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ORIGINAL PAPER

SNX14 inhibits autophagy via the *PI3K/AKT/mTOR* signaling cascade in breast cancer cells

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Abstract

Background: Sorting nexin 14 (SNX14) is a member of the sorting junction protein family. Its specific roles in cancer development remain unclear. Therefore, in this study, we aimed to determine the effects and underlying mechanisms of *SNX14* on autophagy of breast cancer cells to aid in the therapeutic treatment of breast cancer. Methods: In this study, we performed in vitro experiments to determine the effect of SNX14 on breast cancer cell growth. Moreover, we used an MCF7 breast cancer tumor-bearing mouse model to confirm the effect of SNX14 on tumor cell growth in vivo. We also performed western blotting and quantitative polymerase chain reaction to identify the mechanism by which *SNX14* affects breast cancer MCF7 cells. Results: We found that *SNX14* regulated the onset and progression of breast cancer by promoting the proliferation and inhibiting the autophagy of MCF7 breast cancer cells. In vivo experiments further confirmed that *SNX14* knockdown inhibited the tumorigenicity and inhibited the growth of tumor cells in tumor tissues of nude mice. In addition, western blotting analysis revealed that SNX14 modulate the autophagy of MCF7 breast cancer cells via the phosphoinositide 3-kinase/protein kinase B/mechanistic target of rapamycin kinase signaling pathway. Conclusion: Our findings indicate that *SNX14* is an essential tumor-promoting factor in the development of breast cancer.

Keywords Breast cancer · *SNX14* · Apoptosis · Autophagy · *PI3K/AKT/mTOR*

Introduction

Breast cancer is the most common malignancy following ovarian and cervical cancers in women worldwide(Zou 2004). These years breast cancer is the second leading cause of cancer-related fatalities in women in the United States(Rezaeeyazdi et al. 2022). Early breast cancer detection and therapy have benefited greatly from rapid progress in molecular biology. However, treatment of patients with advanced stage of the disease, especially breast cancer, or extensive invasion and metastasis is often ineffective(Neugut et al. 2014). Mechanisms targeting breast cancer cell metastasis have not yet been fully elucidated. Therefore, it is necessary to identify new molecular biological targets for breast cancer.

Sorting junction proteins (SNXs) are a class of PX domain-containing proteins that play key roles in intracellular protein sorting and protein transport(Teasdale and Collins 2011; Thomas et al. 2014). PX specifically binds to phosphatidyl alcohol groups and affects the activity of related organelles. Based on their structural domain composition, 33 SNXs, including SNX3, SNX4, SNX10, SNX12, SNX22, and SNX-PX-BAR, have been identified in mammals(Gallon and Cullen 2015; Teasdale and Collins 2011; Verges 2007). SNX-PX-BAR has been reported to regulate sorting and transport processes in the body. During evolution, the biochemical role of SNX14 seems to have been largely conserved, and the consequences of its loss-of-function vary from species to species(Verges 2007). SNX14 belongs to the G protein signaling pathway, which is involved in intracellular signal transduction. The onset and progression of cancer are closely correlated with abnormal

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G protein signaling(Ha et al. 2015). Phosphatidylinositol phosphate-related SNX14 localizes to lysosomes and is a key component of lysosomes. SNX14 is associated with autophagosome accumulation and activation of apoptosis in zebrafish. Mutations in *Snx14* cause intracellular lysosome–autophagosome dysfunction syndrome(Akizu et al. 2015). Moreover, overexpression of *SNX14* promotes the biosynthesis of lipid droplets, which are nutrient stores used by cells to maintain a stable internal environment(Datta et al. 2019). *SNX14* mutations or deletions in human fibroblasts disrupt autophagy; however, its exact function remains unclear(Bryant et al. 2018). Loss of *SNX14* can also lead to autophagy(Sait et al. 2022). Therefore, *SNX14* may be closely associated with autophagy. Autophagy is crucial for the development, metastasis, and invasion of breast cancer cells(Akkoc et al. 2023).

In this study, we determined the roles and underlying mechanism of *SNX14* as well as its effects on autophagy in breast cancer cells. Our findings may aid in the development of effective breast cancer therapies.

Materials and methods

Expression analysis of SNX14 in breast Cancer

Analysis of SNX14 expression in breast cancer tissues ($n=1104$) and normal tissues ($n=113$) was conducted using the starBase database (https://rnasysu.com/encori/index.php). Immunohistochemical detection results of SNX14 protein in breast tissues ($n=3$) and breast cancer tissues ($n=12$) were obtained from The Human Protein Atlas database (https://www.proteinatlas.org/), followed by analysis of SNX14 expression in breast cancer tissues. Utilizing a best expression cutoff value of 10.91 from The Human Protein Atlas database, breast cancer patients were stratified into SNX14 low-expression group ($n=702$) and SNX14 high-expression group ($n=373$), and Kaplan-Meier survival curves were generated.

Cell culture

HEK293T and human breast cancer (MCF7) cells were preserved in our laboratory. HEK293T and MCF7 human breast cancer cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and 1% penicillin–streptomycin at 37 °C in a 5% humidified CO_2 incubator.

Plasmid construction

Full-length human *SNX14* cDNA (NM_001350550.2) was inserted into the pLV-CMV-MCS-PGK puromycin vector to construct a plasmid encoding *SNX14* (*SNX14*-OE). The pLV-CMV-MCS-PGK puro vector was used as a negative control for *SNX14*-OE. Short hairpin RNA (shRNA) for SNX14 (shSNX14) or a scrambled sequence (as a negative control, shNC) was cloned into PLKO.1-TRC-puro to create a plasmid encoding *shSNX14*. All primer sequences used in this study are listed Table 1.

