Molecules and Cells

RhoBTB3 Regulates Proliferation and Invasion of Breast Cancer Cells via Col1a1

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Breast cancer is the leading cause of cancer-related death in women worldwide, despite medical and technological advancements. The RhoBTB family consists of three isoforms: RhoBTB1, RhoBTB2, and RhoBTB3. RhoBTB1 and RhoBTB2 have been proposed as tumor suppressors in breast cancer. However, the roles of RhoBTB3 proteins are unknown in breast cancer. Bioinformatics analysis, including Oncomine, cBioportal, was used to evaluate the potential functions and prognostic values of RhoBTB3 and Col1a1 in breast cancer. qRT-PCR analysis and immunoblotting assay were performed to investigate relevant expression. Functional experiments including proliferation assay, invasion assay, and flow cytometry assay were conducted to determine the role of RhoBTB3 and Col1a1 in breast cancer cells. RhoBTB3 mRNA levels were significantly up-regulated in breast cancer tissues as compared to in adjacent normal tissues. Moreover, RhoBTB3 expression was found to be associated with Col1a1 expression. Decreasing RhoBTB3 expression may lead to decreases in the proliferative and invasive properties of breast cancer cells. Further, Col1a1 knockdown in breast cancer cells limited the proliferative and invasive ability of cancer cells. Knockdown of RhoBTB3 may exert inhibit the proliferation, migration, and metastasis of breast cancer cells by repressing the expression of Col1a1, providing a novel therapeutic strategy for treating breast cancer.

Keywords: Col1a1, human breast cancer, RhoBTB3

INTRODUCTION

Breast cancer is the most common form of cancer and leading cause of cancer-related death for women worldwide (Koh et al., 2020; Waks and Winer, 2019). Previous studies revealed that hormones such as estrogen and progestin as well as genetic mutation and various other molecules can cause malignancy in breast tumors (Anastasiadi et al., 2017; Desreux, 2018; Harbeck and Gnant, 2017; Lu and Pfeffer, 2013). Despite significant advances in cancer prevention and targeted chemotherapy, the incidence of breast cancer and associated mortality continue to increase (Jafari et al., 2018; Odle, 2017; Pucci-Minafra et al., 2008). Therefore, more effective therapeutic targets are required to optimize the clinical management of breast cancer.

The RhoBTB (Rho-related Broad-complex, Tramtrack, and Bric-à-brac) proteins constitute a subfamily of atypical members within the Rho family of small guanosine triphosphatases (GTPases). The RhoBTB family consists of three isoforms: RhoBTB1, RhoBTB2, and RhoBTB3 (Berthold et al., 2008b; Ji and Rivero, 2016). These molecules have a unique domain architecture in which a GTPase domain is followed by a proline-rich region, tandem of 2 BTB domains, and conserved C-terminal region. Two of the three RhoBTB proteins, RhoBTB1 and RhoBTB2, differ substantially from RhoBTB3 (Berthold et al., 2008b; Ji and Rivero, 2016; Woldu et al., 2018). RhoBTB3 is a Golgi- associated protein that is critical in keeping structure of Golgi and homeostasis of cells (Lu and

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Pfeffer, 2013; Zhang et al., 2015). The deficiency of RhoBTB3 could result in accumulation of cellular substrates which is important for cell degradation, causing an instability of cellular homeostasis, thus ultimately leading to cancers (Lu and Pfeffer, 2013; Zhang et al., 2015). The correlation between RhoBTB3 and cell cycle regulation has been reported recently (Lu and Pfeffer, 2013). However, RhoBTB3 precise functions and underlying mechanisms in breast cancer are poorly understood.

