec), and dissolution curve procedures (95˚C for 5 sec, 60˚C for 60 sec and 95˚C for 1 sec). The relative expression levels of mRNA were calculated using the 2^-ΔΔCq method (19). The qPCR primers are listed in Table II with 18S used as the reference gene.

Western blotting. MCF-10A, MDA-MB-436, MDA-MB-231, MDA-MB-468, MDA-MB-453 and MCF-7 cells; stable OTUD7B-knockdown MDA-MB-468, MDA-MB-453 and MCF7 cells; stable OTUD7B-knockdown and FOXM1-overexpressing MDA-MB-468, MDA-MB-453 and MCF7 cells were lysed in RIPA buffer containing protease and phosphatase inhibitors (Beyotime Institute of Biotechnology). Protein quantification was performed using the bicinchoninic acid method. A total of 20 µg protein/lane was separated by SDS-PAGE on 10% gels and transferred to polyvinylidene difluoride membranes (Bio-Rad Laboratories, Inc.). After blocking with 5% skimmed milk at 25˚C for 1 h, the membranes were incubated with primary antibodies against OTUD7B (cat. no. 16605-1-AP; 1:1,000), Nanog (cat. no. 14295-1-AP; 1:1,000), SOX2 (cat. no. 11064-1-AP; 1:1,000), Flag (cat. no. 20543-1-AP; 1:20,000), HA (cat. no. 51064-2-AP; 1:5,000), His (cat. no. 10001-0-AP; 1:1,000), β-actin (cat. no. 10094-1-AP; 1:2,000), FOXM1 (cat. no. 13147-1-AP; 1:2,000) or GAPDH (cat. no. 10494-1-AP; 1:5,000) (all from Proteintech Group, Inc.) at 25˚C for 2 h, followed by incubation with horseradish peroxidase-conjugated goat anti-rabbit
IgG (cat. no. SA00001-2; 1:2,000; Proteintech Group, Inc.) at 25°C for 1 h. An enhanced chemiluminescence kit (Thermo Fisher Scientific, Inc.) was used to visualize the membranes. ImageJ v1.48 (National Institutes of Health) was used for densitometry. β-actin, β-tubulin and GAPDH were used as internal reference proteins.

**Co-IP.** MDA-MB-468 cells were seeded in 6-well plates (2×10⁶ cells/well) overnight at 37°C and then transfected with 1.6 µg pCDNA3.1 vector, pCDNA3.1-HA-OTUD7B, pCDNA3.1-Flag-FOXM1 or His-Ub per well with Lipofectamine 2000 following the manufacturer's instructions for 24 h at 37°C. Subsequently, cells were harvested and lysed in immunoprecipitation buffer (150 mmol/l NaCl; 50 mmol/l Tris-HCl, Ph 7.4; 40 mmol/l β-glycerophosphate; 1 mmol/l Na₄OV₃; 10 mmol/l NaF; 2 mmol/l EDTA) supplemented with 1 mmol/l PMSF and protease inhibitor on ice for 30 min. The cells in immunoprecipitation buffer were centrifuged at 4°C at 14,000 x g for 15 min, and the supernatant was immediately transferred to a new centrifuge tube to obtain the cell lysate. Protein A/G agarose beads (50 µl; Beyotime Institute of Biotechnology) were incubated with anti-HA (cat. no. 66006-2-1g; 1 µg) or anti-Flag (cat. no. 66008-4-1g; 1 µg) (both from Proteintech Group, Inc.) at 4°C overnight, followed by incubation with 500 µl cell lysates at 4°C for 6 h. The mixture was centrifuged at 14,000 x g at 4°C for 15 min and the supernatant was discarded. The agarose bead-antigen-antibody complex was then cleaned three times with immunoprecipitation buffer. After the supernatant was discarded, the precipitation was added to 60 µl 2X loading buffer, heated at 100°C for 5 min, and then subjected to western blotting.

**Detection of the effect of OTUD7B on FOXM1 degradation.** MDA-MB-468 cells were seeded in 6-well plates (2×10⁶ cells/well) overnight at 37°C and then transfected with 1.6 µg pCDNA3.1 vector, pCDNA3.1-HA-OTUD7B or pCDNA3.1-Flag-FOXM1 with Lipofectamine 2000 for 24 h at 37°C, according to the manufacturer's instructions. Subsequently, the cells were cultured in medium containing 50 µg/ml cyclohexane (MilliporeSigma) for 0, 2, 4, 8 and 12 h at 37°C. Subsequently, the cells were collected for western blotting. Subsequently, ImageJ v1.48 was used to calculate FOXM1 protein levels at different time points.

