Original Article

USP41 promotes breast cancer via regulating *RACK1*

Meiling Huang[#], Jingjing Xiao[#], Changjiao Yan, Ting Wang, Rui Ling

Department of Thyroid, Breast, and Vascular Surgery, Xijing Hospital, The Fourth Military Medical University, Xi'an, China

Contributions: (I) Conception and design: T Wang, M Huang; (II) Administrative support: R Ling; (III) Provision of study materials or patients: M Huang, J Xiao; (IV) Collection and assembly of data: J Xiao, C Yan; (V) Data analysis and interpretation: M Huang, J Xiao; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work and should be considered as co-first authors.

Correspondence to: Rui Ling; Ting Wang. Department of Thyroid, Breast, and Vascular Surgery, Xijing Hospital, The Fourth Military Medical University, 127 Changle West Road, Xi'an 710032, China. Email: lingruiaoxue@126.com; ting_w100@126.com.

Background: Breast cancer (BC) is the most common cancer diagnosed among women and is the second leading cause of cancer death. It is of great significance to explore potential candidate targets for BC.

Methods: The expression of ubiquitin-specific protease 41 (*USP41*) and its prognosis prediction function was firstly evaluated by TCGA database analysis. Using BC cell lines and specimens from 10 patients with primary BC, the upregulation of *USP41* in BC was ensured. By *USP41* overexpression or knockdown, its function was studied by cell function assays, small interfering RNA (*siRNA*), western blot, mass spectrometry, and flow cytometry. The potential mechanism of *USP41* was explored via Co-Immunoprecipitation mass spectrometry, and western blot.

Results: TCGA database analysis revealed that in metastatic BC, *USP41* expression was upregulated and negatively correlated with BC prognosis. In BC cancer cells and cancer specimens, *USP41* was also upregulated. Overexpression of *USP41* greatly enhanced BC colony-forming ability, proliferation, and migration. In contrast, *USP41* knockdown significantly inhibited BC colony-forming ability, proliferation, and migration. Moreover, Co-Immunoprecipitation mass spectrometry results indicated that *USP41* could interact with *RACK1*. *USP41* promoted the protein expression of *RACK1*. The expression of *RACK1* in BC tissues was upregulated. Knockdown of *RACK1* inhibited cell growth and migration, and reversed the oncogenic function of *USP41* in BC cells.

Conclusions: *USP41* can be a potential therapeutic target against BC via *RACK1*.

Keywords: Ubiquitin-specific protease 41 (*USP41*); breast cancer (*BC*); receptor for activated C kinase 1 (*RACK1*)

Submitted Aug 26, 2021. Accepted for publication Oct 19, 2021.
doi: 10.21037/atm-21-4921
View this article at: https://dx.doi.org/10.21037/atm-21-4921

Introduction

Breast cancer (BC) is the most common cancer diagnosed among women and is the second leading cause of cancer death (1). In 2019, approximately 268,600 new cases of invasive BC and 48,100 cases of ductal carcinoma in situ (DCIS) were diagnosed among US women, and 41,760 women died from this disease (2). Similarly, in China, the incidence rate of BC has increased obviously both in urban and rural areas (3); however, the 5-year survival rate of BC in China is 73%, significantly lower than that in developed countries (4). With the development of precision medicine and targeted therapeutics, BC treatment has also begun to evolve toward a more individualized, precise, and targeted approach (5). As a highly heterogeneous disease, BC involves a complex etiology, including genetic alterations, reproductive factors, and environmental factor (6). Hence, it is of great significance to gain a better understanding of the mechanisms underlying BC and to develop novel and effective therapeutic strategies (7).

Ubiquitination can regulate a variety of complex cellular processes and may lead to the activation or deactivation

of tumorigenic pathways in cancer (8,9). Deubiquitinating enzymes (DUBs), reversing the ubiquitination process by removal of ubiquitin, have emerged as promising drug targets for cancer therapy. The DUBs can be divided into 6 subclasses: ubiquitin C-terminal hydrolases (UCHs), ubiquitin-specific proteases (USPs or UBPs), ovarian tumor proteases (OTUs), Machado-Joseph disease proteases (MJDs), JAB1/MPN/Mov34 metalloenzymes (JAMMs), and monocyte chemotactic protein-induced deubiquitin family (MCPIPs). Among the 6 structurally different DUB families, the largest group comprises the ubiquitin-specific proteases (USPs) that belong to the cysteine protease family (10,11). Many studies have indicated that USPs regulate tumor formation by modulating the proliferation and death of cancer cells. In 2019, Zhang *et al.* showed that *USP22* plays critical roles in the malignancy and progression of non-small cell lung cancer (12). In 2020, ubiquitin-specific protease 41 (*USP14*) was shown to promote prostate cancer progression through deubiquitinating the transcriptional factor *ATF2* (13). Lai *et al.* found that low expression of *USP4* was associated with poor survival among lung cancer patients (14). Yun *et al.* considered *USP21* an attractive therapeutic target in metastatic colorectal cancer with high Fra-1 expression (15). In 2004, *USP41* was first identified in human prostate, brain, lungs, aorta, and kidneys (16). In 2021, Ji *et al.* reported the role of USP41 in promoting lung cancer cell proliferation and migration (17). Until now, no data has been reported about the function of *USP41* in cancer development, including BC (18). Moreover, the mechanism of USP41 in cancer progression remains unclear.

In this study, we reported for the first time that *USP41* was upregulated in BC and interpreted its possible signaling for BC as oncogene. We found that *USP41* upregulation enhanced the growth, proliferation, and invasion of BC cells. The potential mechanism of *USP41* in BC was also explored, and it was shown that receptor for activated C kinase 1 (*RACK1*)-related signaling may participate in this progression. Therapy targeting *USP41* and *RACK1* may provide a novel and promising approach to BC treatment.

We present the following article in accordance with the MDAR reporting checklist (available at https://dx.doi.org/10.21037/atm-21-4921).

Methods

Clinical samples

We obtained BC specimens and paired adjacent normal breast tissue specimens from 10 patients with primary BC. All participants were female aged 45.7±12.50 and received no therapy before sample collection. The study was approved by an independent ethics committee of Fourth Military Medical University (KY20213157-1). The study was undertaken in accordance with the Good Clinical Practice guidelines and the Declaration of Helsinki (as revised in 2013). All participants were asked to provide written informed consent before enrollment. All tissues were collected immediately upon resection and transported in liquid nitrogen.