The extracellular matrix is an important component of tumor microenvironment and plays critical roles in cancer development and metastasis, in which collagen is the major structural protein (Chen et al., 2019; Martins Cavaco et al., 2020; Nissen et al., 2019). Collagen type I alpha 1 (Col1a1) is reportedly associated with the development of several human cancer. Col1a1 gene encodes the pro-alpha 1 chains of type I collagen whose triple helix comprises two alpha 1 chains and one alpha 2 chain (Ma et al., 2019). Type I collagen is a fibril-forming collagen, and 3D type I collagen-rich culture is a common method to investigate the proliferation and/or metastasis of cancers (Dudley et al., 2014). Col1a1 was reported to be associated with a variety of cancers, and its overexpression was observed in tissues and cells of breast, lung, and renal cancers (Chen et al., 2019; Fang et al., 2019; Li et al., 2016; Ma et al., 2019; Nissen et al., 2019; Shi et al., 2019; Zhang et al., 2018). Moreover, increased collagen deposition is associated with breast cancer cell proliferation and invasion (Liu et al., 2018; Slocum and Germain, 2019; Zhu et al., 2015). However, the regulatory mechanism of Col1a1 in breast cancer remains unclear despite numerous recent investigations in human oncology.

In this study, we examined whether RhoBTB3 regulates collagen synthesis and secretion in breast cancer. We show that increased mRNA levels of RhoBTB3 and Col1a1 are associated with poor prognosis of patients with breast cancer. Knockdown of RhoBTB3 regulates breast cancer cell proliferation and invasion, accompanied by reduced Col1a1. Furthermore, expression of RhoBTB3 and Col1a1 is significantly correlated in breast cancer. These results suggest that RhoBTB3 regulates breast cancer progression by controlling collagen deposition and may serve as a therapeutic target for breast cancer.

MATERIALS AND METHODS

Plasmid and siRNA

Col1a1 ORF-containing plasmid pECFP-N2-Col1a1 was purchased from Addgene (USA; plasmid 66603). The siGENOME SMART pools siRNA used to target human RhoBTB3 (M-010224-02), human Col1a1 (M-015890-02), and the non-targeting siRNA control (D-001206-13-05) were all purchased from Dharmacon (USA). The individual siRNA used to target human RhoBTB3 (#1:5'-CUGUGUUAGUACAACUGAA-3', #2:5'-CUGACAUCAUUGUGAUCAA-3'), human Col1a1 (#1:5'-CACCAAUCACCUGCGUACA-3', #2:5'-CUGUUCUGUUCCUUGA-3') and the non-targeting siRNA control (5'-CCUACGCCACCAAUUUCGU-3') were all purchased from Bioneer (Korea).

Cell culture and transfection

Human breast cancer cell line (MDA-MB-231, MDA-MB-468, and HCC1143) was purchased from American Type Culture Collection (ATCC, USA). Cells were maintained in high-glucose Dulbecco's modified Eagle medium (DMEM; Hyclone, USA) supplemented with 10% fetal bovine serum (FBS; HyClone) and 1% penicillin–streptomycin at 37°C in a humidified 5% CO_2 incubator. Plasmid DNA and siRNA were transfected using Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer's protocol.

Immunoblotting

For immunoblotting analysis, control and siRhoBTB3 cells were trypsinized and counted; equal amounts of conditioned medium (normalized to cell number) were precipitated using PEG8000 (Promega, USA). Equal amounts of protein were electrophoresed on 6%-10% SDS-PAGE, transferred to PVDF membranes (Millipore, USA) and probed with Anti-Collagen Alpha-1(I) Chain Carboxy-Telopeptide antibody (LF-68, 1:3,000; Kerafast, USA).

Immunofluorescence microscopy

Cells were seeded in a 6-well plate with acid-washed glass coverslips. After 24 h, the cells were fixed with 4% paraformaldehyde in DPBS for 30 min, washed five times with phosphate-buffered saline (PBS), and permeabilized with 0.1% Triton X-100 in DPBS for 15 min at room temperature (RT) or 25°C. Thereafter, the cells were incubated with blocking buffer (0.5% bovine serum albumin [BSA] in PBS) for 30 min at RT, followed by 1 h incubation at RT with rabbit anti-LF68 (1:500; prepared in 0.5% BSA in DPBS) and Alexa Fluor 488 goat anti-rabbit IgG secondary antibody (1:500; Invitrogen). The cells were subsequently subjected to five washes with PBS. Images were obtained using Axio Observer Z1 fluorescence microscope (Carl Zeiss, Germany) and merged using the Zeiss Zen 2.3 software.

