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ORIGINAL ARTICLE

CUL1 Knockdown Attenuates the Adhesion, Invasion, and Migration of Triple-Negative Breast Cancer Cells via Inhibition of Epithelial-Mesenchymal Transition

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Abstract

Cullin-1 (CUL1) is an important factor for tumor growth and a potential therapeutic target for breast cancer therapy, but the molecular mechanism in triple-negative breast cancer (TNBC) is unknown. In the present study, CUL1 shRNA was transfected into BT549 and MDA-MB-231 breast cancer cells. Cell morphology, adhesion, invasion, and migration assays were carried out in the CUL1 knockdown cells. Additionally, protein expression levels of epithelial-mesenchymal transition (EMT)-related factors, Akt phosphorylation at S473 (pAkt), glycogen synthase kinase-3β phosphorylation at ser9 (pGSK3β), cytoplasmic and nuclear β-catenin, and epidermal growth factor receptor phosphorylation at Tyr1068 (pEGFR) were detected by Western blot analysis. CUL1 knockdown significantly suppressed the adhesion, invasion and migration capabilities of the cells, and decreased the expression of Snail1/2, ZEB1/2, Twist1/2, Vimentin, and increased the expression of Cytokeratin 18 (CK18). Moreover, CUL1 knockdown significantly downregulated the phosphorylated levels of Akt, GSK3β, and EGFR, inhibiting the translocation of β-catenin from the cytoplasm to the nucleus. The results indicate that CUL1 knockdown prohibited the metastasis behaviors of breast cancer cells through downregulation (dephosphorylation) of the EMT signaling pathways of EGFR and Akt/GSK3β/β-catenin in breast cancer. These results strongly suggested that reinforcement of the EMT might be a key for CUL1 to accelerate TNBC metastasis.

Keywords CUL1 · Breast Cancer · Epithelial-mesenchymal transition · Akt · GSK3β · EGFR

Introduction

Breast cancer makes up the most substantial number of carcinomas diagnosed in women globally, which accounts for 23% of all female cancers and 14% of total cancer mortality [1]. However, approximately 15–20% of breast cancers are triple-negative [2], which is characterized by low or no expression of estrogen receptor (ER), progesterone receptor (PR), or human epidermal growth factor receptor 2 (HER2) [3]. Women diagnosed with triple-negative breast cancer (TNBC) have poorer prognosis than those diagnosed with other breast cancer subtypes [2]. The lack of an established receptor target lessens the therapeutic options and remains to be a critical challenge in treating patients with TNBC.

Much effort has been placed into searching for cancer-related biomarkers. Cullin-1 (CUL1), which serves as a skeleton of the Skp1-CUL1/Rbx1-F-box protein (SCF) ubiquitin E3 ligase complex [4], plays an important role in signal transduction, cell cycle progression, and ubiquitin-dependent proteolysis [5]. Considerable lines of evidence indicate that CUL1 participates in tumor progression and its overexpression has been linked to poor prognoses in many cancers. CUL1 overexpression improves the proliferation abilities of melanoma cells by regulating p27 expression [6], along with promoting the migration and invasion of human trophoblast [7]. Additionally, CUL1 overexpression is significantly associated with poor prognoses and more advanced tumor-node-metastasis (TNM) staging in gastric cancer, non-small-cell lung cancer, and breast cancer [8–10]. However, the

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mechanism by which CUL1 leads to TNBC metastasis remains to be elucidated.

Metastasis is a complex process through which tumor cells invade the surrounding stroma and blood vessels to transport from the primary tumor mass to distant sites to set up series discontinuous colonies. Most scholars believe that epithelial tumor cells have the abilities of invasion and dissemination through the epithelial-mesenchymal transition (EMT) mechanism [10]. Studies have shown that the EMT process is characterized by the downregulation of epithelial markers, including Cytokeratin 18 (CK18) and E-cadherin, and the upregulation of mesenchymal markers, such as Vimentin and Snail [11, 12]. Several signaling pathways can be activated during the EMT process in breast cancer cells, such as the Src/focal adhesion kinase (FAK) pathway [13], mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway [14], and Akt/glycogen synthase kinase-3β (GSK3β)/β-catenin pathway [15, 16]. Also, epidermal growth factor receptor (EGFR), as an essential part of the ErbB receptors family, can activate downstream signaling pathways, including the phosphoinositide-3 kinase (PI3K)/Akt/GSK3β pathway, to control the proliferation, survival, and migration of cells [16]. Moreover, the overexpression or inappropriate activation of EGFR was reported to relate to poor patient outcomes [17, 18]. Thus, it remains unclear whether the anti-tumor mechanisms of CUL1 knockdown in TNBC are associated with the EGFR/Akt/GSK3β/β-catenin signaling pathway.

In this study, we knocked-down the CUL1 in two TNBC cells, including MDA-MB-231 and BT549, by shRNA to evaluate their effects on the metastatic characteristics of the cells, as well as the process of EMT and the possible signaling pathway during metastasis.

Materials and Methods

Antibodies and Plasmids

Rabbit anti-CUL1, anti-Snail antibody, anti-Slug antibody, anti-ZEB1 antibody, anti-ZEB2 antibody, anti-Twist2 antibody, anti-Vimentin antibody, anti-CK 18 antibody, anti-pAkt (Ser475) antibody, anti-Akt antibody, anti-pGSK3β (Ser9) antibody, anti-GSK3β antibody, anti-β-catenin antibody, anti-pEGFR (Ser1068) antibody, anti-EGFR antibody, anti-Lamin A antibody, and anti-β-actin antibody were purchased from Abcam Biotechnology (Cambridge, UK). Rabbit anti-Twist1 antibody was obtained from Cell Signaling Technology (CST, Boston, USA). Interleukin 1 beta (IL-1β) was purchased from Peprotech (Rocky Hill, NJ, USA). shRNA control targeting GFP (SHC004), shRNA Targeting CUL1–1 (TRCN0000318414), and shRNA Targeting CUL1–2 (TRCN0000318413) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Cells and Cell Culture

To reveal the metastatic mechanism of breast cancer, BT549 and MDA-MB-231 cells were purchased from American Tissue Culture Collection (ATCC, Rockville, MD, USA),