Construction of stable *SNX14*-knocked down and -overexpressing cell lines

HEK293T cells were co-transfected with 9 μg of plasmids encoding *shSNX14* or *SNX14*-OE in 10-cm plates using Lipofectamine 2000 (Invitrogen) along with the appropriate packaging plasmids (3 μg of pMD2G and 6 μg of pspax2). The lentivirus-containing supernatant was collected after 48 h. After enrichment, the titer was calculated as previously described(Li et al. 2015a). MCF7 cells were transduced with 10^8 $TCID_{50}$/mL lentivirus in the presence of 8 μg/mL polypropylene. Puromycin was added at a final concentration of 2.0 μg/mL after replacing the medium with a fresh medium after 48 h. Cells were collected for the determination of *SNX14* expression after two weeks.

Table 1 Sequences of primers used in the paper

Primer name	Sequences (5'-3')
SNX14 OE-F	GAAATGTACAAGGAATTCATGGTGCCCTGGGTGCGGAC
SNX14 OE-R1	ACTTATCGTCGTCATCCTTGTAATCCATCCAAGATGTCACAGAGG
SNX14 OE-R2	GAATTATCTAGGGATCCTTACTTATCGTCGTCATCCTTG
shSNX14 -F	CCGGAGGGATGTGACAGATGATGAACTCGAGTTCATCATCTGTCACATCCCTTTTTT
shSNX14 -R	AATTAAAAAAGGGATGTGACAGATGATGAACTCGAGTTCATCATCTGTCACATCCCT
18 S rRNA-QF	CGACGACCCATTCGAACGTCT
18 S rRNA-QR	GCCTCACTAAACCATCCAATCGG
SNX14-QF	TATGATTACCTGATGTATG
SNX14-QR	GAATATAGCATCTCTGAG

F: forward primer; R: reverse primer; QF: forward primer of quantitative PCR; QR: reverse primer of quantitative PCR

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from the cells using TRIzol Reagent (Thermo Fisher Scientific, Inc.) and a Reverse Transcription kit (Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions. RNA was reverse-transcribed into cDNA. RT-qPCR was performed using the SYBR Green PCR kit (TaKaRa, China) on a CFX Connect 96 device (Bio-Rad Laboratories, Inc.). The $2^{-\Delta\Delta Cq}$ approach was used to determine the relative expression(Livak and Schmittgen 2001). All primer sequences are listed in Table 1.

Western blotting

Total cellular protein was extracted using a protein extraction kit (Thermo Fisher Scientific). Total protein concentration was determined using the Bradford assay (Bio-Rad Laboratories, Inc.), according to the manufacturer's instructions. Subsequently, sodium dodecyl sulfate-polyacrylamide gel electrophoresis was used for the separation of proteins or cell lysates, and polyvinylidene difluoride membranes were used to transmit the gel, which were blocked with 5% skim milk in TBST (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, and 0.1% Tween-20) for 1 h and incubated with primary antibodies overnight at 4 °C, followed by incubation with secondary antibodies and detection using an ECL system (Thermo). All antibodies used here are listed in Table 2.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

MCF7 cells stably overexpressing *SNX14* or *shSNX14* were seeded at a density of 10,000 cells/well in a 96-well plate. Briefly, 20 µL of MTT (5 mg/mL) was added to each well after 0, 24, 48, or 72 h, and the cells were incubated at 37 °C for 4 h. After carefully aspirating the supernatant from each well, 150 µL of dimethyl sulfoxide was added to each well, and the cell culture plate was agitated for 10 min to break up the crystals. Data were then recorded after measuring the absorption at 490 nm using a microplate reader (Thermo Fisher Scientific, Inc.). Time was plotted on the x-axis and absorbance was plotted on the y-axis to obtain the cell growth curve.

Electron microscopy analysis

MCF7 cells were collected, centrifuged at 200 × g, and fixed in 0.2 M sodium cacodylate buffer (pH 7.4) containing 2% glutaraldehyde overnight at 4 °C. The samples were subsequently dehydrated and embedded in Epon 812 for ultrathin sectioning post-fixation with cacodylate-buffered 1% osmium tetroxide. Ultrathin sections were stained with uranyl acetate and lead citrate and observed under a transmission electron microscope (H-7500; Hitachi, Tokyo, Japan).

Flow cytometry

MCF7 cells (1×10^6) stably overexpressing *SNX14* or *shSNX14* were placed in a 6-well plate. Following a 24-h culture at 37 °C, adherent and floating cells were separated via centrifugation at 200 × *g* for 5 min at room temperature, washed with phosphate-buffered saline, and processed to detect apoptosis using an Annexin V-FITC/PI Apoptosis Detection Kit (catalog number: A211-02; Vazyme Biotech Co., Ltd.), according to the manufacturer's instructions. NovoCyte setup (ACEA Bioscience Inc.) and NovoExpress software 1.4.1 (ACEA Bioscience Inc.) were used to examine the data. The proportion of apoptotic cells (including early and late apoptotic cells) relative to the total number of cells was calculated.