Real-time polymerase chain reaction (RT-PCR) with reverse Transcription

Total RNA was isolated using RNeasy Mini Kit (Qiagen, USA) according to the manufacturer's protocol. Reverse transcription was performed using 1 mg of the total RNA as template and Super-Script™ III Reverse Transcriptase (Invitrogen). Quantitative RT-PCR (qRT-PCR) was performed in triplicates on a LightCycler 480 (Roche, Switzerland) using LightCycler480 SYBR Green I Master Mix (Roche) and the following primers: human Col1a1, 5′-GTGTTGTGCGSTGSCG-3′ and 5′-TCGGTGGGTGACTCT-3′; human RhoBTB3, 5′-CTGGTGTATCTTTAGGTGGTG-3′ and 5′-GTGCTGGTGGGATGTTG-3′; human GAPDH, 5′-CTCCTCCACCTTTGACGC-3′ and 5′-CCACCACCCTGTTGCTGT-3′. Expression of the housekeeping geneGAPDH was used to normalize the data.

Cell cycle analysis

Cells were harvested, resuspended, and fixed with 70% (v/v) ice-cold ethanol overnight. Fixed cells were washed twice with ice-cold PBS and centrifuged at 300 × *g* for 5 min. The cells were then incubated for 30 min at RT with staining solution (0.1 mg/ml RNase A, 50 μg/ml propidium iodide).

Samples were analyzed using an FACS Calibur cytometer (BD Biosciences, USA).

Proliferation assay

Cells were transfected with siRhoBTB3, siCol1a1, and/or Col1a1 plasmid DNA for 24 h, trypsinized, and resuspended in medium. After 48 h, the cells were seeded in 96-well plates at a density of 4×10^3 cells/well. After 72 h of treatment, a mixture of CyQUANT NF Cell proliferation dye reagent and deliverer (Invitrogen) was added to the wells, and the plates were incubated at 37°C for 30 min. Fluorescence intensity was measured as the ratio of fluorescence at 530 nm to that at 485 nm.

Invasion assay

Transwell chambers (Corning, USA) were coated with Matrigel Basement Membrane Matrix (BD Biosciences). Cells were suspended in serum-free medium and seeded in the upper chamber at a density of 2×10^3 cells/well, whereas serum-containing medium was placed in the lower chamber. After incubating for 24 h, the cells penetrating through the pores were stained with Diff-Quik staining solution (Sysmex, Japan) and observed under a microscope.

Statistical analyses

To assess expression levels of RhoBTB3 and Col1a1 in human breast cancer, we retrieved data profiles from Oncomine (https://www.oncomine.com). We analyzed the expression levels in invasive breast carcinoma, invasive ductal breast carcinoma, and ductal breast carcinoma *in situ* epithelia. We used cBioPortal (Breast Invasive Carcinoma, TCGA, [Cancer Genome Atlas Network, 2012]) to analyze correlation between the expression levels of RhoBTB3 and Col1a1 (http://www.cbioportal.org). All experiments were repeated at least three times. Results are reported as mean ± SEM. Significance of difference was assessed by independent Student's *t*-test. Value of $P < 0.05$ was considered statistically significant.

RESULTS

Upregulation of RhoBTB3 in breast cancer tissue

To investigate whether the expression levels of RhoBTB3 are associated with breast cancer tissue, we assessed the mRNA expression of RhoBTB3 in TCGA human breast cancer using