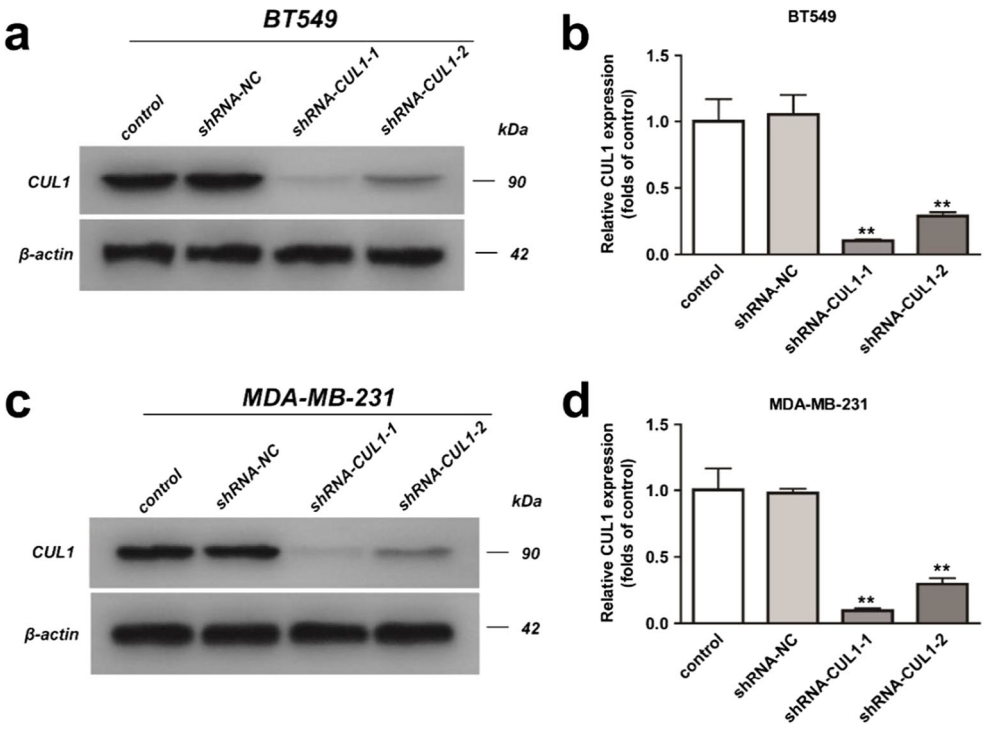

Fig. 1 Knock-down of CUL1 in breast cancer cells. Western blot analysis reveals the efficiency of CUL1 knocking-down in BT549 (**a**) and MDA-MB-231 cell lines (**c**). CUL1 levels were indicated as fold differences of the control (**b**, **d**). The results were presented as mean ± SD ($n = 3$). $^{**}P < 0.01$, vs. control group

both of which are triple-negative breast cancer cell lines characterized as invasive/metastatic phenotype and poor prognosis [19, 20]. Roswell Park Memorial Institute (RPMI) medium 1640 and Complete Dulbecco's Modified Eagles Medium (DMEM) (GIBCO, Grand Island, NY, USA) were employed to routinely maintain BT549 and MDA-MB-231 cells, respectively, in an incubator at 37 °C with 5% CO_2. All the complete mediums contained 10% fetal bovine serum (FBS) (GIBCO, Grand Island, NY, USA), 100 IU/ml penicillin, and 100 μg/ml streptomycin (Sigma-Aldrich, St. Louis, MO).

Cell Transfection

GFP shRNA or CUL1–1 shRNA, or CUL1–2 shRNA was transfected to BT549 or MDA-MB-231 cells using Lipofectamine 2000 (Invitrogen, CA, USA). The day before transfection, the cells were seeded in a 6-well plates at 2×10^5 cells per well in 2 mL of antibiotic-free medium. The medium was changed to 1.5 mL of Opti-MEM serum-free medium for each well half an hour before transfection. Next, 5 μg GFP shRNA control, CUL1–1 shRNA, and CUL1–2 shRNA were added to 250 μL of Opti-MEM serum-free medium, respectively, and gently mixed with Solution A. Then, 10 μL of the Lipofectamine 2000 was added to 250 μL of Opti-MEM serum-free medium, and gently mixed with Solution B. Solution B was added into Solution A and mixed gently, and allowed to react at room temperature for 20 min. The mixed transfection reagent was uniformly dispersed and slowly added dropwise to a 6-well plate at 500 μL/well. After incubation at 37 °C for 4 h, the medium was replaced with RPMI-1640 medium for BT549 cells and DMEM medium for MDA-MB-231 cells. At 48 h, the transfected cells were screened for future experiments.

Cell Adhesion Assay

HUVEC cells were seeded in a 24-well plates at 2×10^4 cells/well with fresh medium (without Endothelial Cell Growth Supplement). Once the cells reached 80% attachment, the medium was exchanged with DMEM containing 1 ng/mL of IL-1β and 1% FBS for 4 h. Meanwhile, the BT549 and MDA-MB-231 cells were