Table 2 Information about antibodies

Antibody name	Manufacturers	Catalog number	Dilution rate
TFAR19 antibody	Proteintech	12456-1-AP	1:1000
Beclin1 antibody	Proteintech	11306-1-AP	1:3000
P62 antibody	Proteintech	18420-1-AP	1:5000
LC3 antibody	Proteintech	14600-1-AP	1:3000
Beta actin antibody	Proteintech	20536-1-AP	1:5000
Phospho-AKT antibody	Proteintech	80455-1-RR	1:5000
AKT antibody	Proteintech	10176-2-AP	1:5000
SNX14 antibody	Invitrogen	PA5-53615	1:100
Pro-caspase 3 antibody	Proteintech	19677-1-AP	1:1000
cleaved-caspase-3 antibody	Cell signaling technology	9661	1:1000
GAPDH antibody	Proteintech	10494-1-AP	1:5000
BAX antibody	Proteintech	50599-2-Ig	1:5000
Bcl-2 antibody	Proteintech	12789-1-AP	1:5000
horse radish peroxidase-conjugated Goat Anti-Rabbit IgG	Proteintech	SA00001-2	1:10,000

Treatment with inhibitors

MCF7 cells (1×10^6) stably overexpressing *SNX14* or *shSNX14* were seeded in a 6-well plate overnight. MCF7 cells stably overexpressing *SNX14* were treated with 0.5 ng/mL rapamycin, an inhibitor of mechanistic target of rapamycin kinase (mTOR), and those stably overexpressing *shSNX14* were treated with 5 mM 3-methyladenine, an inhibitor of phosphoinositide 3-kinase (PI3K). After 24 h, cells were collected for western blotting analysis.

Animal experiments

Briefly, 1×10^6 MCF7 cells stably overexpressing *shSNX14* were subcutaneously injected into six-week female BALB/c nude mice procured from the Shanghai Laboratory Animal Research Center. Tumor volumes were measured on days 0, 22, 26, 30, 34, 38, and 42 post-injection. On day 42 post-injection, all mice were euthanized with carbon dioxide at a flow rate of 30–70% of the euthanasia chamber volume. Immunohistochemistry (IHC), hematoxylin and eosin (HE) staining, and western blotting were performed on the obtained tumors. Animal Care and Use Committee of Zhejiang university school of medicine endorsed this study.

HE staining

After fixing in 4% formaldehyde solution and dehydration with ethanol, tumor tissue was embedded in paraffin and cut into sections of 4 μm. After deparaffinization, the sections were stained using a hematoxylin-eosin staining kit (catalog number: G1076; Servicebio) and observed under a light microscope (Nikon).

IHC assay

Tumor sample sections were deparaffinized, rehydrated, and treated with 3% H_2O_2 before treatment with citrate buffer. The sections were treated with diluted Ki67 antibody (catalog number: 27309-1-AP; Proteintech) for 12 h at 4 °C after blocking with 5% goat serum. After incubation with horseradish peroxidase-conjugated goat anti-rabbit IgG (catalog number: SA00001-2; Proteintech), the sections were incubated with DAB for color development. Subsequently, the sections were treated with hematoxylin to stain the nuclei and dehydrated. Finally, clear glue was used to seal the sections, which were observed under an Olympus light microscope (BX51; Olympus Corporation).

Statistical analysis

Data were analyzed using SPSS 17.0, a statistical program. Means and standard deviations were used to express the statistical data. The data were compared across groups using Student's *t*-test, and the Shapiro–Wilk test was used to determine if the data were normal. One-way analysis of variance and Tukey's test were used to determine the significant differences between groups. Differences were considered statistically significant at $P < 0.05$.

Results

Expression and prognostic implications of SNX14 in breast Cancer

Analysis from the starBase database revealed a significant upregulation of SNX14 expression in breast cancer tissues compared to normal breast tissues (Fig. 1a). Additionally, immunohistochemical examination from The Human Protein Atlas database indicated that SNX14 protein was undetectable in normal breast tissues, whereas in breast cancer tissues, 1/12 of samples showed no SNX14 protein detection, 1/4 exhibited low levels, and 1/3 displayed moderate levels of SNX14 protein (Fig. 1b), indicating an elevation of SNX14 protein in breast cancer tissues relative to normal breast tissues. Kaplan-Meier survival curves from The Human Protein Atlas database further illustrated that breast cancer patients with high SNX14 expression exhibited significantly poorer survival compared to those with low SNX14 expression (Fig. 1c). These findings collectively underscore the overexpression of SNX14 in breast cancer tissues, with higher SNX14 expression correlating with lower survival rates among breast cancer patients.

Identification of stable cells overexpressing SNX14 or shSNX14

Initially, we separately cultured various breast cancer cell lines (MDA-MB-231/436/457/468 and MCF7) along with normal mammary epithelial cells (MCF10A) to observe the expression profile of SNX14. As depicted in Fig. 2a, SNX14 expression levels were notably elevated in breast cancer cells, with the most pronounced increase observed in MDA-MB-436 and MCF7 cells. For ease of experimentation, subsequent studies primarily focused on MCF7 cells. After that, we constructed stable cell lines overexpressing either *SNX14* or *shSNX14*. RT-qPCR revealed a significant increase in *SNX14* mRNA levels in cells stably overexpressing *SNX14* (Fig. 2b) and a significant decrease in *SNX14* mRNA levels in cells stably overexpressing *shSNX14*