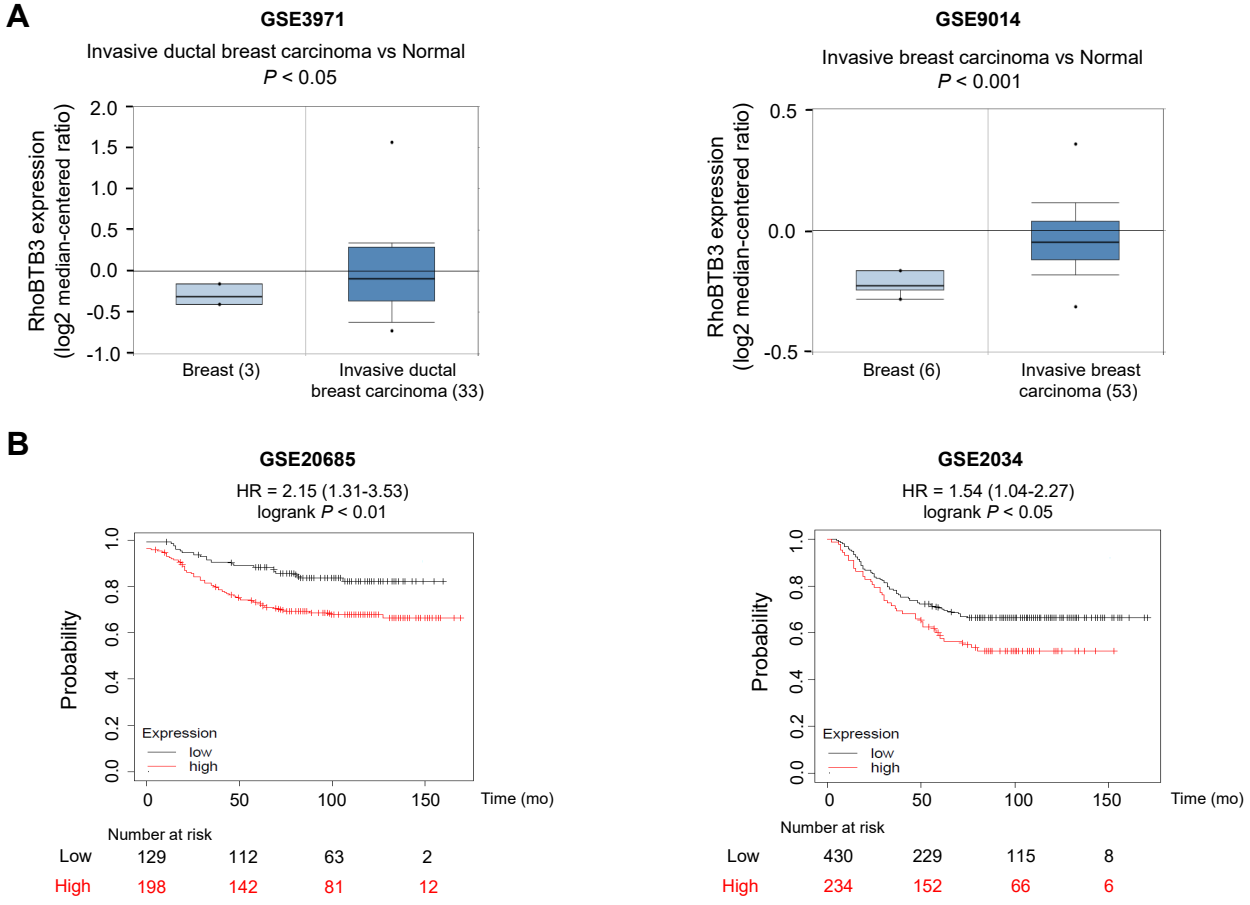

Fig. 1. RhoBTB3 analysis in breast cancer. (A) The box plot comparing specific RhoBTB3 expression in normal (left plot) and cancer tissue (right plot) was derived from the Oncomine database. The analysis was shown in invasive breast carcinoma and invasive ductal breast carcinoma relative to in normal breast tissue. (B) The survival curve comparing patients with high (red) and low (black) expression of RhoBTB3 in breast cancer was plotted from the Kaplan-Meier-plotter (202975_s_at, Split patients by auto select best cutoff).

the Oncomine database. RhoBTB3 expression was significantly upregulated in invasive breast carcinoma ($P < 0.05$) and invasive ductal breast carcinoma ($P < 0.001$) as compared to in normal breast tissue, indicating that RhoBTB3 is an oncogene in breast cancer (Fig. 1A). Furthermore, we examined whether RhoBTB3 expression is associated with clinical outcomes in patients with human breast cancer. Kaplan–Meier survival analysis showed that breast cancer patients with high RhoBTB3 expression had significant shorter relapse-free survival (Fig. 1B). Thus, our systematic analysis based on a bioinformatics database may help researchers determine the role of RhoBTB3 in breast cancer and can be targeted as potential oncogenic markers for breast cancer treatment.