**Statistical analysis.** The experiments were independently repeated three times. Data are presented as the mean ± standard deviation. Unpaired Student's t-test was used for comparisons between unpaired groups, paired Student's t-test was used for comparisons between paired groups, and one-way ANOVA followed by Tukey's post-hoc test was used for comparisons among multiple groups. Prior to statistical analysis, the normality of data was verified by the Shapiro-Wilk test. P<0.05 was considered to indicate a statistically significant difference. All statistical analyses were performed using GraphPad Prism v5.0 (Dotmatics).
Results

**OTUD7B is upregulated in breast cancer tissues and cells.** To clarify the role of OTUD7B in breast carcinogenesis, first the expression levels of OTUD7B in breast cancer tissues and cells were examined. Western blotting showed that the expression levels of OTUD7B were higher in breast cancer tissues than those in adjacent normal tissues (Fig. 1A). Next, the mRNA and protein expression levels of OTUD7B were analyzed in several breast cancer cell lines. OTUD7B mRNA and protein expression levels were increased in breast cancer cell lines compared with those in MCF-10A cells (Fig. 1B and C). Among the breast cancer cell lines, MDA-MB-468, MDA-MB-453 and MCF-7 cells showed relatively higher OTUD7B expression levels; therefore, these three cell lines were selected for further analysis.

**OTUD7B knockdown reduces the stemness and proliferation of MDA-MB-468 cells.** A previous study has shown that OTUD7B maintains the stemness of neural progenitor cells by removing polyubiquitin conjugates from SOX2 protein, increasing its stability (20). Thus, whether OTUD7B affects the stemness of MDA-MB-468 cells was investigated. Western blotting showed that shOTUD7B was successfully transfected into MDA-MB-468 cells and reduced the protein expression levels of OTUD7B (Fig. 2A). The protein expression levels of SOX2 and Nanog, two stemness-associated proteins, were markedly suppressed by OTUD7B knockdow

![Image of Western blotting results for OTUD7B, SOX2, and Nanog in MDA-MB-468 cells, showing knockdown effects.](image)
OTUD7B affects breast cancer stemness by regulating FOXM1

Furthermore, the mean fluorescence intensity of CD44 and EpCAM were also significantly decreased in MDA-MB-468 cells with stable knockdown of OTUD7B (Fig. 2B and C). Sphere formation assays showed that the tumorsphere diameters of MDA-MB-468 cells were significantly reduced by OTUD7B knockdown (Fig. 2D). The impact of OTUD7B on cell proliferation was investigated and it was found that OTUD7B knockdown inhibited the proliferation and colony formation abilities of MDA-MB-468 cells (Fig. 2E and F). These data indicated that OTUD7B knockdown may suppress the stemness and proliferation of MDA-MB-468 cells.

**OTUD7B interacts with and stabilizes FOXM1 via deubiquitination.** FOXM1 is a transcriptional activator that is involved in the stemness of several types of human cancer cells (21), and its degradation rates are accelerated in OTUD7b-depleted cells (22). Whether FOXM1 is involved in the effects of OTUD7B on the stemness of breast cancer cells was explored in the present study, as was whether OTUD7B interacts with FOXM1 in breast cancer cells. HA-OTUD7B and Flag-FOXM1 plasmids were co-transfected into breast cancer cells and the interaction between OTUD7B and FOXM1 was detected by co-IP experiments. FLAG-tagged FOXM1 was detected in the HA-OTUD7B immunoprecipitates and vice versa (Fig. 3A).

OTUD7B is a deubiquitinase; therefore, whether OTUD7B affects FOXM1 ubiquitination and stability was explored. As shown in Fig. 3B, in the immunoprecipitate pulled down by the Flag antibody, the His-Ub level in the group with overexpression of HA-OTUD7B was markedly lower than that in the group without overexpression of HA-OTUD7B, indicating that OTUD7B reduced the ubiquitination level of Flag-FOXM1.