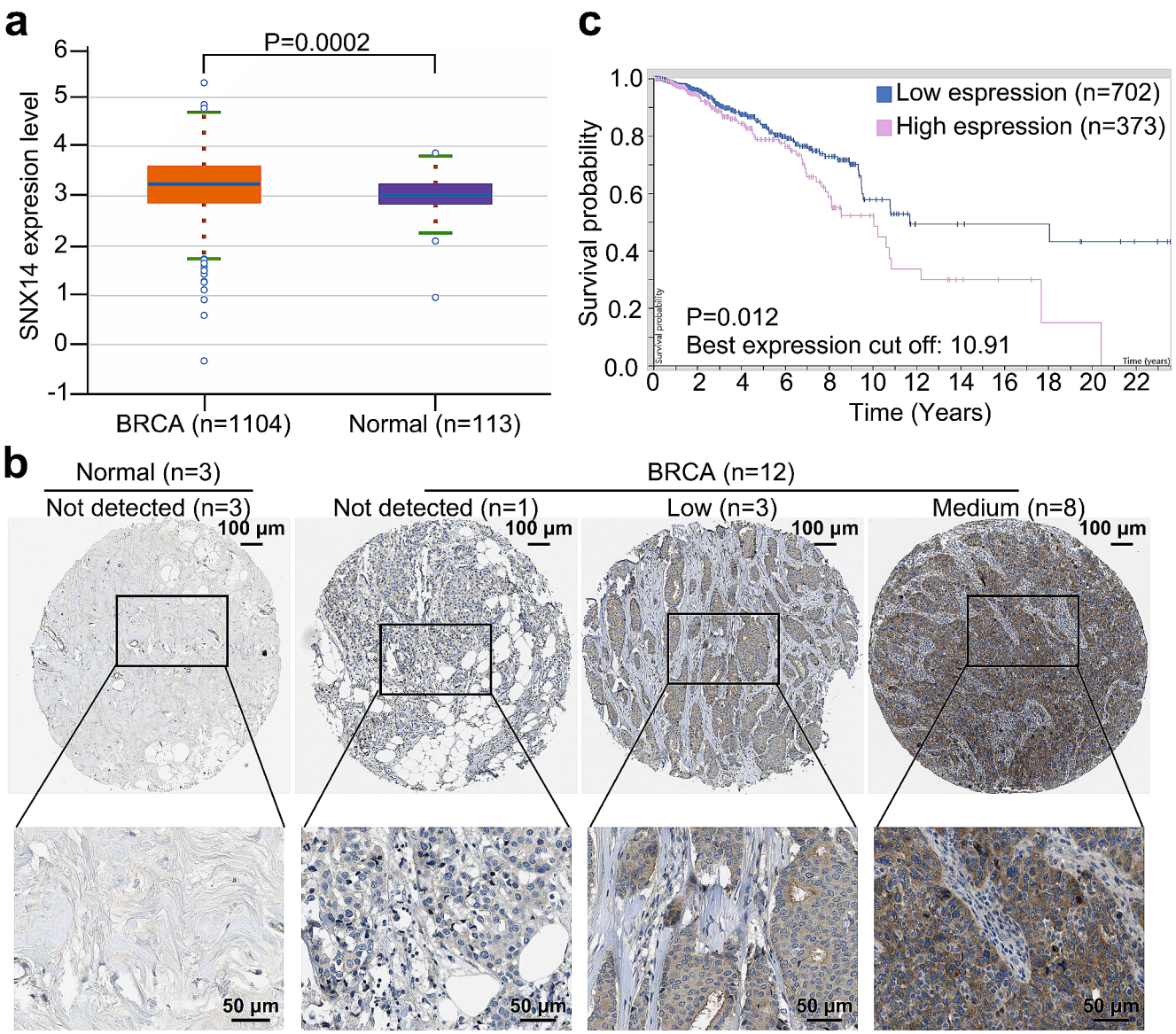

Fig. 1 Expression analysis of *SNX14* in breast cancer. (**a**) Analysis of *SNX14* expression levels in breast cancer using starBase (https://rnasysu.com/encori/index.php). (**b**) Immunohistochemical detection of *SNX14* in breast tissue and breast cancer tissue obtained from The Human Protein Atlas. (**c**) Analysis of the impact of *SNX14* expression levels on the survival of breast cancer patients using The Human Protein Atlas (https://www.proteinatlas.org/)

(Fig. 2c). In addition, SNX14 protein levels were significantly increased and decreased in cells stably overexpressing SNX14 and shSNX14, respectively (Fig. 2d and e).

SNX14 knockdown increases the autophagy and apoptosis and inhibits the survival of MCF7 cells

SNX14 knockdown significantly inhibited the growth of MCF7 cells (Fig. 3a). In contrast, overexpression of *SNX14* promoted the proliferation of MCF7 cells compared to the vector group (Fig. 3a). Western blotting revealed that, compared to the control group, the LC3-II/LC3-I ratio and pro-autophagy protein Beclin 1 levels were decreased and p62 protein levels were increased in the *SNX14*-OE group (Fig. 3b and c). In contrast, the LC3-II/LC3-I ratio and Beclin 1 expression levels were increased and p62 levels were decreased after *SNX14* knockdown (Fig. 3b and c). These results suggest that *SNX14* has the potential to regulate autophagy in MCF7 cells. Electron microscopy revealed that the *SNX14* knockdown group had a much larger number of autophagic lysosomes than the shNC group (Fig. 3d). In summary, *SNX14* inhibited autophagy in MCF7 cells. Furthermore, *SNX14* knockdown significantly promoted the apoptosis of breast cancer cell MCF7 ($P < 0.0001$), but its overexpression had no significant effect on the apoptosis of MCF7 cells (Fig. 3e and f).