RhoBTB3 modulates breast cancer cell growth and invasion

To assess the role of RhoBTB3 in regulating the proliferation and invasive ability of breast cancer cells, breast cancer cells, MDA-MB-231, MDA-MB-468, and HCC1143 were transfected with sicon and siRhoBTB3 as demonstrated by qPCR and Western blot analysis (Fig. 2A, Supplementary Figs. S1A and S2A). The cell proliferation assay showed that knocked-down of RhoBTB3 significantly decreased in breast cancer cells as compared to in control cells (Fig. 2B). In addition, we examined whether RhoBTB3 affects the invasive ability of breast cancer cells. As shown in Fig. 2C, the invasive ability of RhoBTB3 knockdown cells was significantly reduced as compared to that of control cells.

Since RhoBTB3 can effectively inhibit the growth of human breast cancer cells, we expected that this inhibitory activity was due to its ability to interfere with cell cycle progression. Therefore, we investigated the cell cycle phase after transfection with siCon and siRhoBTB3 using flow cytometry. Quantitative data revealed that deficient of RhoBTB3 cells had reduced distribution in the G0/G1 phase but increased their distribution in both S and G2/M phases (Fig. 2D). These results suggest that the proliferation-promoting function of RhoBTB3 is mediated by promoting S and G2/M phase transitions in breast cancer cells. We obtained similar results using individual siRhoBTB3. (Supplementary Fig. S3).

Col1a1 is high expressed in breast cancer tissue, while knockdown of Col1a1 inhibit breast cancer cell growth and invasion

Similar to RhoBTB3, we retrieved the expression profiles of Col1a1 in human breast cancer (Oncomine database). Col1a1 was found to be upregulated in breast cancer tissues compared to in normal tissues. These data are consistent with those of previously published studies on Col1a1 expression in breast cancer cells (Fig. 3A). Furthermore, Kaplan–Meier survival analysis showed that patients with breast cancer with high RhoBTB3 expression had significant shorter relapse-free