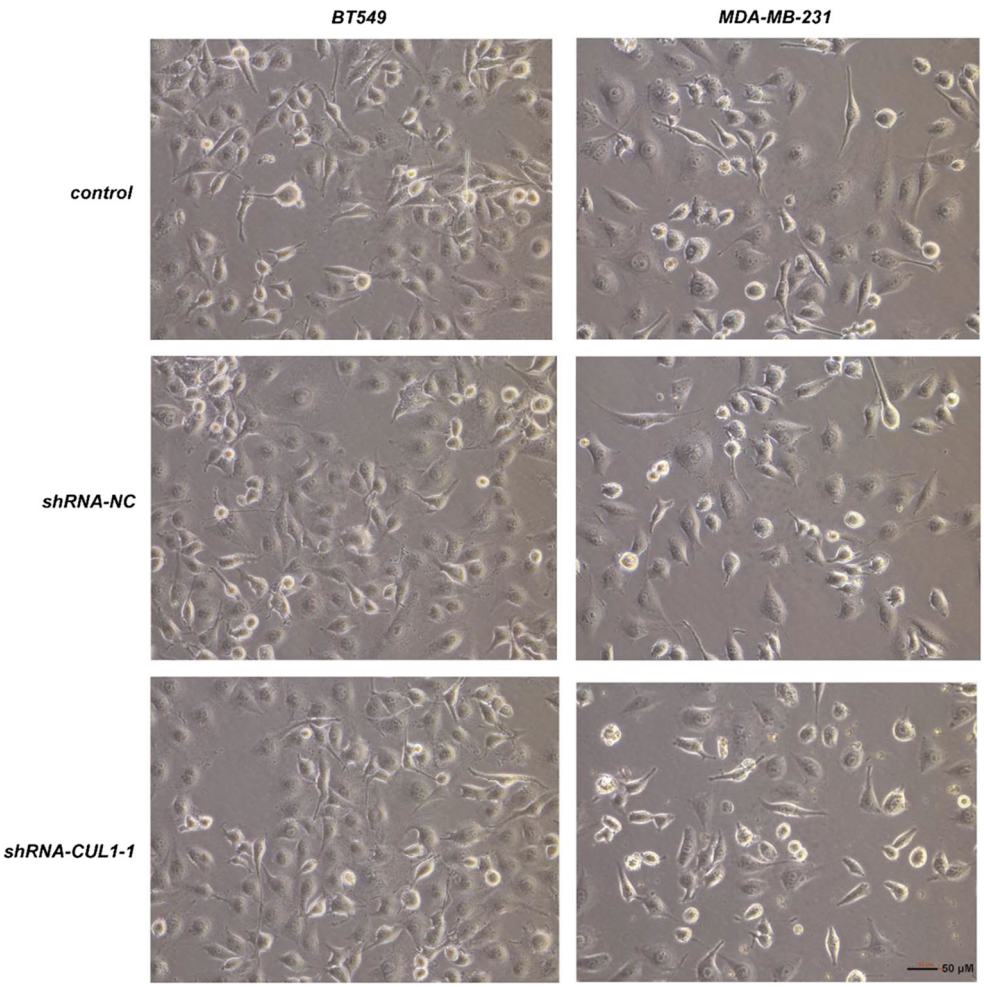

Fig. 2 Phase-contrast microscopy images of BT549 or MDA-MB-231 cells. Pictures show the morphological changes of BT549 and MDA-MB-231 cells after being transfected with GFP shRNA or CUL1 shRNA

incubated in PBS containing Rhodamine 123 (5 μg/mL) for 1 h. The rhodamine 123-labeled breast cancer cells were then seeded at 5×10^4 in 24-well plates containing HUVEC cells in serum-free medium for 1 h. Subsequently, after washing with 1 mL of PBS 3-times, the cells were observed under a fluorescence microscope. Twenty fields of view were randomly selected from each group, and the average number of the BT549 cells or MDA-MB-231 cells was recorded.

Transwell Invasion Assay

The breast cancer cell invasion abilities were assessed using Transwell-based invasion assays [21]. Briefly, the Transwell filters (8-μm pore size, Corning) were coated with Matrigel (Corning, NY, USA). Then, 2.5×10^5 cells/mL suspended in serum-free medium were added to the upper chambers. After supplementing with 600 μL of 10% FBS medium (BT549: RPMI-1640 medium, MDA-MB-231: DMEM medium) to the lower chamber of the 24-well plate, the cells were cultured in a 37 °C incubator for 24 h. Next, the top chamber was removed, washed 3-times with PBS at room temperature, and placed into a new 24-well plate with formaldehyde for 30 min. The formaldehyde was then discarded, and 700 μL of 1% crystal violet was added to the lower chamber. After staining for 15 min, the upper chamber was gently rinsed with water. The cells that did not pass through the chamber were gently wiped off with a wet cotton swab. The cells passed through the up chamber were counted under a microscope. The counting field of view was randomly selected.

Wound-Healing Assay

The culture medium was discarded when the cells were 80–90% confluent in 6-well plates. Next, the cells were treated with mitomycin, incubated at 37 °C for 1 h, and washed with 1 mL of PBS. The plate was scratched with a 10 μL pipet tip to create a wound space and the space borders were marked immediately. After rinsing with PBS for three times, the cells were added with 600 μL of a medium containing 1% FBS and placed in