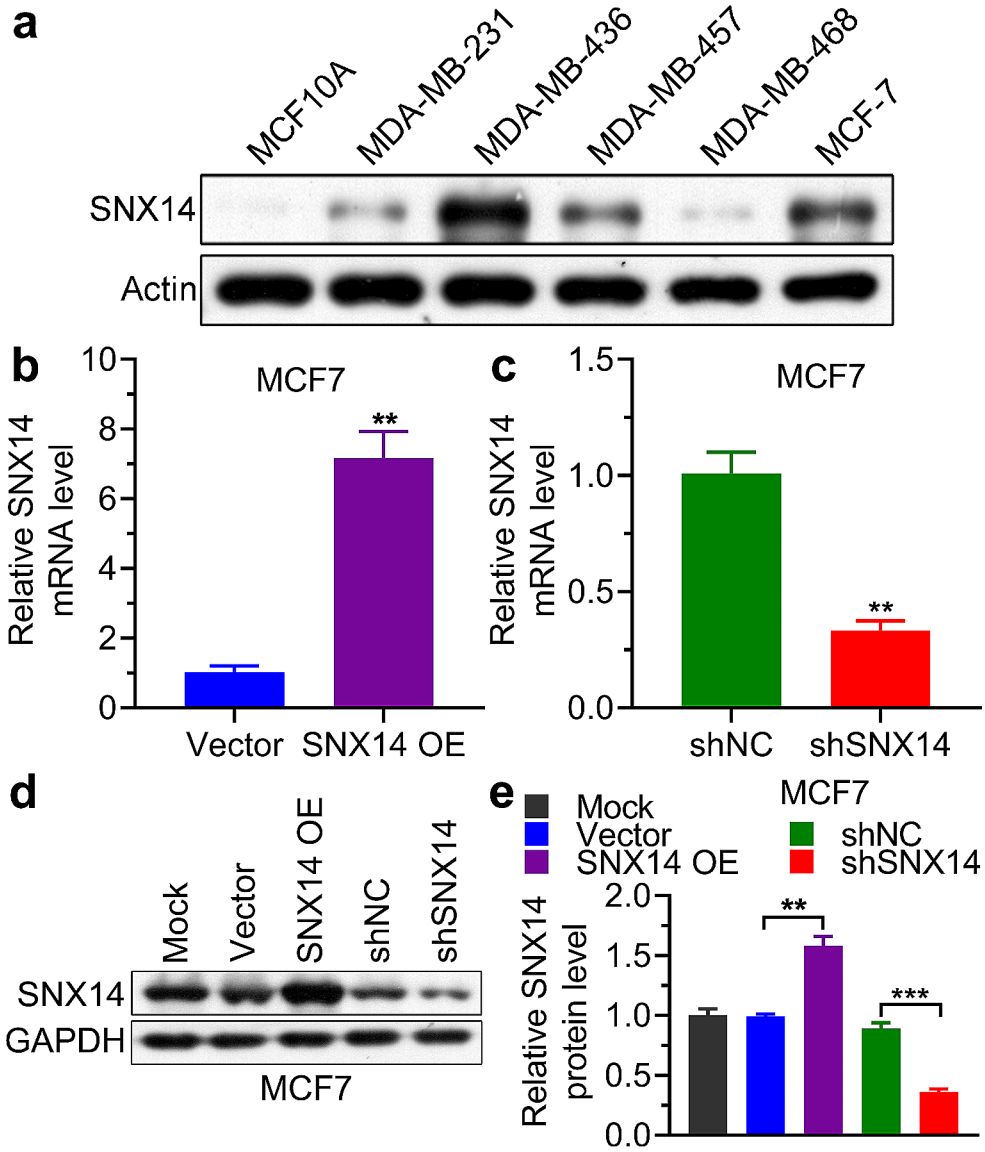

Fig. 2 Identification of stable cells overexpressing sorting nexin 14 (*SNX14*) or short hairpin RNA (shRNA) for *SNX14* (*shSNX14*). (**a**) Expression of *SNX14* in different cell lines. Among them, MCF10A is human normal breast epithelial cells. (**b**) Reverse transcription quantitative polymerase chain reaction (RT-qPCR) analysis of *SNX14* mRNA levels in MCF7 cells overexpressing *SNX14*. (**c**) RT-qPCR analysis of *SNX14* mRNA levels in MCF7 cells overexpressing *shSNX14*. (**d**) SNX14 protein expression levels in MCF7 cells overexpressing *SNX14* or *shSNX14* determined via western blotting. Housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), served as the baseline for all samples. (**e**) Relative quantification of SNX14 protein levels by calculating the gray scale of western blotting bands

SNX14 may regulate the expression levels of apoptosis-related molecules by activating the *mTOR* signaling pathway in MCF7 cells

To explore the potential molecular mechanisms by which *SNX14* regulates apoptosis in MCF7 cells, we treated *SNX14*-OE or knockdown cells with rapamycin and 3-methyladenine, respectively. We found that overexpression of *SNX14* decreased the protein levels of cleaved caspase 3 and Bcl-2 and decreased the protein levels of BAX (Fig. 4a and b). Rapamycin treatment alleviated the effects of *SNX14* overexpression (Fig. 4a and b). Cleaved caspase 3 and Bcl-2 protein levels were decreased and BAX protein levels were increased after *SNX14* knockdown in cells (Fig. 4a and b). However, 3-methyladenine treatment exacerbated the effects of *SNX14* knockdown (Fig. 4a and b). These data suggest that *SNX14* knockdown promotes apoptosis by inhibiting the *PI3K* signaling pathway.

SNX14 promotes tumor growth in mice

Subcutaneous tumors formed from MCF7 cells overexpressing *shSNX14* in mice were smaller (Fig. 5a), slower in growth (Fig. 5b), and lighter in weight (Fig. 5c) than those formed from MCF7 cells overexpressing shNCs. The ratio of p-AKT/AKT was low and beclin 1 and TFAR19 protein expression levels were increased in the shSNX14 group than in the shNC group (Fig. 5d and e). HE staining revealed increased eosinophilic activity in subcutaneous tumors formed by MCF7 cells with *SNX14* knockdown (Fig. 5f). IHC revealed that the shSNX14 group had lower levels of Ki67 than the shNC group (Fig. 5g and h). These findings indicate that *SNX14* promotes the growth and proliferation