Cells, antibodies, and reagents

Human BC cell lines (MDA-MB-231, MCF-7) were obtained from American Type Culture Collection (ATCC; Rockville, MD, USA). The MDA-MB-231 cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS). The MCF-7 cells were grown in DMEM supplemented with 10% FBS. All the cells were cultured in a humidified atmosphere with 5% CO_2 at 37 ℃. RACK1 (Proteintech, USA, 1:1,000), USP41 (Invitrogen, USA, 1:1,000), β-actin (Servicebio, China, 1:1,000), goat anti-mouse IgG (1:5,000, Sigma-Aldrich, St. Louis, MO, USA) and GAPDH (Proteintech, USA, 1:1,000) were used for western blot.

The Cancer Genome Atlas (TCGA) database analyses

We used TCGA database (http://tcga-data.nci.nih.gov/tcga/) to investigate the expression of *USP41* in BC. The BC patients were enrolled regardless of hormonal status and human epidermal growth factor receptor 2 (*HER2*) status. There were 1,104 cancer samples and 113 normal samples included. These participants had received chemotherapy, including adjuvant and neoadjuvant chemotherapy, but the specific regimen was unknown. The participants were segregated into "low" and "high" level of *USP41* messenger RNA (mRNA) by median and we analyzed its relationship with survival.

Knockdown and overexpression assay

Knockdown assay was performed by transfecting the cells with siRNAs. siRNAs were obtained from HIPPOBIO (Huzhou, China). Lipofectamine® RNAiMAX Reagent (Invitrogen) was used to transfect the siRNAs in indicated cells, according to the manufacturers' protocols.

The sequences of siRNAs were as follow. siCtrl, 5'-UUCUCCGAACGUGUCACGU-3'; siUSP41#1, 5'-GGCUCAUCAGUGUCAGUACGU-3'; siUSP41#2, 5'-GCUCACCCUCCGACUUUCUUU-3'; siRACK1#1, 5'-CCAUGUUAUGGGAUCUCAA-3'; siRACK1#2, 5'-CCAACAGCAGCAACCCUAU-3'.

Overexpression assay was performed by transfecting the cells with pCDH vectors. Negative controls cells were transfected with empty pCDH vectors. Overexpressed cells were transfected with pCDH vectors containing the coding sequence of indicated genes.

Cell proliferation analysis

Cell Counting Kit-8 (CCK-8) assay was performed to measure proliferation of BC cells according to manufacturer's instructions. Briefly, 6,000 cells/well were seeded in 96-well plates in medium containing 10% FBS and incubated under 37 ℃, 5% CO_2. After treatment, 10 μL CCK-8 reagent was added and incubated for 1 h, the absorbance under 450 nm was measured with a microplate reader. The same experiments were repeated after a defined incubation period.

Colony formation assay

The different cells were seeded in 6 cm dishes at a density of 300 cells/dish. Following incubation for 2 weeks in a humidified incubator at 37 ℃ in an atmosphere of 95% air and 5% CO_2, the cells were washed with phosphate-buffered saline (PBS), and colonies were fixed with methanol for 10 min and stained with 0.5% crystal violet for 15 min. The number of colonies was counted under a microscope (CKX53; Olympus, Tokyo, Japan). All experiments were performed in triplicate dishes in 3 independent experiments.

Transwell assay

Transwell migration assay was performed using Transwell inserts. In serum-free medium, 8 ×10^4 cells were seeded into the upper chamber of the insert and the bottom of the chamber contained the DMEM with 10% FBS. After 36 h incubation, the cells were fixed with methanol and stained with Giemsa. Then cells on the top surface of the membrane were wiped off, and cells on the lower surface were examined under an inverted light microscope. The number of migrated or invaded cells was quantified by counting the number of cells from 10 random fields at ×100 magnification.

Apoptosis assays

Additionally, cellular apoptosis was determined by flow cytometry (FAC). Cell lines cultured in DMEM supplemented with 10% FCS were seeded in 96-well plates (2×10^4 cells/well). After treatment with *USP41* overexpression or knockdown, cellular apoptosis was determined by FAC after a stain with Annexin v-FITC or propidium iodide (PI) or both for 15 min in the dark at room temperature following the instructions of the Annexin v-FITC apoptosis detection kit.

Cell cycle analysis

Cell cycle distribution was analyzed using flow cytometric analysis. Briefly, after treatment with *USP41* overexpression or knockdown, cells were harvested and fixed with precooled 75% (v/v) ethanol for 24 h. Afterwards, ethanol was discarded by centrifugation. Fibroblasts were rehydrated with PBS at room temperature and resuspended in PI DNA staining buffer and incubated for 30 min at room temperature in the dark. Detection was performed on a flow cytometer (FACSAria; Becton, Dickinson and Co. Biosciences, Franklin Lakes, NJ, USA).

RNA extraction and reverse transcription quantitative polymerase chain reaction

Total RNA was isolated from the cells and tissues using TRIzol reagent, according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). Complementary DNA (cDNA) was generated from 1 μg total RNA using SuperScript III (Invitrogen, USA) and polyN primers. qPCRwas performed using the StepOne and StepOne Plus real-time PCR system (Applied Biosystems, Foster City, CA, USA). The relative levels of gene expression were represented as $2^{-\Delta\Delta Ct}$. The experiments were repeated in triplicate. The sequence of indicated primers was listed as follow: USP41 forward, 5'-TGGAGGGCAGTATGAGCTTTTT-3', and reverse, 5'-ATGACCGGAGTCTGCCATTC-3'; RACK1 forward, 5'-CCACCACGAGGCGATTTGT-3', and reverse, 5'-CCCAGGGTATTCCATAGCTTGAT-3'; β-actin forward, 5'-CATGTACGTTGCTATCCAGGC-3', and reverse, 5'-CTCCTTAATGTCACGCACGAT-3'.

Western blot

Cells were homogenized and lysed with radioimmunoprecipitation assay (RIPA) lysis buffer [100 mM NaCl, 50 mM Tris-HCl pH 7.5, 1% TritonX-100, 1 mM ethylenediamine tetraacetic acid (EDTA), 10 mM b-glycerophosphate, 2 mM sodium vanadate and protease inhibitor]. Protein concentration was assayed using the micro-bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL, USA). A quantity of 40l g of protein per lane was separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electroblotted onto nitrocellulose (Amersham Pharmacia, Freiburg, Germany). Then, the membrane was blocked with 5% non-fat milk and incubated with antibodies against proteins. Incubation with the primary antibody was carried out overnight in a cold room. The membrane was then incubated with a secondary antibody conjugated to goat anti-mouse IgG (1:5,000, Sigma-Aldrich, St. Louis, MO, USA) and developed using enhanced chemiluminescence (ECL; Amersham Pharmacia, Piscataway, NJ, USA).