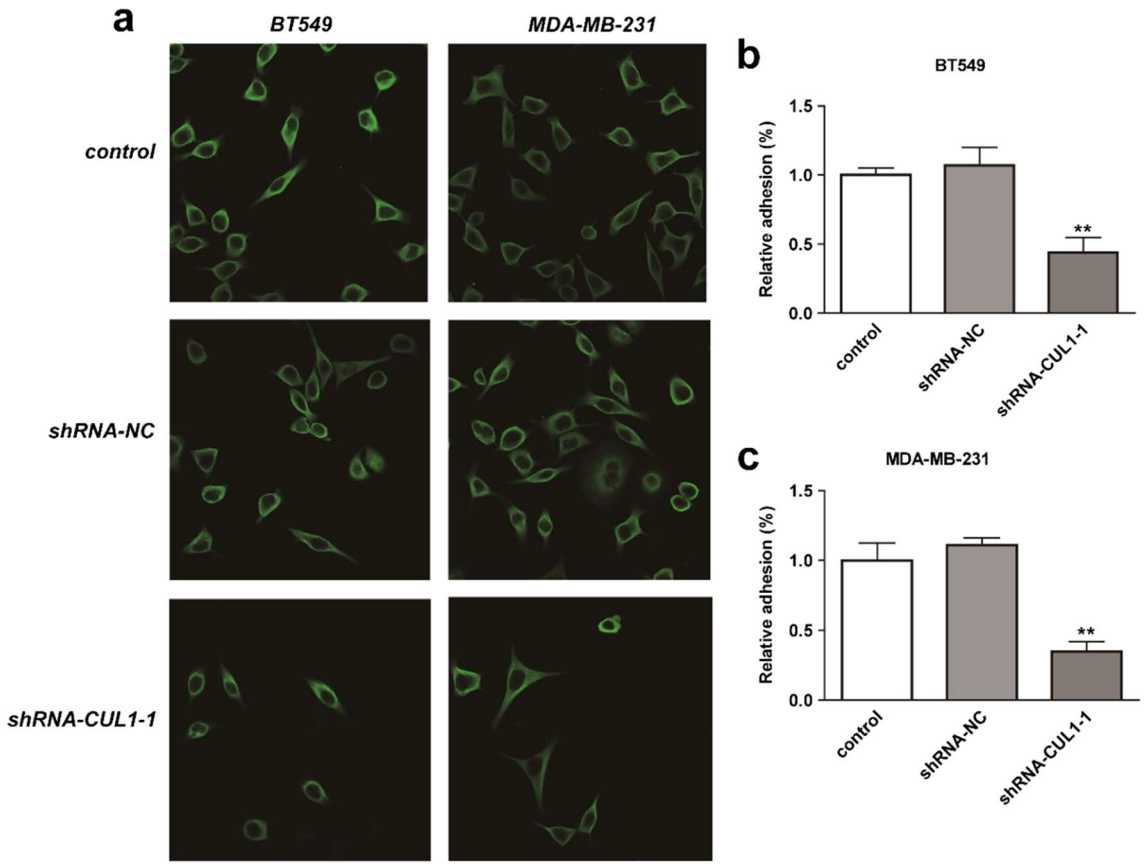

Fig. 3 Knockdown of CUL1 inhibited adhesion of breast cells to IL-1β-HUVECs. Rhodamine 123-labeled BT549 cells or MDA-MB-231 cells were added to the HUVEC monolayers stimulated by IL-1 β and transfected with or without CUL1 shRNA or the control vector (**a**). Fluorescence microscopy showed the BT549 (**b**) cells or the MDA-MB-231 cells (green) (**c**) adhered to the HUVECs. Data were expressed as the mean ± SD of each group of cells. $^{**}P < 0.01$, vs. control group

a 37 °C incubator for 48 h. Pictures were taken under the microscope, and the wound healing area was analyzed by Image J software and calculated. Each sample was measured in triplicate.

Western Blotting Analysis

First, 40 μg protein from each sample was loaded onto an SDS-PAGE gel and electrophoresed, transferred on a PVDF membrane, blocked in a 5% skim milk buffer, and reacted with a primary antibody at 4 °C for overnight. After washing with TBST three times, the membrane was reacted with secondary antibody for 1 h at room temperature, washed with TBST, and the probed protein was visualized by chemiluminescent reagents. The blot was imaged with the Tanon 6600 Luminescence Imaging Workstation, and the probed protein level was estimated by densitometry analysis using Image Pro Plus 6.0 software (Media Cybernetics, Inc., MD, USA).

Statistical Analysis

SPSS version 16.0 (IBM, Chicago, IL, USA) was used to perform statistical analyses. Results are expressed as the means ± SD, and each experiment was repeated at least three times. Between-group comparisons were was analyzed using one-way analyses of variance (ANOVA) and Tukey's test. $P < 0.05$ was considered a significant difference.

Results

Knocking-Down of CUL1 by shRNA in TNBC Cell Lines

shRNA control targeting GFP and Two CUL1 shRNA (shRNA Targeting CUL1–1 and shRNA Targeting CUL1–2) were transfected into BT549 cells and MDA-MB-231, respectively. CUL1 expression in the cells was detected by Western blot. CUL1 protein expression declined by 95% and 76% in the BT549 cells transfected with shRNA Targeting CUL1–1 and shRNA Targeting CUL1–2, respectively, as compared with the shRNA-NC group (Fig. 1a, b) ($P < 0.01$). CUL1 levels decreased by 89% and 68% in the MDA-MB-231 cells transfected with shRNA Targeting CUL1–1 and shRNA Targeting CUL1–2, respectively, as compared with the control group (Fig. 1c, d) ($P < 0.01$). The results implied that the shRNA Targeting CUL1–1 was more effective

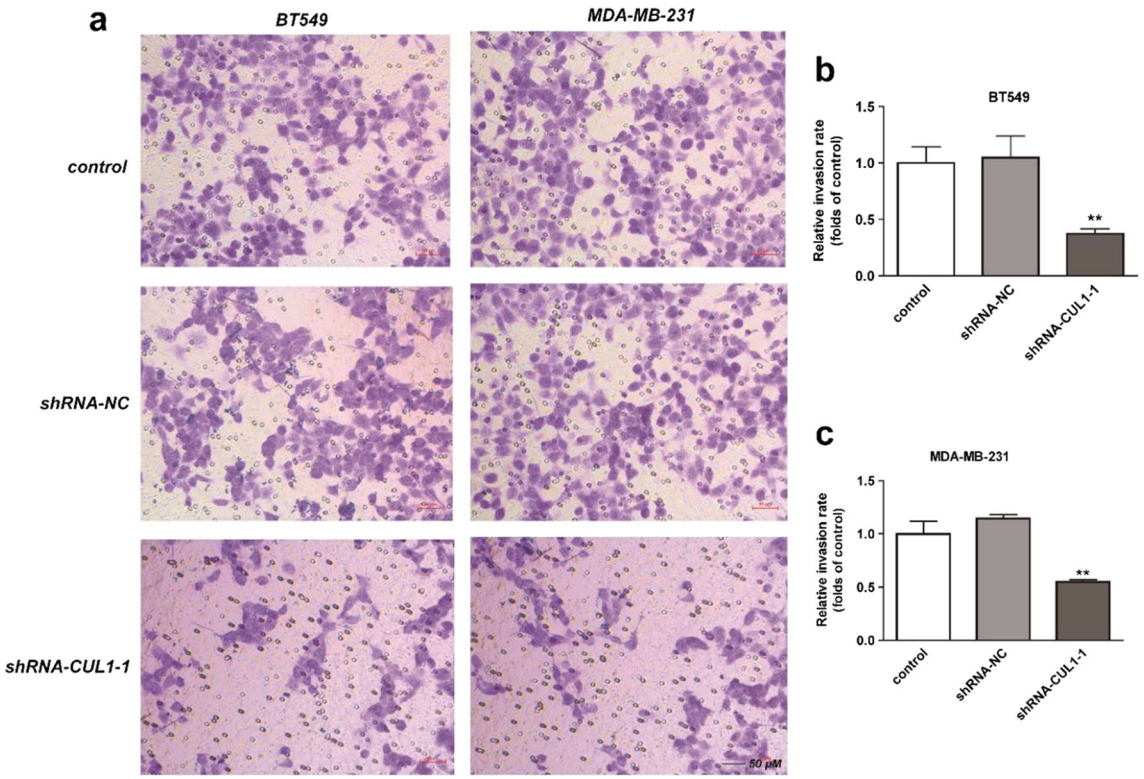

Fig. 4 Knockdown of CUL1 inhibited the invasive ability of breast cancer cells in vitro. The Transwell invasion assay showed that knocking-down of CUL1 inhibited the cellular invasion of the BT549 and the MDA-MB-231 (**a**). Graphical representation of the number of invasive BT549 (**b**) and MDA-MB-231 (**c**) cells per microscopic field. Data were shown as the mean ± SD from three independent experiments. $^{**}P < 0.01$, vs. control group

compared with shRNA Targeting CUL1–2. The control group and shRNA-NC group have no differences. Thus, shRNA Targeting CUL1–1 was employed in the next experiments.