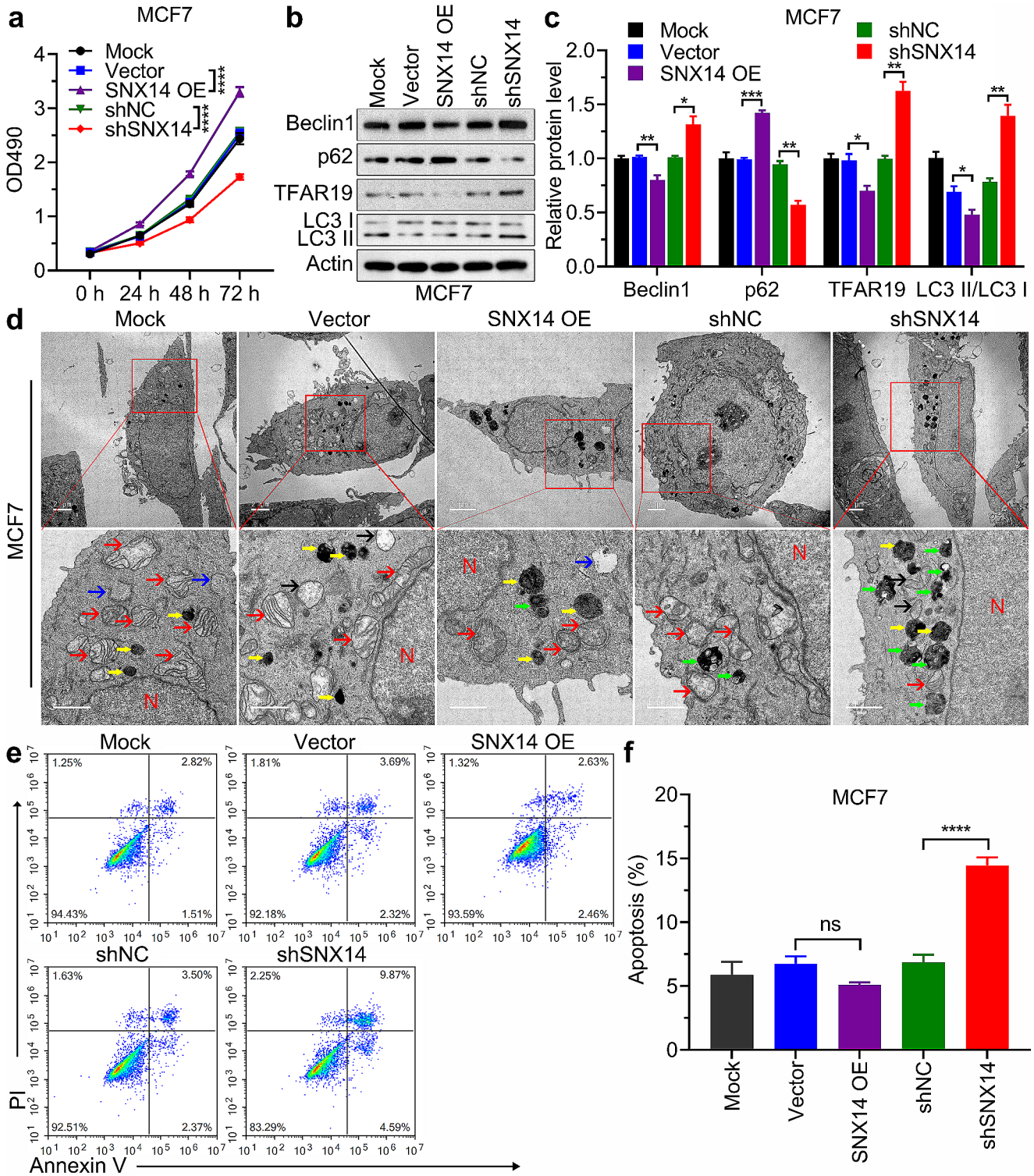

Fig. 3 *SNX14* knockdown increases the autophagy and apoptosis and inhibits the survival of MCF7 cells. (**a**) 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay of *SNX14*-overexpressing and knocked-down cells. (**b**) Beclin 1, p62, TFAR19, and LC3A/B protein levels determined using western blotting in MCF7 cells overexpressing *shSNX14* or *SNX14*. (**c**) Relative quantification of Beclin 1, p62, TFAR19, and LC3A/B protein levels. (**d**) Representative image of the electron microscopic analysis of *SNX14*-overexpressing or knocked-down cells. Magnified areas indicate the autophagosomes. N: cell nucleus; red arrow: mitochondrion; yellow arrow: lysosome; blue arrow: phagocytic vacuole; black arrow: autophagic vacuole; green arrow: autophagic lysosome. (**e**) Representative image of apoptosis analysis of *SNX14*-overexpressing or knocked-down cells. (**f**) Statistical histogram of the apoptosis rate of *SNX14*-overexpressing or knocked-down cells