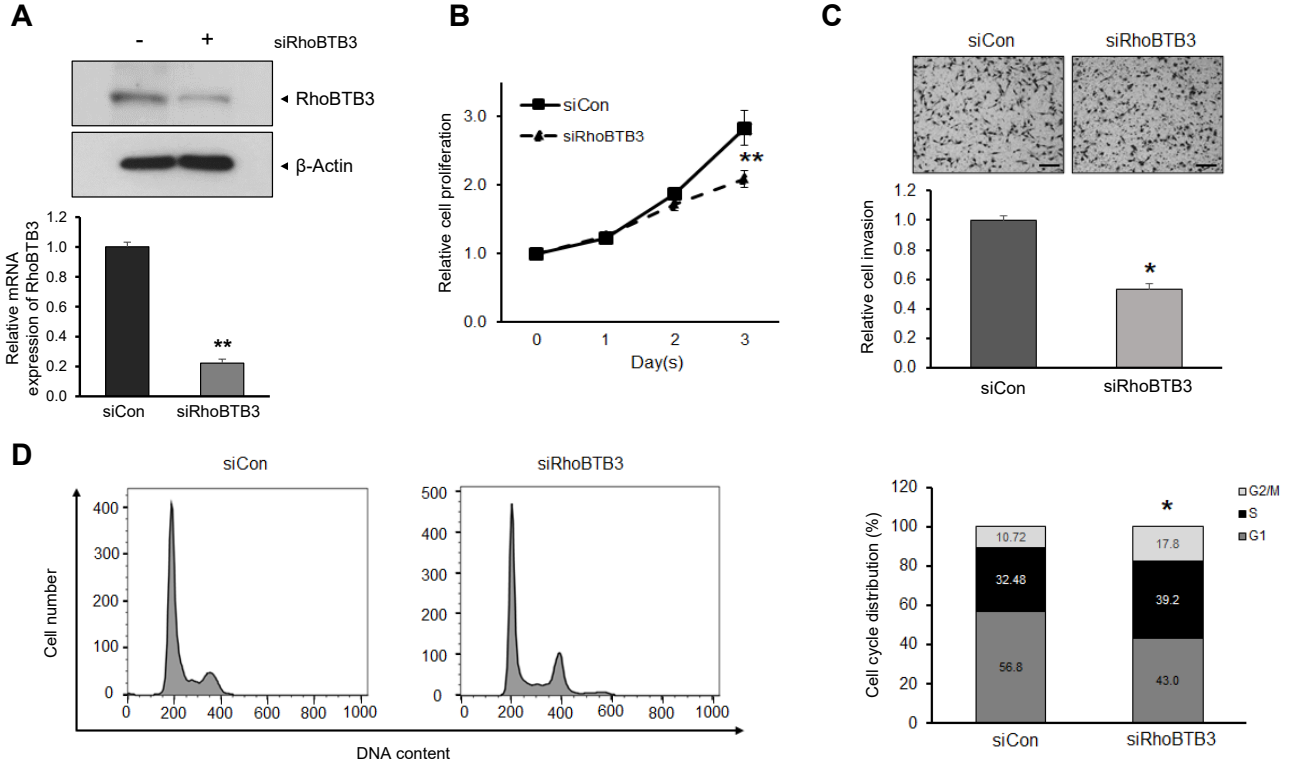

Fig. 2. Loss of RhoBTB3 suppresses growth and invasion of MDA-MB-231cells. (A) qRT-PCR analysis of RhoBTB3 expression after RhoBTB3 knockdown (Smart-pool siRNA). (B) Cell proliferation was assessed at the indicated times by CyQUANT NF Cell Proliferation Assay Kit. (C) The effects of RhoBTB3 knockdown (Smart-pool siRNA) in breast cancer cells on cell invasion were analyzed by Matrigel-coated Transwell invasion analyses. Scale bars = 200 μm. (D) Cell cycle progression was measured using flow cytometry after RhoBTB3 knockdown. Data are representative of three independent experiments. Error bars represent ± SEM. $*P < 0.05$, $**P < 0.01$.