Co-immunoprecipitation and mass spectrometry

Co-immunoprecipitation and mass spectrometry (CoIP-MS) was used to explore the interacting proteins with *USP41*. The process consisted of 5 main steps: (I) incubation of cell lysates with antibodies, (II) binding of immune complexes to protein A/G agarose resin, (III) removal of non-interacting proteins, (IV) elution to obtain protein interacting complexes, (V) mass spectrometry identification of protein interacting complexes. The enriched co-immunoprecipitation products were analyzed by mass spectrometry. Peptides with scores b20 were removed, and higher scores meant a better degree of matching with the secondary atlas. Peptides were searched and compared qualitatively in UniProt (https://www.uniprot.org/). The UniquePep Count and the Cover Percent were also evaluated as auxiliary metrics for the final identification results.

Statistical analysis

The data were presented as means ± SD. Student's *t*-test was used for statistical analysis, unless otherwise indicated. GraphPad Prism 8 (GraphPad Software Inc., La Jolla, CA, USA) and SPSS 23.0 Software (IBM Inc., Armonk, NY, USA) were used for all statistical analyses. Statistically significant differences were indicated when $P<0.05$.

Results

Overexpression of USP41 in BC cell lines and tissues

To investigate the role of *USP41* in BC progression, we determined its expression in BC. Our TCGA database analysis revealed that in metastatic BC, *USP41* expression was upregulated (*Figure 1A*). The overexpression of *USP41* was negatively correlated with BC prognosis (*Figure 1B*). The protein levels of *USP41* were higher in tumor tissues than their adjacent non-tumorous tissues in 10 pairs of clinical BC specimens (*Figure 1C*).

Establishment of stable USP41 expression in BC cell lines

To further explore the function of *USP41*, we used small interfering RNA (siRNA) to generate a stable cell line (MCF7 and MDA-MB-231 BC cells) in which *USP41* was knocked down. We also established *USP41* overexpression cell lines by transfecting MCF7 and MDA-MB-231 cells. Transfection efficiency was confirmed by western blot analysis and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Compared with the cells transfected with the control siRNA, the cells that had been transfected with the *USP41* siRNA plasmid exhibited a significantly decreased expression of *USP41* at both the protein and mRNA level (*Figure 2A,2B*). On the contrary, the MCF7 and MDA-MB-231 BC cells that had been transfected with the *USP41* overexpression plasmid exhibited obviously increased expression of *USP41* at both the protein and mRNA level (*Figure 2C,2D*).

Effects of USP41 gene on BC growth

The CCK-8 and colony formation assays were used to exam the effects of *USP41* expression on BC cell proliferation. As shown in *Figure 3*, knockdown of *USP41* suppressed the proliferation of MCF-7 and MDA-MB-231 cells (*Figure 3A,3C*). In contrast, the overexpression of *USP41* significantly enhanced the proliferation of MCF-7 cells and MDA-MB-231 cells (*Figure 3B,3D*). Knockdown of *USP41* reduced the colony-forming ability of MCF 7 cells and MDA-MB-231 cells (*Figure 3E*), while overexpression of *USP41* promoted the colony-forming ability of MCF-7 cells and MDA-MB-231 cells (*Figure 3F*).

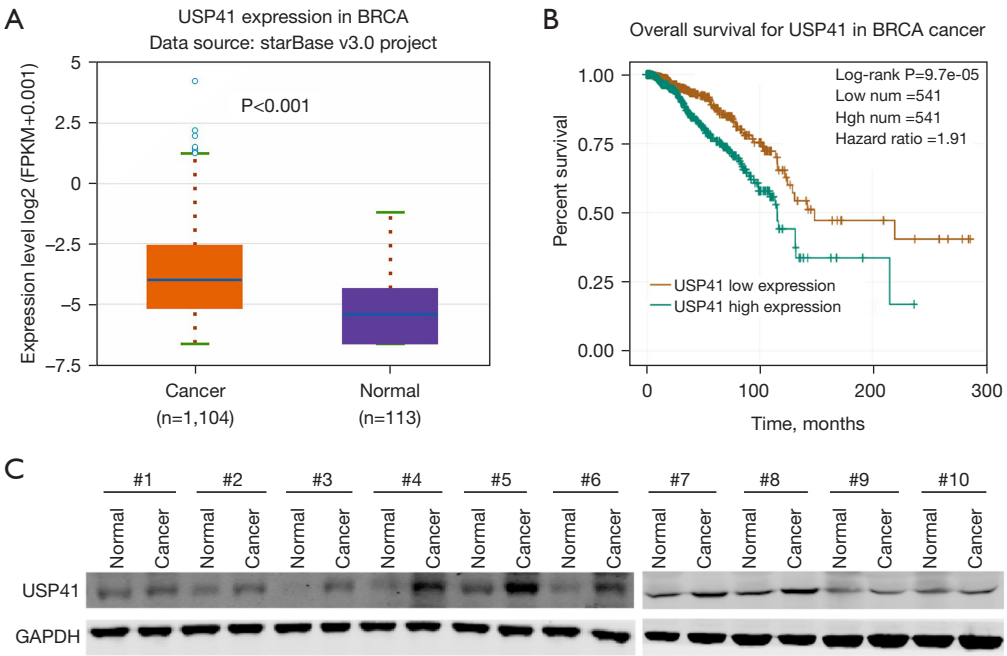

Figure 1 Overexpression of *USP41* in BC. (A) Overexpression of *USP41* in BC by TCGA database analysis. (B) Association of *USP41* with BC prognosis by TCGA database analysis. (C) The protein levels of *USP41* in clinical BC specimens (n=10) (P<0.05, Wilcoxon's signed-rank test). *USP41*, ubiquitin-specific protease 41; N, non-tumorous tissues; C, breast cancer specimens; BC, breast cancer; TCGA, The Cancer Genome Atlas.

Effects of USP41 gene on BC migration

Transwell assay indicated that *USP41* inhibition inhibited the migration compared to control cells in MCF-7 cell line (*Figure 4A*) and MDA-MB-231 cell line (*Figure 4B*). Meanwhile, the overexpression of *USP41* enhanced cell invasion in MCF-7 cells (*Figure 4C*). Identically, *USP41* overexpression promoted cell migration in MDA-MB-231 cell line (*Figure 4D*).

Effects of USP41 gene on BC cell cycle and apoptosis

We next confirmed the role of *USP41* in BC cell cycle and apoptosis. As shown in *Figure 5A,5B*, knockdown of *USP41* enhanced cell apoptosis in MCF-7 and MDA-MB-231 cells. Cell cycle arrests of MCF-7 and MDA-MB-231 were also studied. It was shown that *USP41* knockdown could arrest cells in G0/G1 phase (*Figure 5C,5D*).