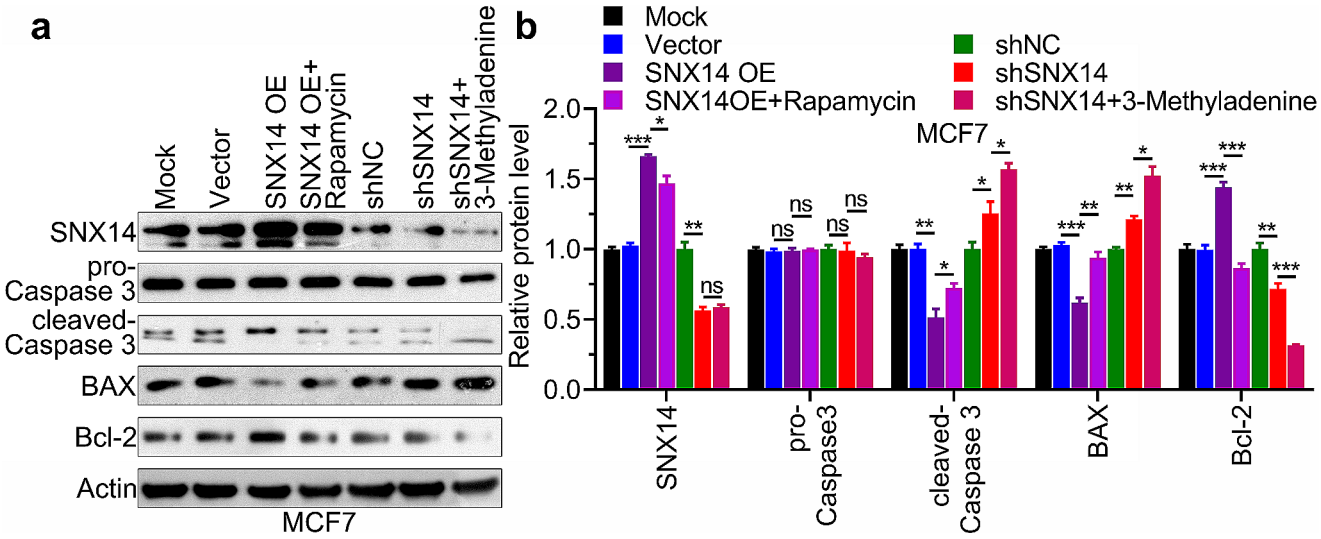

Fig. 4 *SNX14* regulates the expression levels of apoptosis-related molecules by activating the mechanistic target of rapamycin kinase (*mTOR*) signaling pathway in MCF7 cells. (**a**) Western blotting of *SNX14*-overexpressing or knocked-down MCF7 cells after rapamycin or 3-methyladenine treatment. (**b**) Relative quantification of protein by calculating the gray scale of western blotting bands

of breast cancer cells via the activation of the *AKT* signaling pathway.

Discussion

Kurten et al. discovered SNXs as a class of proteins containing phagocyte oxidase homology (phoxhomology, PX) in yeast two-hybrid experiments in 1996(Kurten et al. 1996). Currently, 33 members of SNX family have been identified in mammals. Sorting nexin 3 (*SNX3*) plays a key role in the occurrence and progression of non-small cell lung cancer(Pan et al. 2019). *SNX4*, another important member of the SNX family, is usually expressed at high levels only in invasive cell lines, indicating its role as a tumor-promoting factor(Leprince et al. 2003). *SNX16* is crucial for endocytosis, protein sorting, and cell signal transduction and is strongly associated with the development of various diseases(Wassmer et al. 2009). Zhang et al. reported that the loss of *SNX27* inhibits the proliferation of highly aggressive breast cancer MDA-MB-231 cells(Zhang et al. 2019). *SNX14* is another important member of SNXs, whose role in cancer development, especially in vivo, remains unknown. Here, we found that the overexpression of *SNX14* considerably promoted, whereas its knockdown significantly inhibited the proliferation of breast cancer MCF7 cells. Therefore, variations in the expression levels of *SNX14* may considerably and time-dependently control the proliferation of MCF7 human breast cancer cells. We further explored the tumor-promoting effect of *SNX14* in vivo by establishing an MCF7 breast cancer tumor-bearing mouse model. Our findings revealed that the suppression of *SNX14* expression may drastically slow down the growth of MCF7 breast cancer tumor-bearing mice and decrease the relative expression of Ki67 protein, indicating that the inhibition of proliferation of the tumor cells(Elkablawy et al. 2016; Li et al. 2015b; Pathmanathan and Balleine 2013; Yerushalmi et al. 2010). Ki67 exists only in the active phase of the cell cycle (G1/S/G2/M phase). Given that the expression of Ki67 is closely related to the proliferation and growth of tumor cells, Ki67 is often used as a proliferation marker in routine cancer pathology research(Li et al. 2015b; Pathmanathan and Balleine 2013). Moreover, HE staining revealed that *SNX14* knockdown significantly reduced the tumor growth in MCF7 cells.

Autophagy acts as a “double-edged sword” as it is crucial for both carcinogenesis and tumor control(Degenhardt et al. 2006; Gozuacik and Kimchi 2004; Takamura et al. 2011). Autophagy promotes cancer cell survival and metastasis recurrence in breast cancer(Abedin et al. 2007). *PI3K/AKT/mTOR* pathway is a vital signaling system for regulating autophagy, and *mTOR* is a major autophagy regulator(Morgensztern and McLeod 2005; Porta et al. 2014). *PI3K/AKT* controls the action of many inflammatory mediators and activates several intracellular signaling cascades. As a member of the family of heterodimeric lipid kinases, PI3K is activated by various upstream cell surface receptors, phosphorylated by PIP2, and further activated by AKT. AKT regulates downstream effector molecules via phosphorylation cascades and participates in cell proliferation, survival, and other cellular processes(Morgensztern and McLeod 2005). Several malignancies, including breast cancer, are induced by the *PI3K/AKT* pathway(Paplomata and O’Regan 2014). In this study, we found that, *SNX14* overexpression inhibited, whereas *SNX14* knockdown