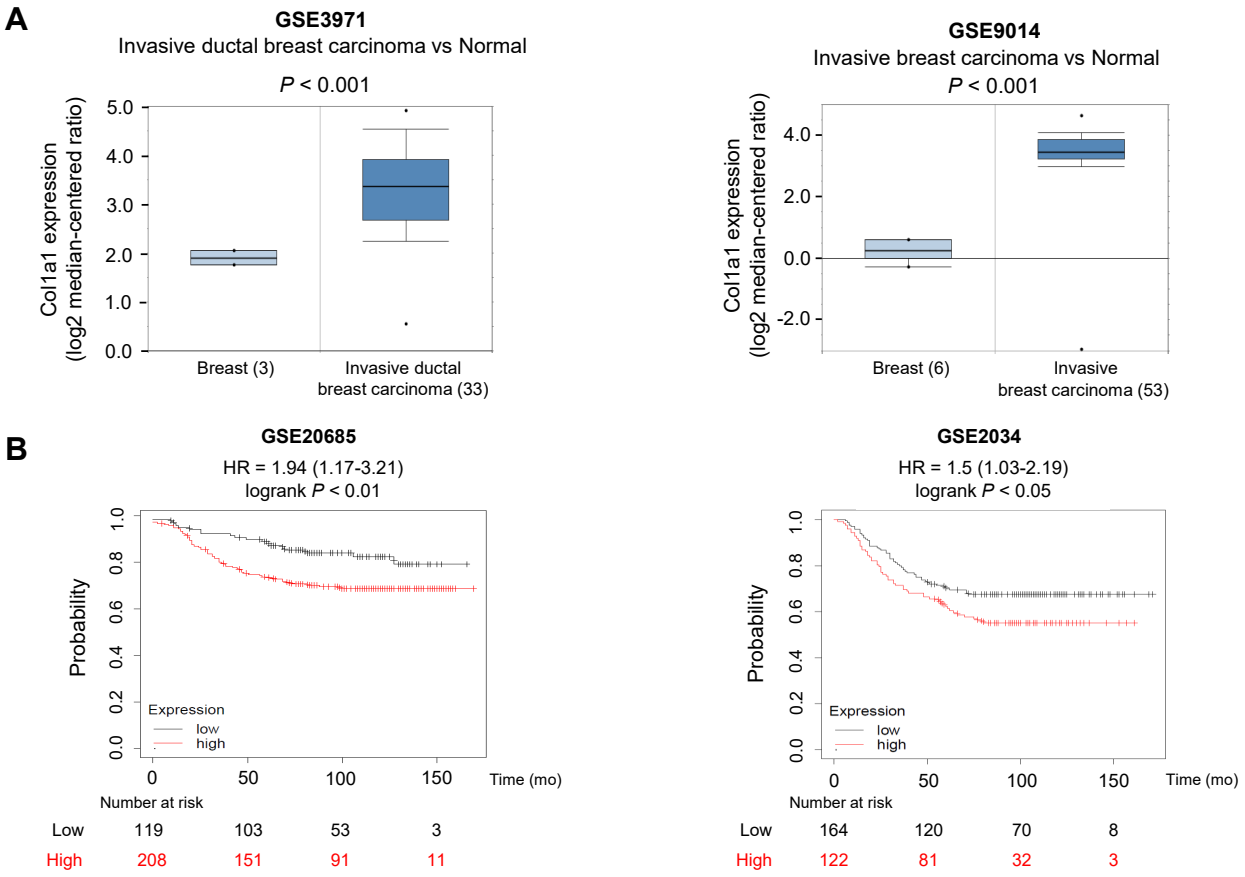

Fig. 3. RhoBTB3 is associated with Col1a1 expression in MDA-MB-231cells. (A) The boxplot comparing specific Col1a1 expression in normal (left plot) and cancer tissue (right plot) was derived from the Oncomine database. The analysis was shown in invasive breast carcinoma and invasive ductal breast carcinoma relative to in normal breast tissue. (B) The survival curve comparing patients with high (red) and low (black) expression of Col1a1 in breast cancer was plotted from the Kaplan-Meier-plotter (202311_s_at, Split patients by auto select best cutoff).

survival (Fig. 3B). These results may help researchers determine the role of Col1a1 in breast cancer and identify potential oncogenic markers for breast cancer treatment.

RhoBTB3 reduces breast cancer cell growth and invasion by down regulating Col1a1

We investigated whether RhoBTB3 regulates proliferation and invasion of breast cancer cells via Col1a1. RhoBTB3 interference models were constructed. As shown in Fig. 4A, Col1a1 expression in breast cancer cells was decreased significantly following transfection with siCol1a1. To identify the effects of Col1a1 on the proliferation and invasion of breast cancer cells, we performed various assays. The proliferation assay revealed that knockdown of Col1a1 significantly decreased the growth rate of breast cancer cells (Fig. 4B). Additionally, reduced invasive ability was observed in Col1a1 knockdown cells as compared to in control cells. The number of invaded Col1a1 knockdown cells decreased to 60% of that of control cells when MDA-MB-231 cells were used (Fig. 4C). Furthermore, we examined the effect of RhoBTB3 depletion on collagen synthesis. Interestingly, qPCR and immunoblotting analysis showed that knockdown of RhoBTB3 in breast cancer cells reduced mRNA and secreted Col1a1 levels (Figs. 4D and 4E, Supplementary Figs. S1D-S1E and S2D-S2E). In addition, cytoplasmic levels of Col1a1 were reduced in RhoBTB3-silenced MDA-MB-231 cells compared to in control cells, as indicated via immunofluorescence microscopy (Fig. 4F). We obtained similar results using individual siRhoBTB3 and siCol1a1 (Supplementary Fig. S4).

Indeed, RhoBTB3 inhibition led to decreased cell proliferation as compared to that of control cells, which was rescued upon Col1a1 overexpression (Fig. 5A, Supplementary Figs. S1B and S2B). Consistent with the proliferation data, RhoBTB3-depleted cells exhibited a reduced invasive ability as compared to control cells. This reduction was rescued by simultaneous Col1a1 overexpression (Fig. 5B, Supplementary Figs. S1C and S2C). These results suggest that RhoBTB3 promotes cancer growth and invasion by regulating collagen deposition. Notably, expression of RhoBTB3 correlated positively with the expression of Col1a1 according to analysis using cBioPortal (Breast Invasive Carcinoma, TCGA) (Fig. 5C). In summary, these data indicate that RhoBTB3 expression plays a critical role in human breast cancer, possibly by modulating collagen-associated cancer development and progression.