USP41 interacted with RACK1 in BC cells

Study of protein-protein interactions (PPIs) is an essential process to understand the biological functions of proteins and its underlying mechanism (19). Here, we performed CoIP-MS to discover novel proteins interacting with *USP41*. The CoIP-MS analyses identified a list of genes that interacted with *USP41*, including *RACK1*. The Co-IP results indicated that *USP41* could interact with *RACK1* (*Figure 6A*). Immunoblotting results showed that *USP41* promoted the protein expression of *RACK1* (*Figure 6B*). We also found the overexpression of *RACK1* in BC tissues, compared with those in the adjacent normal tissue samples (*Figure 6C*). Next, we inhibited the expression of *RACK1* by gene knockdown (*Figure 6D*). Colony-forming assay demonstrated that knockdown of *RACK1* reduced the colony-forming ability of BC cells (*Figure 6E,6F*). Transwell assay indicated that knockdown of *RACK1* inhibited the migration compared to control cells (*Figure 6G*).

Knockdown of RACK1 reversed the oncogenic function of USP41 in BC cells.

At last, we knocked down *RACK1* in *USP41* overexpressed BC cells (*Figure 7A*). When *USP41* overexpression significantly promoted the proliferation, colony formation,

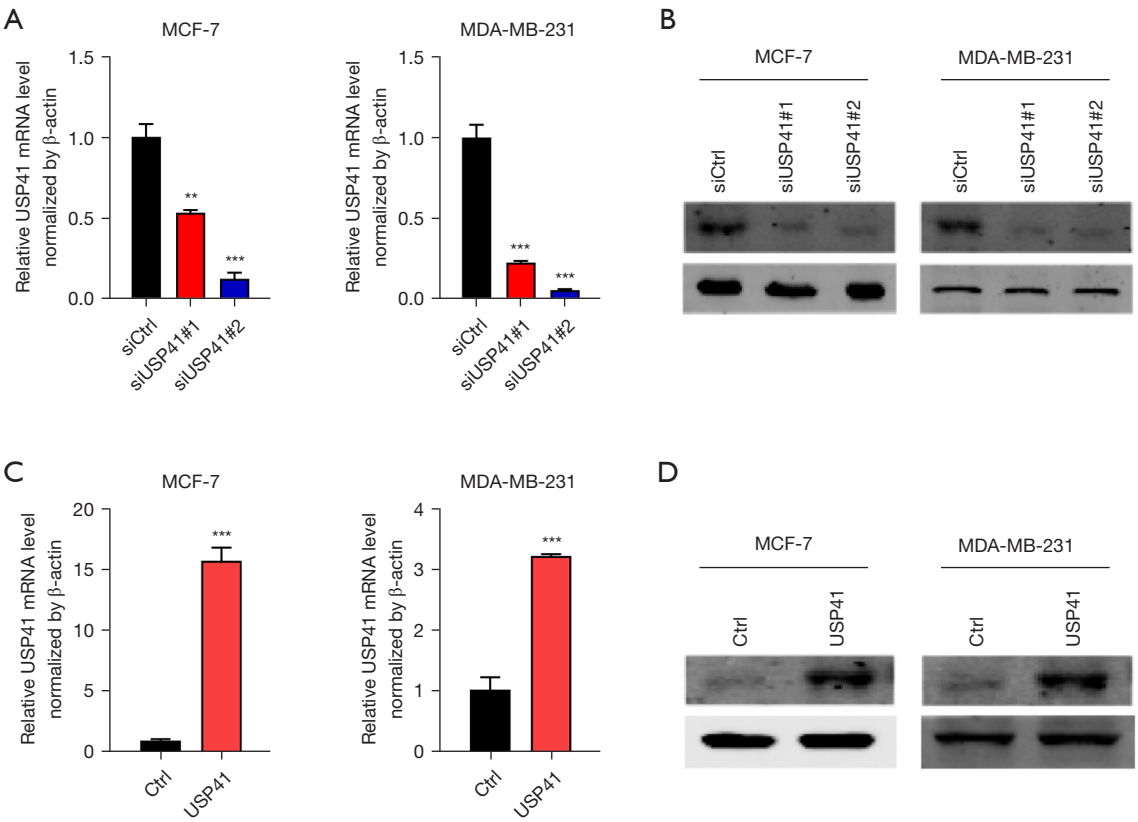

Figure 2 Establishment of *USP41* gene overexpression and knockdown. (A) Knockdown of *USP41* mRNA level in MCF-7 and MDA-MB-231 cell line was assessed by RT-qPCR. (B) Protein levels of *USP41* knockdown in MCF-7 and MDA-MB-231 BC cells. (C) Up-regulation of *USP41* mRNA level in MCF-7 and MDA-MB-231 cell lines was assessed by RT-qPCR. (D) Protein levels of *USP41* overexpression in MCF-7 and MDA-MB-231 BC cells. **, P<0.05; ***, P<0.01. *USP41*, ubiquitin-specific protease 41; mRNA, messenger RNA; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; BC, breast cancer.

and cell invasion of MCF-7 and MDA-MB-231 cells, knockdown of *RACK1* could obviously reduce the growth and invasion ability (*Figure* 7*B*-7*D*). Thus, *USP41* promoted BC function through upregulating *RACK1*.

Discussion

The USPs are emerging as potential therapeutic targets in many diseases. In BC, several USPs were overexpressed, such as *USP9X*, *USP15*, *USP32*, *USP9Y*, *USP10*, *USP25*, *USP4*, and *USP11* (20). In 2016, Li *et al.* showed that *USP4* could inhibit BC cell growth through the upregulation of PDCD4 (21). Both *USP14* and *USP21* were also involved in the progression of BC (22,23). In 2018, *USP2-69* was found over-expressed in breast invasive ductal carcinoma, and was closely related to proliferation promoting effects (24). Qiu *et al.* demonstrated that *USP8* might predict better clinical characteristics and might be a protective factor for patients with BC (25). In 2020, *USP8* was proved as a positive regulator of Notch signaling, offering a therapeutic target for BC (26). Pal *et al.* claimed that targeting USPs as BC treatment is especially promising (27). However, no results have been reported for *USP41* in BC, even in tumors. In this study, *USP41* was found overexpressed in BC and shown to be associated with the cancer progression for the first time.