Knocking-Down of CUL1 Results in Morphological Changes in TNBC Cells

The cellular morphology was observed with a phase-contrast microscope in the cells transfected with GFP shRNA or CUL1–1 shRNA for 36 h. The GFP shRNA-transfected cells (shRNA-NC group) showed as spindle-shaped with long thin cytoplasm shape. However, the CUL1–1 shRNA transfected cells, including the BT549 and MDA-MB-231 cells, displayed as closely arranged with rounded or irregular shape (Fig. 2).

Knocking-Down of CUL1 Inhibits the Adhesion of TNBC Cells to HUVECs

The strength of cellular adhesion affected by CUL1–1 knocking-down was determined using a cellular adhesion assay. The ability of cellular adhesion effected by CUL1 knocking-down was determined using a cell adhesion assay. The Rhodamine 123-labeled BT549 cells or MDA-MB-231 cells were added to the HUVEC monolayers that were stimulated by IL-1β and transfected with CUL1 shRNA. CUL1 knocking-down significantly inhibited the cellular adhesion ability of HUVECs and BT549 cells (63%) or MDA-MB-231 cells (86%), respectively, compared with the shRNA-NC and control groups ($P < 0.01$; Fig. 3). No significant difference was found between the control and shRNA-NC groups.

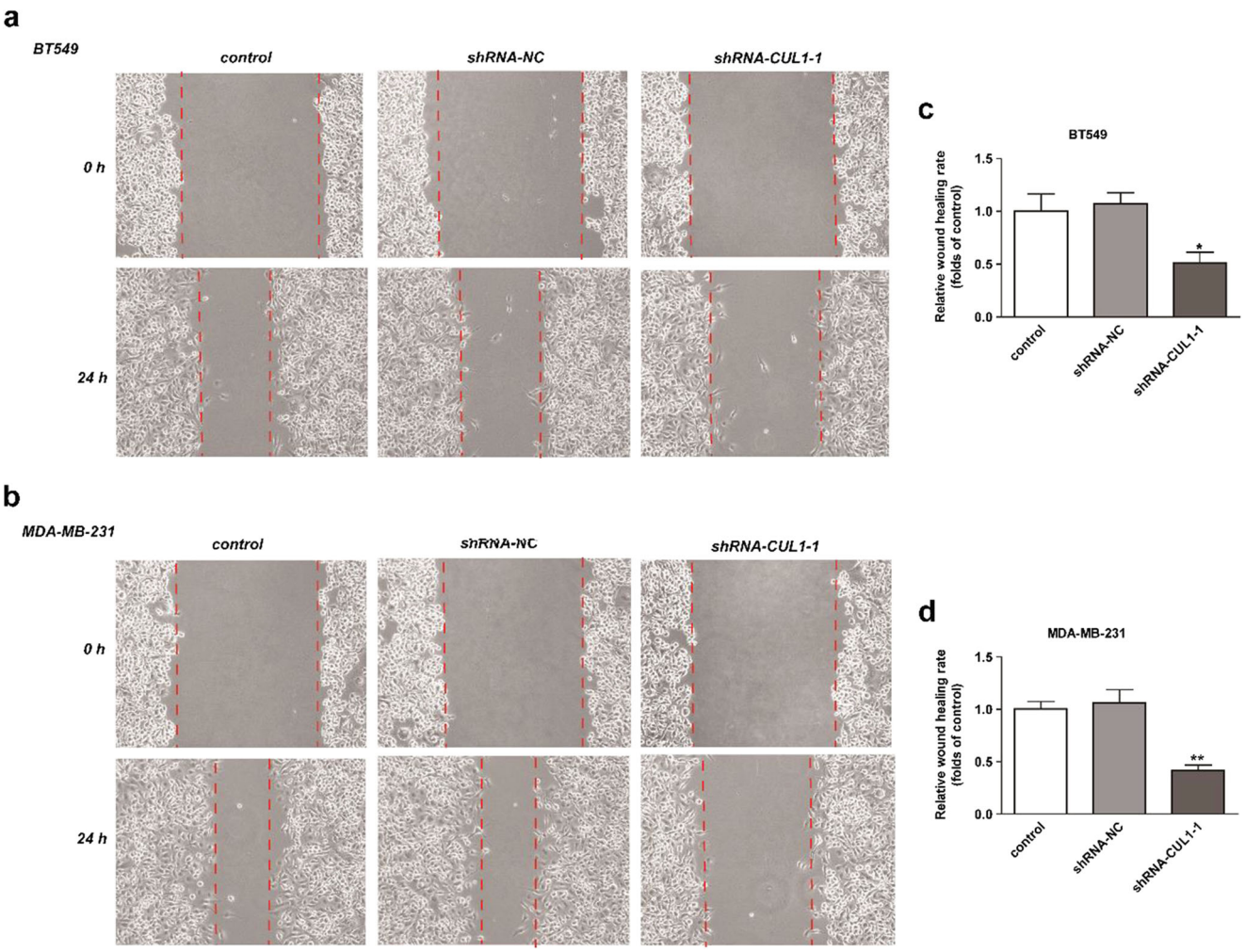

Fig. 5 Knocking-down of CUL1 inhibited wound healing in breast cells. BT549 and MDA-MB-231 cells were transfected with shRNA-NC or shRNA-CUL1. Movement of BT549 (**a**) and MDA-MB-231 (**b**) cells into the wound area were shown at 0 and 24 h. The lines indicated the boundary lines of scratch. Cell migration was assessed by recovery of the scratch area (**c**, **d**). Data were shown as the mean ± SD from three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, vs. control group

Knocking-Down of CUL1 Limits the Invasion of TNBC Cells

The cellular invasive activity was estimated by Transwell invasion assays in the CUL1-knocked-down cells. CUL1 knocking-down significantly prevented the cellular invasion activities of BT549 cells (68%) and MDA-MB-231cells (59%), respectively, compared with the control group ($P < 0.01$, Fig. 4). The control group and shRNA-NC group showed no differences.

Knocking-Down of CUL1 Restricts Wound Healing in TNBC Cells

An important property of tumor cells is their high migratory ability. To explore the inhibitive effects of cellular migration by CUL1 knocking-down, wound healing assays were performed in BT549 cells and MDA-MB-231 cells. CUL1 knocking-down significantly inhibited the wound healing abilities of the BT549 (56%) and MDA-MB-231(65%) cells as compared with the control group ($P < 0.05$ and 0.01, respectively; Fig. 5). The control group and shRNA-NC group showed no differences.