Protein biosynthesis was pharmacologically inhibited using cycloheximide in MDA-MB-468 cells and FOXM1 protein levels over 12 h with or without OTUD7B overexpression

![Figure 3](image-url)

**Figure 3.** OTUD7B interacts with and stabilizes FOXM1. (A) Interaction between OTUD7B and FOXM1 analyzed using co-IP. (B) Effect of OTUD7B on FOXM1 ubiquitination analyzed using co-IP. (C) Effect of OTUD7B on FOXM1 degradation detected by western blotting. ***P<0.0001. OTUD7B, OTU domain-containing 7B; CHX, cycloheximide; FOXM1, forkhead box protein M1; HA, hemagglutinin; co-IP, co-immunoprecipitation; His-Ub, His-ubiquitin; TCL, total cell lysate.
were evaluated. It was found that Flag-FOXM1 protein levels consistently decreased from 0 to 12 h after cycloheximide treatment and this decrease was significantly alleviated by OTUD7B overexpression in MDA-MB-468 cells (Fig. 3C). These findings suggested that OTUD7B stabilizes FOXM1 protein by reducing its polyubiquitination.

**FOXM1 overexpression rescues the OTUD7B knockdown-induced inhibition of stemness and proliferation in breast cancer cells.** To examine the role of FOXM1 in the OTUD7B-mediated effects on the proliferation and stemness of breast cancer cells, the effects of FOXM1 overexpression on the expression of stemness markers were analyzed. It was revealed that the protein expression levels of SOX2, Nanog, CD44, CD24, and EpCAM were increased by FOXM1 overexpression in OTUD7B-knockdown breast cancer cells (Figs. 4 and 5). The reduced tumorsphere diameter of OTUD7B-knockdown breast cancer cells was also reversed by FOXM1 overexpression (Fig. 6).

To determine the role of FOXM1 in OTUD7B knockdown-mediated cell proliferation inhibition, FOXM1 was overexpressed in OTUD7B-knockdown breast cancer cells and the proliferation of the cells was analyzed by CCK8 assay. As shown in Fig. 7A-C, OTUD7B knockdown inhibited the...

Figure 4. Overexpression of FOXM1 increases the protein expression levels of SOX2 and Nanog in OTUD7B-knockdown breast cancer cells. (A) Western blotting of OTUD7B, FOXM1, SOX2, and Nanog proteins in MDA-MB-468, MDA-MB-453, and MCF7 cells. Statistical analysis of relative protein expression levels of OTUD7B, FOXM1, SOX2, and Nanog in (B) MDA-MB-468, (C) MDA-MB-453, and (D) MCF7 cells. *P<0.05, **P<0.001, and ***P<0.0001. OTUD7B, OTU domain-containing 7B; FOXM1, forkhead box protein M1; NC, negative control; sh, short hairpin; ns, not significant.
proliferation of MDA-MB-468, MDA-MB-453 and MCF-7 cells, whereas the overexpression of FOXM1 blocked the inhibitory effect induced by OTUD7B knockdown. Similar results were observed in colony formation assays, which showed that overexpression of FOXM1 alleviated the inhibitory effect of OTUD7B silencing on the colony formation ability of MDA-MB-468, MDA-MB-453 and MCF7 cells (Fig. 7D).

Discussion

In the present study, it was revealed that OTUD7B interacts with and deubiquitinates FOXM1 in breast cancer cells, leading to the stabilization of FOXM1. Furthermore, knocking down OTUD7B inhibited the proliferation and stemness of breast cancer cells by enhancing FOXM1 degradation.

OTUD7B was first identified as a negative regulator of NF-κB that inhibits the polyubiquitination of receptor-interacting protein 1 signal adapter proteins (23-25). OTUD7B dysregulation has been found to participate in the development of numerous types of cancer, but studies on the role of OTUD7B in oncogenesis have yielded conflicting results. In breast, pancreatic and endometrial cancer, OTUD7B may play an oncogenic role by facilitating the proliferation, progression and metastasis of tumor cells (9,13,26). However, in hepatocellular carcinoma (HCC), OTUD7B may function as a tumor suppressor, which is evidenced by the fact that OTUD7B knockdown accelerates the migration and invasion of HCC cells in vitro and promotes metastasis in vivo. Furthermore, reduced OTUD7B expression is associated with poor prognosis in patients with HCC (10,12). The opposite results have been shown in lung cancer. Zhang et al (11) reported that OTUD7B expression was negatively correlated with tumor size, lymph node metastasis and tumor-node-metastasis stage in patients with non-small cell lung cancer. However, patients with high OTUD7B expression had favorable prognoses. Additionally,
Lin et al (27) showed that OTUD7B augmented EGF-induced Akt signal transduction, promoting lung cancer cell proliferation and migration. These results indicated that the functions of OTUD7B in cancer may be cancer dependent.