A member of the tryptophan-aspartate-40 (WD-40) family of proteins, *RACK1* is an evolutionarily conserved a 36 kDa scaffold protein with 7 WD-40 repeats, containing the subunit of G-proteins (28,29). The WD40 repeats of *RACK1* provides the complex protein-protein bio-interactions among signaling molecules such as integrins, phosphodiesterase 4D5, and Src tyrosine kinase, as well as protein kinase C (PKC) (30). It seems that *RACK1*

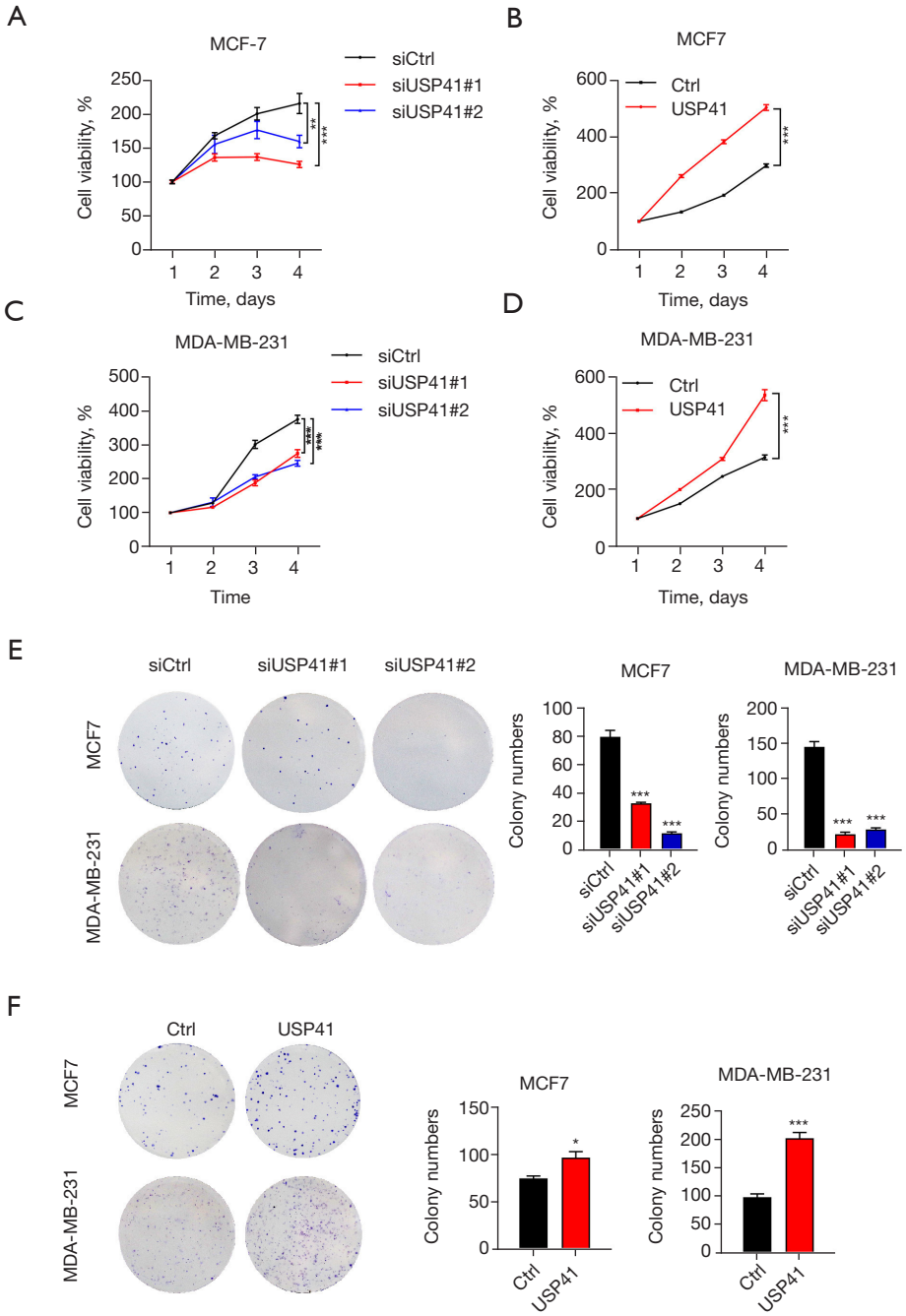

Figure 3 Effects of *USP41* gene on BC growth. (A) The results of CCK-8 assay that were conducted after *USP41* knockdown in MCF-7 cells. (B) Cell proliferation after *USP41* overexpression in MCF-7 cells was measured by CCK-8 assays. (C) Cell proliferation after *USP41* knockdown in MDA-MB-231 cells. (D) The result of CCK-8 assay that were conducted after the overexpression of *USP41* in MDA-MB-231 cell. (E) The result of colony formation assays that were conducted after *USP41* knockdown in MCF7 and MDA-MB-231 cells. (F) The result of colony formation assays that were conducted after the overexpression of *USP41* in MCF-7 and MDA-MB-231 cell. *, P>0.05, **, P<0.05; ***, P<0.01. *USP41*, ubiquitin-specific protease 41; BC, breast cancer; CCK-8, Cell Counting Kit-8.