Knocking-Down of CUL1 Alters the EMT-Related Markers in TNBC Cells

Next, we examined the effects of CUL1 silencing on the EMT in the BT549 and MDA-MB 231 cells. We studied the expression of EMT-related proteins (Fig. 6). Next, we examined the effects of CUL1 silencing-induces on the EMT in the BT549 and MDA-MB-231 cells. Western blot analysis showed that silencing of CUL1 suppressed the expression of transcription factors including Snail, Slug, ZEB1, ZEB2, Twist1, and Twist 2 in both BT549 and MDA-MB-231 cells compared with the control group ($P < 0.05$ or $P < 0.01$).

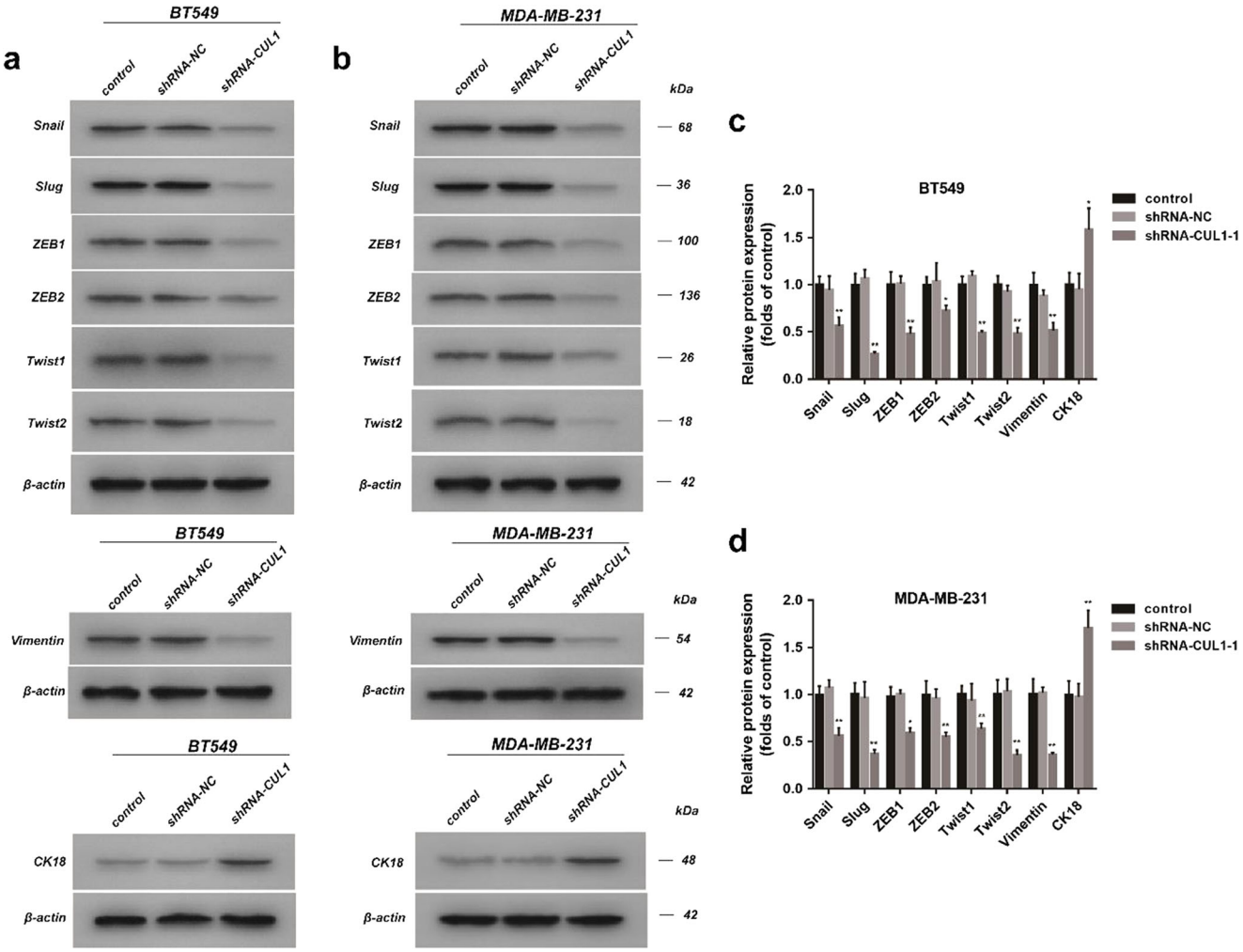

Fig. 6 CUL1 knocking-down weakened EMT in BT549 and MDA-MB-231 cells. After transfection with shRNA-CUL1, the expression of Snail, Slug, ZEB1, ZEB2, Twist1, Twist2, Vimentin, and CK18 was detected in BT549 and MDA-MB-231 cells by Western blot. The corresponding bands are shown in (**a**, **b**, **c**, **d**). The levels of Snail, Slug, ZEB1, ZEB2, Twist1, Twist2, Vimentin, and CK18 were normalized to control. The result was presented as mean ± SD (n = 3). $^{*}P < 0.05$, $^{**}P < 0.01$, vs. control group

Moreover, the silencing of CUL1 suppressed the mesenchymal proteins Vimentin and upregulated the epithelial marker CK18 ($P < 0.05$ or $P < 0.01$) compared with the control group in both cell lines. The control group and shRNA-NC group showed no differences.

Knocking-Down of CUL1 Modulates the Akt/GSK3β/β-Catenin Pathway in TNBC Cells

Akt/GSK3β/β-catenin pathway activation was previously shown to induce EMT and cytoskeleton reconstruction, ultimately leading to the enhanced invasiveness of cancer cells [15]. In the present study, we examined the cause-effect connections of the Akt/GSK3β/β-catenin pathway and CUL1 knock-down by analysis of the phosphorylation status of Akt and GSK3β, and the level of cytoplasmic and nuclear β-catenin expression. Compared to the control group, the phosphorylation of Akt and GSK3β were significantly attenuated, the expression of cytoplasmic β-catenin was increased, as well as the nuclear β-catenin. However, the total β-catenin levels were suppressed by CLU1 knockdown in BT549 and MDA-MB-231 cells ($P < 0.05$, Fig. 7). The control group and shRNA-NC group showed no differences.