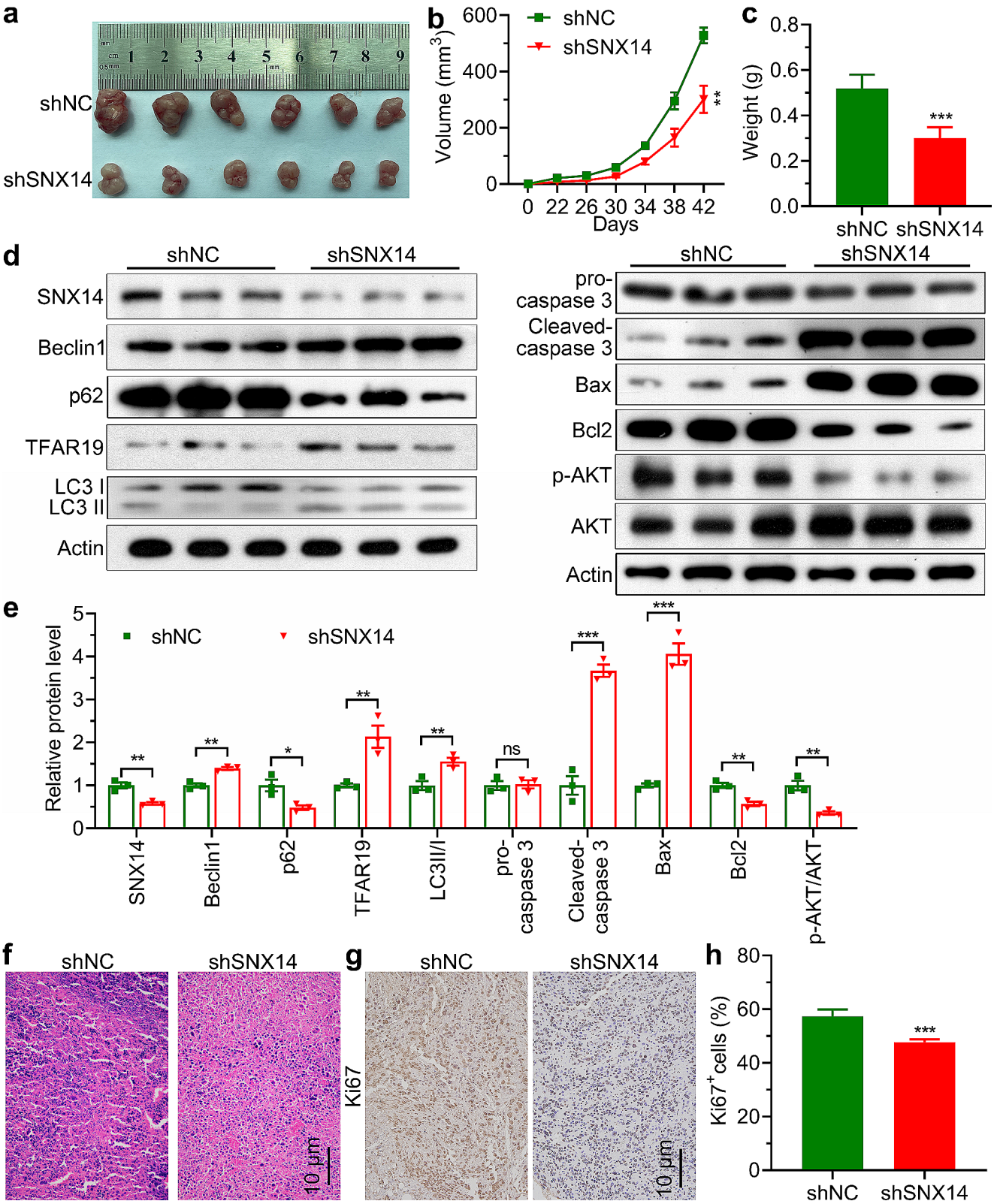

Fig. 5 Silencing of *SNX14* inhibits the growth of breast cancer tumors in mice. (**a**) Images of tumors harvested from shNC and shSNX14 groups. (**b**) Tumor volumes of shNC and shSNX14 groups. (**c**) Tumor weights of shNC and shSNX14 groups. (**d**) Western blotting analysis of the harvested tumors. (**e**) Relative quantification of protein by calculating the gray scale of western blotting bands. (**f**) Hematoxylin and eosin (HE) staining images of tumors from shNC and shSNX14 groups. (**g**) Immunohistochemistry (IHC) images of tumors from shNC and shSNX14 groups. (**h**) Percentage of Ki-67-positive cells in shNC and shSNX14 groups

promoted autophagy in MCF7 cells. *SNX14* overexpression activated the *PI3K/AKT* signaling pathway. Consequently, we hypothesized that *SNX14* overexpression prevents the autophagy of MCF7 cells by stimulating the *PI3K/AKT/mTOR* signaling pathway. To confirm this, we used the PI3K (3-methyladenine) and mTOR (rapamycin) inhibitors to inhibit the *PI3K/AKT/mTOR* signaling pathway and found that inhibition of the *PI3K/AKT* and *AKT/mTOR* signaling pathways significantly affected *SNX14*-mediated autophagy. In summary, *SNX14* prevents autophagy by activating the *PI3K/AKT/mTOR* signaling pathway in MCF7 cells.

In conclusion, we determined the role of *SNX14* and its underlying molecular mechanism in controlling the development of breast cancer at the cellular, tissue, and molecular levels in this study. In vitro studies revealed that *SNX14* controls the emergence and progression of breast cancer by promoting the proliferation and inhibiting the autophagy of MCF7 breast cancer cells. In vivo experiments in nude mice further confirmed that knocking down the expression of *SNX14* inhibited the tumorigenicity of MCF7 cells and decreased the growth of tumor cells in tumor tissues in nude mice. Moreover, we found that *SNX14* modulates the *PI3K/AKT/mTOR* signaling pathway to facilitate the autophagy of breast cancer MCF7 cells. Therefore, *SNX14* may be a useful therapeutic candidate for the management and treatment of breast cancer.

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Author contributions J and S designed the experiments; S, H and L performed the experiments; Y and Li prepared figures; J, S and Y were responsible for statistical analysis and provided helpful suggestions; S and H wrote the manuscript. All authors read and approved the final version of the article.

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Data availability All data in this study are available upon request from the corresponding author.

Declarations

Competing interests The authors declare no competing interests.

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