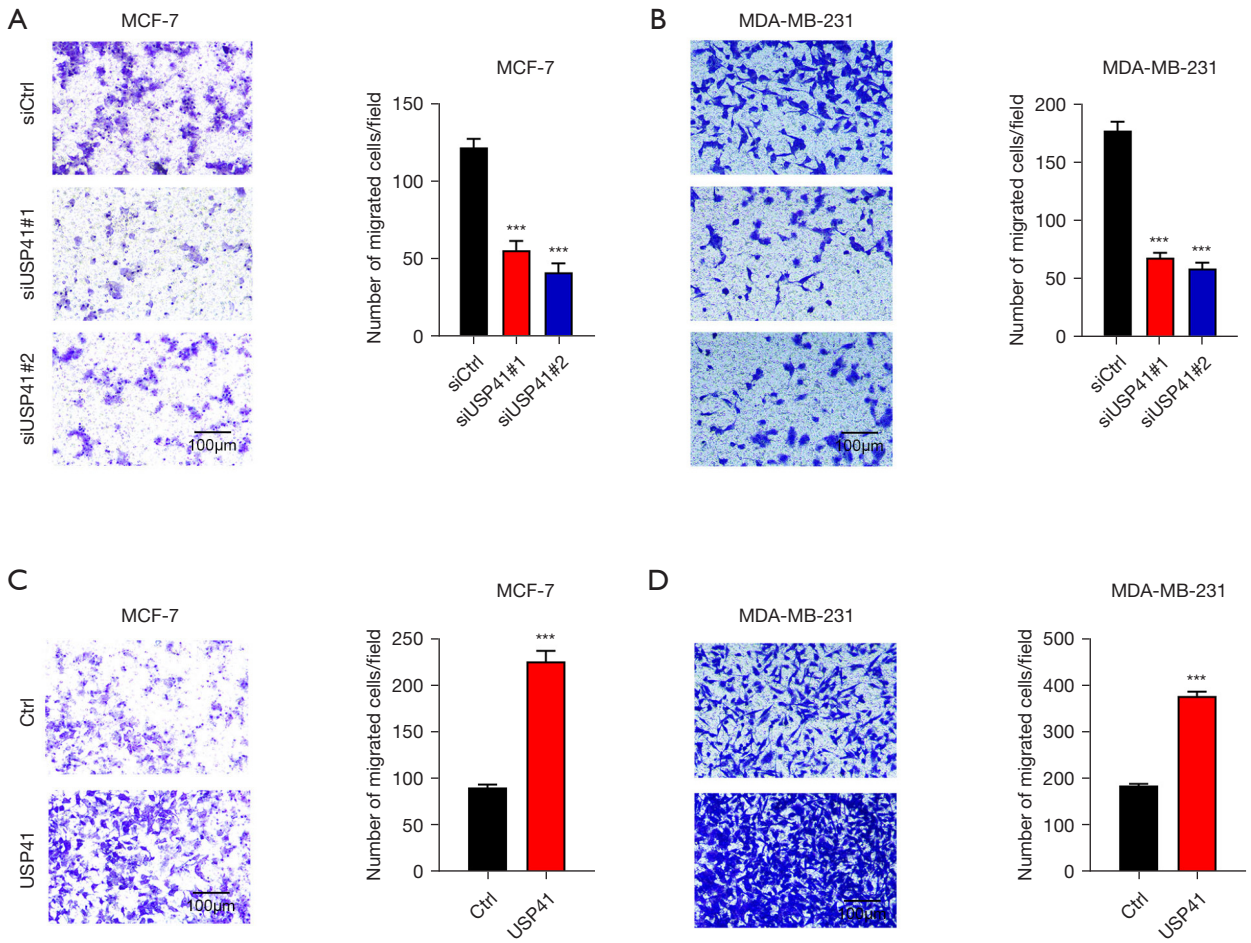

Figure 4 Effects of *USP41* gene on BC migration. (A,B) Cell invasion analysis after *USP41* knockdown in MCF-7 and MDA-MB-231 cells. (C,D) The result of cell invasion assay that was conducted after the overexpression of *USP41* in MCF-7 and MDA-MB-231 cell. Transwell staining results are shown as a reference. ***, P<0.01. *USP41*, ubiquitin-specific protease 41.

is essential for cellular functions because its amino acid sequence in human is 100% consistent with that in rats, chickens, mice, and cows (31). As reported, *RACK1* can interact with numerous signaling proteins and is regarded as a scaffolding, anchor, or adaptor protein, participating into multiple intracellular signal transduction, immune defense, cell growth, and migration pathways (32). In 2018, *RACK1* was verified as an oncogene in colon cancer (33). In 2020, *RACK1* overexpression was demonstrated to dampen the progression of cervical squamous cell carcinoma (34). Wu *et al.* showed that *RACK1* stimulates tumor invasion and lymph node metastasis of cervical cancer, providing promising means for cervical cancer treatment (35). Dan *et al.* found that *RACK1* promotes cancer progression by increasing the M2/M1 macrophage ratio in oral squamous cell carcinoma (36). For BC, *RACK1* was firstly identified as a superior independent biomarker for diagnosis and prognosis in 2010. Comparing with conventional diagnostic index [*Ki67*, estrogen receptor (*ER*), progesterone receptor (*PR*) and *HER-2*] in BC, *RACK1* possessed superiority in sensitivity and specificity as biomarker (37). In this study, we also found the overexpression of *RACK1* in BC and its potential role in proliferation and migration. However, the mechanisms through which *RACK1* regulates BC progression are still undetermined

The USPs-regulated signaling pathways implicated in BC are more likely to be transforming growth factor beta (*TGF-β*) signaling, which has a well-documented role in mediating epithelial-to-mesenchymal transition (EMT), tumor progression, and metastasis (20). In 2012, *USP11*