CUL1 Knocking-Down Suppresses the Phosphorylation of EGFR in the TNBC Cells

EGFR is involved in the regulation of cell growth in breast cancer. The active form, phosphorylated EGFR (pEGFR), can trigger signaling processes to promote cell proliferation, migration, adhesion, angiogenesis, correlating with poor prognosis in

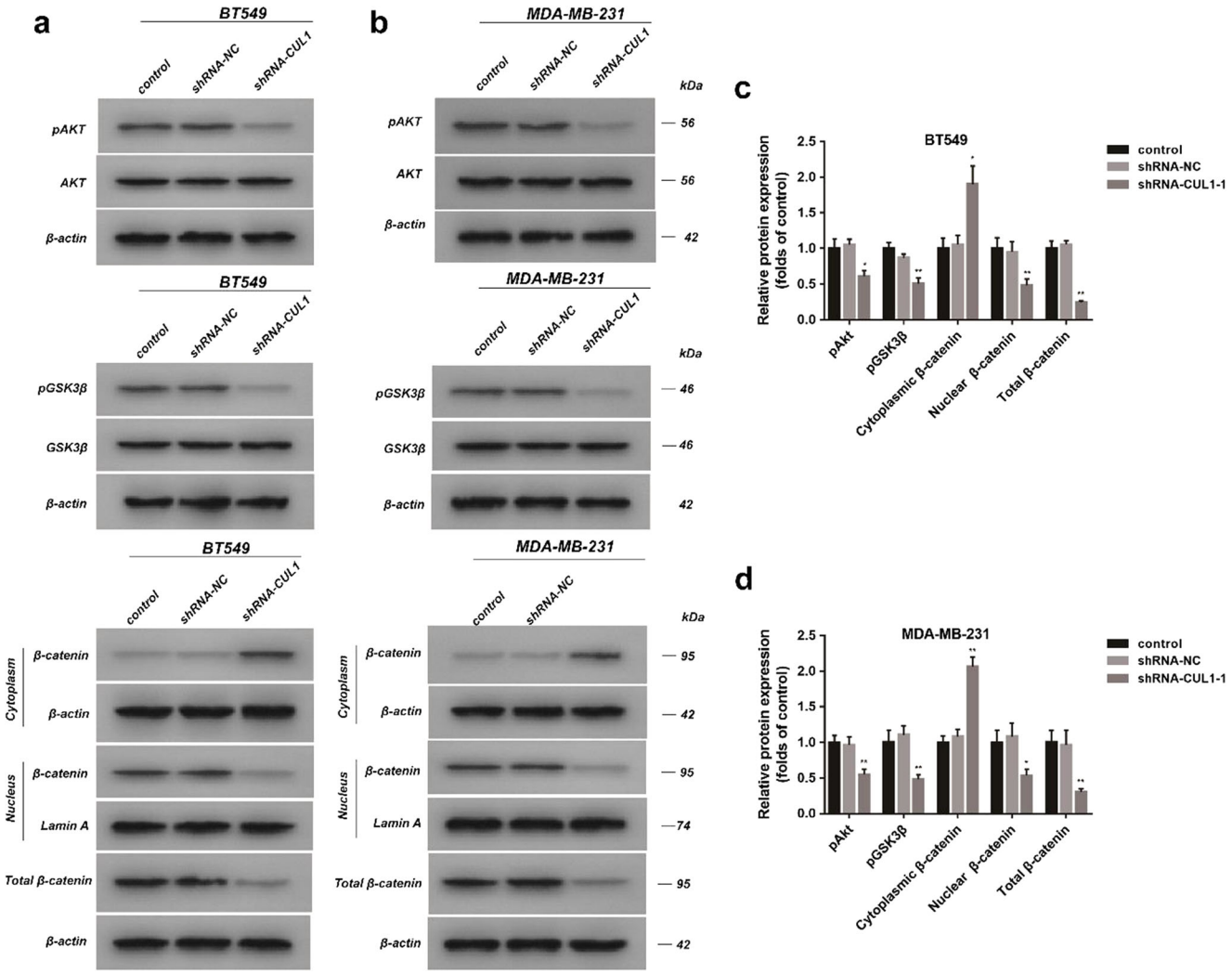

Fig. 7 CUL1 knocking-down modulated Akt/GSK3β/β-catenin pathway in BT549 and MDA-MB-231 cells. After transfection with shRNA-CUL1, the levels of phosphorylated Akt and GSK3β, cytoplasmic and nuclear β-catenin expression, and total β-catenin levels were detected in the BT549 and MDA-MB-231 cells by Western blot. Representative bands were shown in (**a**, **b**, **c**, **d**). Protein expression was normalized to the control. The result was presented as mean ± SD (n = 3). $^{*}P < 0.05$, $^{**}P < 0.01$, vs. control group