In ERα-positive breast cancer, OTUD7B directly interacts with and stabilizes ERα and knocking down OTUD7B inhibits cell proliferation by promoting ERα degradation (15). In ERα-negative breast cancer, silencing OTUD7B inhibits cell migration and invasion in vitro and impairs lung metastasis of cancer cells in vivo by increasing lysine-specific demethylase 1 (LSD1)-Ub and targeting LSD1 for p62-mediated proteolysis (16). In the present study, it was demonstrated that knocking down OTUD7B inhibited the proliferation and stemness of breast cancer cells by enhancing FOXM1 degradation, which indicates that OTUD7B may function as an oncoprotein in breast cancer.

Cancer stem cells are characterized by increased self-renewal and differentiation capacity, leading to metastasis, drug resistance and cancer relapse, which ultimately result in treatment failure (28,29). As a proliferation-specific transcription factor, FOXM1 has been implicated in the initiation, progression, metastasis and drug resistance of breast cancer (22). In agreement with the findings of the present study, several recent studies have also reported that FOXM1 facilitates the stemness of both ER-positive and ER-negative breast cancer cells (30-32).

Due to the important roles played by deubiquitinases in each stage of breast cancer progression, much effort has been put into developing inhibitors for breast cancer treatment, which has led to promising results. A number of small molecule inhibitors of different deubiquitinases including ZRANB1, CSN5, UCHL3, USP14, USP1 and USP2 have been developed, and are beginning to be used to interfere with proliferation, apoptosis, metastasis and DNA repair, and to enhance sensitivity to immunotherapy and chemotherapy in breast cancer (33-38). In the present study, the oncogenic role played by OTUD7B in breast cancer was demonstrated and the results suggested that OTUD7B is a possible target for drug design in this malignancy. MDA-MB-468 and MDA-MB-453 cells are triple-negative breast cancer cells (39,40) and MCF-7 cells are double-positive breast cancer cells (41). The results of the present study showed that OTUD7B and FOXM1 may

![Figure 6. Overexpression of FOXM1 alleviates the reduced tumorsphere diameter of OTUD7B-knockdown breast cancer cells. (A) Tumorsphere diameter analysis of MDA-MB-468, MDA-MB-453 and MCF7 cells. (B) Statistical analysis of tumorsphere diameter of MDA-MB-468, MDA-MB-453 and MCF7 cells. *P<0.05 and **P<0.01. OTUD7B, OTU domain-containing 7B; FOXM1, forkhead box protein M1; NC, negative control; sh, short hairpin.](image)
regulate the proliferation and stemness of these three cell lines and suggested that drugs targeting OTUD7B or FOXM1 may have the potential to treat triple-negative breast cancer and double-positive breast cancer.

Acknowledgements
Not applicable.

Funding
This research was funded by the Natural Science Foundation of Fujian (grant nos. 2020J011265 and 2021J011385), the Sanming Science and Technology Project (grant no. 2019-S-1) and the Fujian Provincial Health Technology Project (grant no. 2021CXA046).

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions
DZ and WC were involved in conceptualization of the present study. XF performed the statistical analysis. HW and SH
performed the experiments. JX obtained the informed consent from the patients and collected the cancer tissues. DZ wrote the original draft. HW, SH, XF, WC and JX revised the manuscript. DZ supervised the study and was involved in project administration. HW and DZ acquired the funding. HW, SH and DZ confirm the authenticity of all the raw data. All authors read and approved the final manuscript and have agreed to be accountable for all aspects of the work.

Ethics approval and consent to participate
The study procedures were approved by The Ethics Committees of Sanming First Hospital Affiliated to Fujian Medical University [Sanming, China, approval no. 2020(12)] and written informed consent was obtained from all of the patients.

Patient consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

References