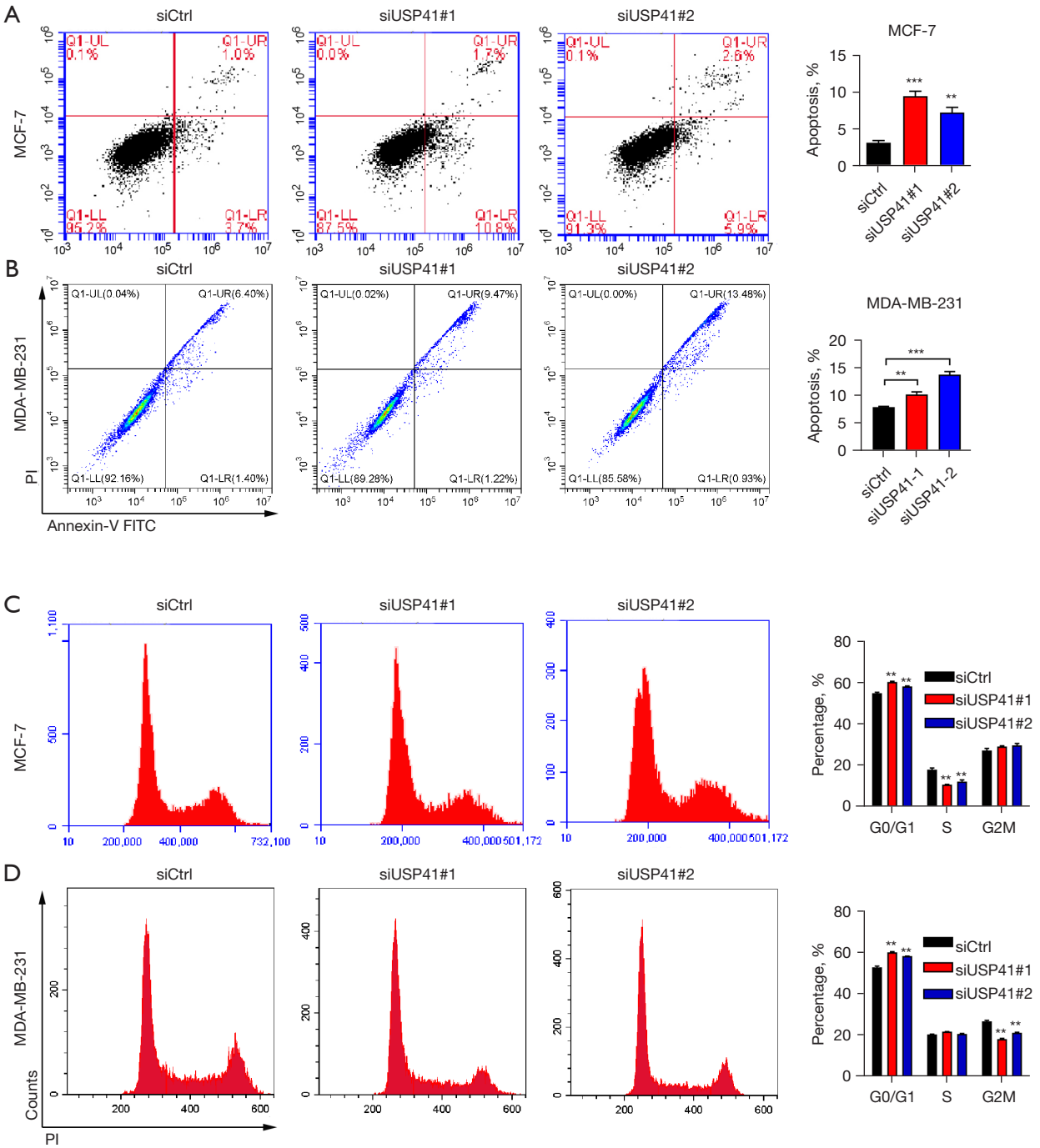

Figure 5 Effects of *USP41* gene on BC cell cycle and apoptosis. (A,B) Cell apoptotic analysis after *USP41* knockdown in MCF-7 and MDA-MB-231 cells. (C,D) The results of cell cycle assay that were conducted after the knockdown of *USP41* in MCF-7 and MDA-MB-231 cell. **, P<0.05; ***, P<0.01. *USP41*, ubiquitin-specific protease 41.

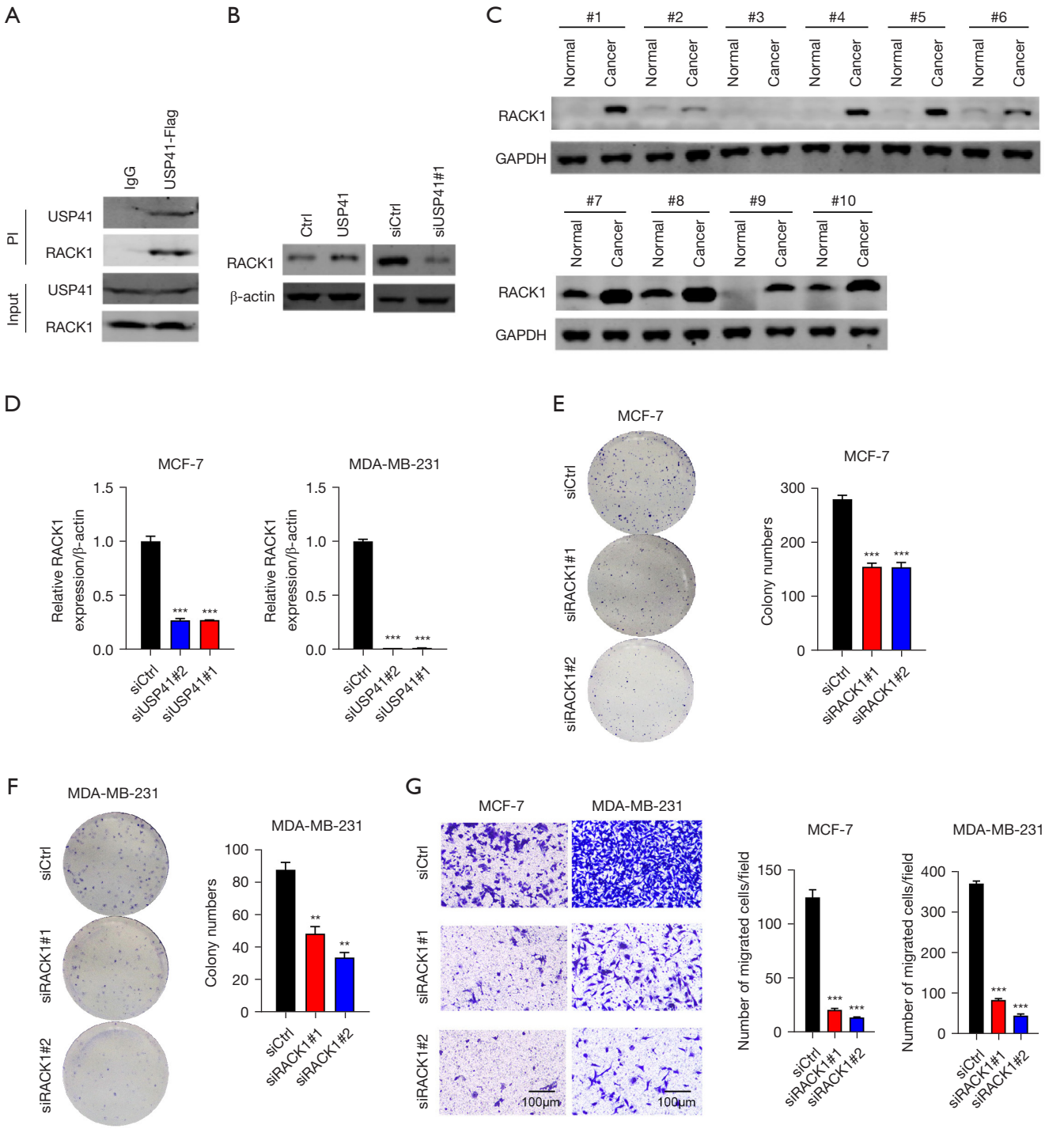

Figure 6 *USP41* combined with *RACK1* in BC cell. (A) Co-IP assay was performed in *USP41*-Flag overexpressing cells. (B) Immunoblotting analysis of *RACK1* in *USP41* overexpressing and knockdown cells. (C) Immunoblotting analysis of *RACK1* in BC and normal tissues. (D) The mRNA level of *RACK1* after gene knockdown. (E,F) Effects of *RACK1* knockdown on BC cell colony formation. (G) Effects of *RACK1* knockdown on BC cell migration. Transwell staining results are shown as a reference. **, P<0.05; ***, P<0.01. *USP41*, ubiquitin-specific protease 41; *RACK1*, receptor for activated C kinase 1; BC, breast cancer; Co-IP, co-immunoprecipitation; mRNA, messenger RNA.