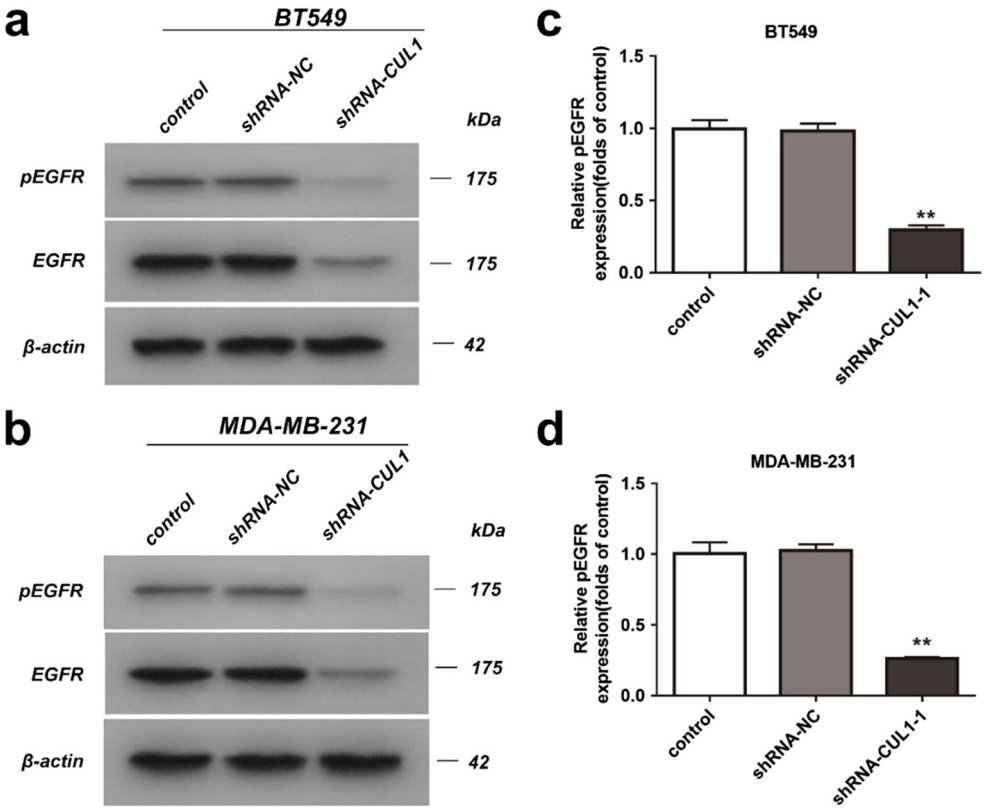

Fig. 8 Effect of CUL1 knocking-down on the phosphorylation of EGFR in BT549 and MDA-MB-231 cells. After transfection with shRNA-CUL1, the levels of phosphorylated EGFR in the BT549 and the MDA-MB-231 cells were detected by Western blot. Representative bands were shown in (**a**, **b**). The levels of phosphorylated EGFR were also normalized to control (**c**, **d**). The results were presented as mean ± SD (n = 3). $^{**}P < 0.01$, vs. control group

breast cancer [22, 23]. Our study showed that EGFR phosphorylation was significantly suppressed by CLU1 knocking-down in BT549 and MDA-MB-231 cells ($P < 0.01$; Fig. 8) after transfected CUL1–1 shRNA, compared to the control group. The control group and shRNA-NC group had no differences.

Discussion

CUL1 has been reported as a novel marker for cancer prognosis, which could be a potential therapeutic target protein for breast cancer [10]. Given that TNBC subtype (ER negative, PR negative and HER2 negative) has the poorer outcomes than other breast cancer subtypes [2], we explored the mechanism of CUL1 in TNBC progression. Our results have demonstrated that silencing of CUL1 regulates EMT through dephosphorylation of EGFR and modulation of the Akt/GSK3β/β-catenin pathways to prohibit cellular metastases in TNBC cells. A limitation of this study is that only one pathway of EMT was investigated. Thus, the complex mechanisms of CUL1 in TNBC require further exploration in the future.

It has been shown that EMT plays a vital role in breast cancer metastasis, especially in the most aggressive and lethal TNBC subtype [24, 25]. EMT is regulated by several transcription factors, including zinc-finger proteins (Snail1/2 and ZEB1/2) and basic helix-loop-helix proteins (Twist1/2). Overexpression of these factors can trigger EMT, resulting in enhanced cell adhesion, invasiveness, and migration of breast cancer cells [26–28]. Alteration in the invasion and migration abilities was reflected by morphological changes. The cuboidal shaped epithelial cells often become more elongated through modifications to the actin-myosin cytoskeleton. This transformation is usually associated with the downregulation of epithelial proteins, such as E-Cadherin and CK18, concordant with the upregulation of mesenchymal proteins, such as N-cadherin and Vimentin [29]. Such changes promote invasion and migration, and could facilitate the TNBC metastasis [25]. In this study, we demonstrated that silencing of CUL1 significantly restrained the EMT process by decreasing the expression of Snail1/2, ZEB1/2, Twist1/2, and Vimentin, while increasing the expression of CK18, which strongly indicates that EMT plays an important role in CUL1-induced TNBC metastases.

GSK3β is a downstream effector of Akt, and a vital component of the destruction complex that facilitates the phosphorylation of β-catenin at Ser33/34/Thr41 residues [30, 31]. The Akt/GSK3β/β-Catenin cascade has been reported to play a fundamental role in inducing expression of ZEB1 and other EMT-related genes in breast cancer [32]. Here, we found that silencing of CUL1 can suppress the phosphorylation of Akt (Ser473), restrain the phosphorylation of downstream GSK3β (Ser9) and the expression of total β-catenin, and alter the expression of EMT related markers in TNBC. We also found that the CUL1 knockdown prevented the

translocation of β-catenin from the cytoplasm to the nucleus in TNBC. Studies have reported that the accumulation of nuclear β-catenin is one of the hallmarks of activated β-catenin signaling in TNBC [33]. This activation is reported to be regulated by Akt-PP2A through phosphorylation of β-catenin at Ser552 and Ser675 [34]. Altogether, these results indicate that CUL1 promotes TNBC cell invasion through the Akt/GSK3β/β-catenin pathway by triggering the EMT process.

EGFR is a tyrosine kinase receptor that belongs to the ErbB receptor family, and is activated by the extracellular binding of several ligands, such as epidermal growth factor (EGF) and transforming growth factor-α (TGF-α) [35]. EGFR auto-phosphorylation can induce oncogenic signaling in TNBC, such as mitogen-activated protein kinases (MPAK) and Akt pathways [36]. Studies have reported that PI3K-Akt survival cascade can be stimulated by EGFR phosphorylation in a Ras-independent manner and can affect the EMT in a variety of ways to influence tumor aggressiveness [37]. In the present work, we detected the suppressed phosphorylation levels of EGFR at Tyr1068 in the BT549 and MDA-MB-231 cells induced by CUL1 knockdown. Phosphorylation of EGFR at Tyr1068 is reported to result in receptor dimerization, recruitment of Grb2 and subsequent activation of the PI3K-Akt signaling pathway [38]. Thus, we suspected that there could be a connection to EGFR and PI3K-Akt signal pathway induced by CUL1 in EMT. We believe that this explanation is helpful to understand the mechanism by which CUL1 regulates endothelial cell adhesion, invasion, and migration in TNBC.

Conclusion

CUL1 knockdown significantly changed cell morphology, decreased cell adhesion, invasion and migration abilities in both MDA-MB-231 and BT549 breast cancer cells. At the molecular level, these alterations occurred through EGFR dephosphorylation and regulation of the Akt/GSK3β/β-catenin signaling pathway to depress the EMT process in TNBC cells. Our study revealed that CUL1 is an oncogene and the targeting of this gene may provide a new way to treat TNBC.

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Compliance with Ethical Standards

Conflict of Interest No potential conflicts of interest were disclosed.

Ethical Approval None.

Informed Consent None.

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