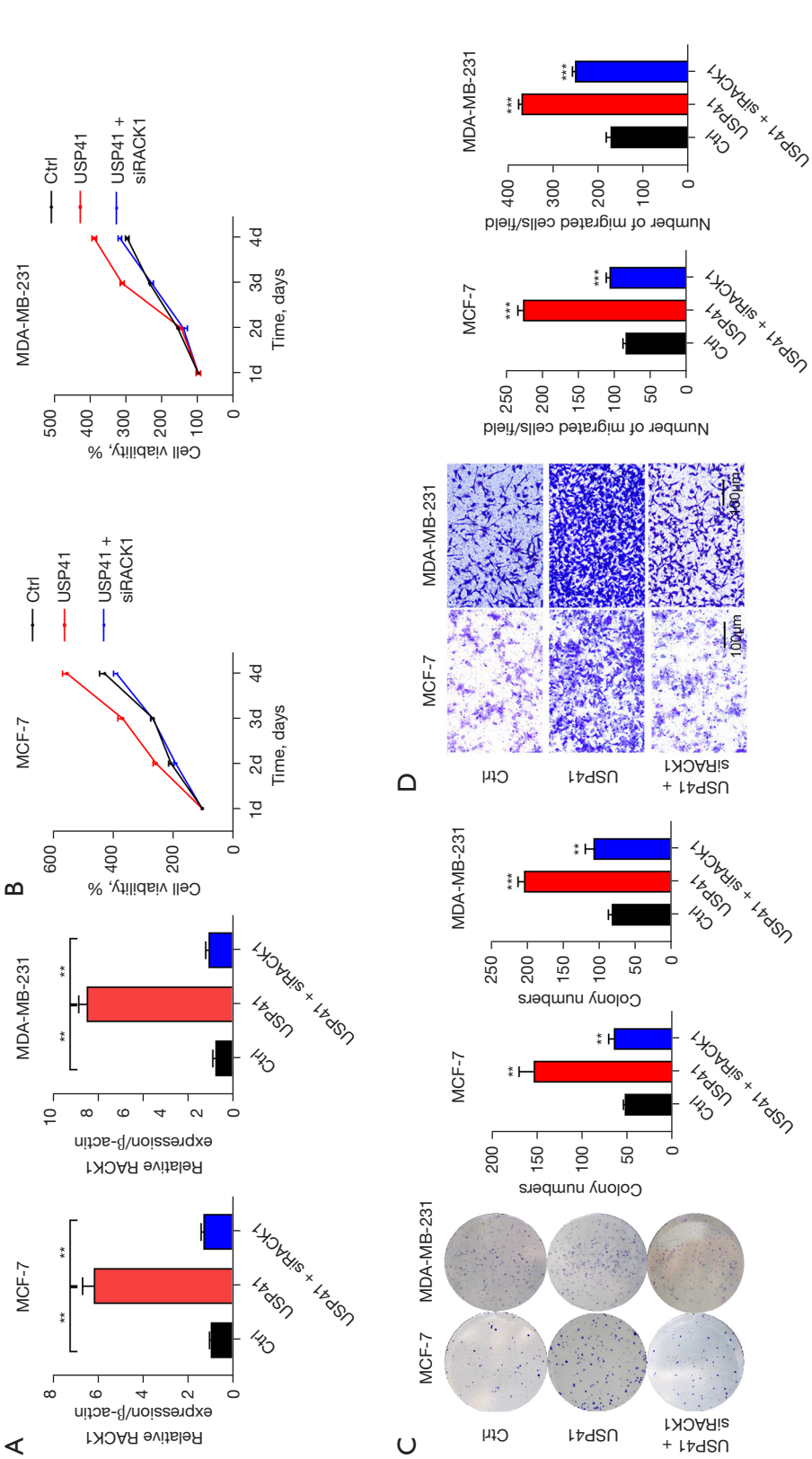

Figure 7 *USP41* promoted BC cell growth and invasion through upregulation of *RACK1*. (A) The mRNA level of *RACK1* was checked in Ctrl, *USP41*, and *USP41*+siRACK1 MCF-7 and MDA-MB-231 cells. (B) CCK-8 assay was performed to analyze cell proliferation in Ctrl, *USP41*, and *USP41*+siRACK1 MCF-7 and MDA-MB-231 cells. (C) Colony formation assay was performed in Ctrl, *USP41*, and *USP41*+siRACK1 MCF-7 and MDA-MB-231 cells. (D) Transwell analysis of cell invasion was performed in Ctrl, USP41, and *USP41*+siRACK1 MCF-7 and MDA-MB-231 cells. Transwell staining results are shown as a reference. **, P<0.05; ***, P<0.01. *USP41*, ubiquitin-specific protease 41; *RACK1*, receptor for activated C kinase 1; BC, breast cancer; mRNA, messenger RNA; CCK-8, Cell Counting Kit-8.

was found to augment *TGF-β* signaling by deubiquitylating *ALK5* (38). Eichhorn *et al.* demonstrated that *USP15* stabilized *TGF-β* receptor I and promoted oncogenesis through the activation of *TGF-β* signaling (39). In 2019, Zhang *et al.* showed that *USP4* inhibition prevented the activity of the *TGF-β/Smad* signaling pathway (40). Galant *et al.* found that overexpression of several USPs amplifies fibrotic responses induced by *TGF-β* (41). In this study, *USP41* was found to be associated with *RACK1* and *RACK1* has been identified as concomitant regulator of *TGF-β1* (42). Zhou *et al.* indicated that *RACK1* can inhibit collagen synthesis in KFs via inhibition of the *TGF-β1/Smad* signaling pathway (43). Silencing of *RACK1*was shown to attenuate renal fibrosis by inhibiting *TGF-β* signaling (44). Above all, *USP41* may exert an oncogenic function via *TGF-β* signaling in combination with *RACK1*. However, the mechanisms through which *RACK1* regulates BC are wide ranging and diverse. Further study is still needed to explore its role in *USP41* mediated BC progression.

In conclusion, *USP41* overexpression promotes the proliferation and invasion of BC by combination with *RACK1*, which may serve as an emerging therapeutic potential target for BC treatment.

Acknowledgments

Funding: This work was supported by The National Natural Science Foundation of China (81572917); Key Project of Natural Science Foundation of Shaanxi Province (2021JZ-29); Key Research and Development Plan of Shaanxi Province (2018ZDXM-SF-066); Project of Xijing Hospital (XJZT18MJ30).

Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://dx.doi.org/10.21037/atm-21-4921

Data Sharing Statement: Available at https://dx.doi.org/10.21037/atm-21-4921

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/atm-21-4921). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was approved by an independent ethics committee of Fourth Military Medical University (KY20213157-1). The study was undertaken in accordance with the Good Clinical Practice guidelines and the Declaration of Helsinki (as revised in 2013). All participants were asked to provide written informed consent before enrollment.

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(English Language Editor: J. Jones)

Cite this article as: Huang M, Xiao J, Yan C, Wang T, Ling R. *USP41* promotes breast cancer via regulating *RACK1*. Ann Transl Med 2021;9(20):1566. doi: 10.21037/atm-21-4921