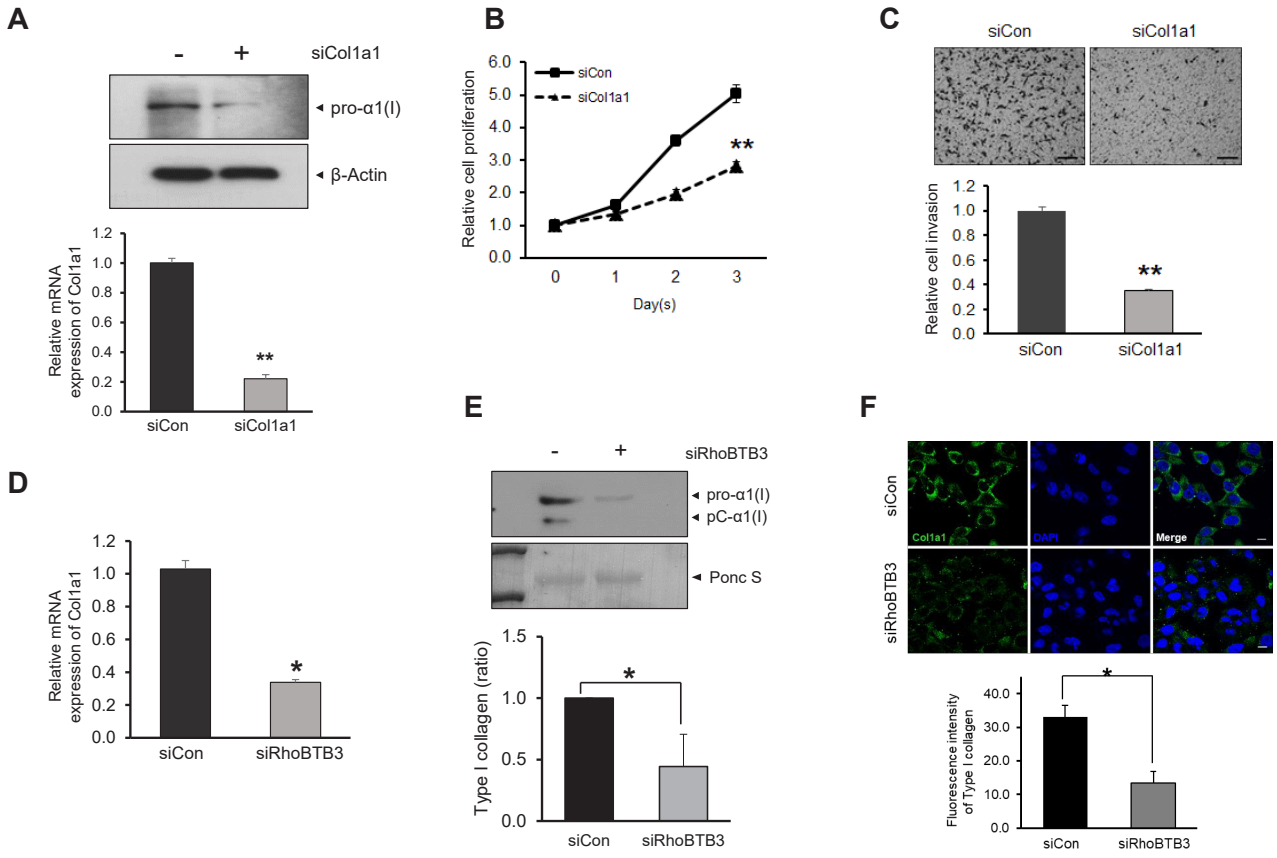

Fig. 4. RhoBTB3 is associated with collagen expression in MDA-MB-231cells. (A) qRT-PCR analysis of Col1a1 expression after Col1a1 knockdown (Smart-pool siRNA). (B) Cell proliferation was assessed at the indicated times by CyQUANT NF Cell Proliferation Assay Kit. (C) The effects of Col1a1 knockdown (Smart-pool siRNA) in breast cancer cells on cell invasion were analyzed by Matrigel-coated Transwell invasion analyses. Scale bars = 200 μm. (D) qRT-PCR analysis of Col1a1 expression after RhoBTB3 knockdown (Smart-pool siRNA). (E) Immunoblot analysis of Col1a1 in conditioned medium after RhoBTB3 knockdown (Smart-pool siRNA). (F) Immunofluorescence staining of Col1a1 (green) and DAPI (blue) after RhoBTB3 knockdown (Smart-pool siRNA). Error bars represent ± SEM. $*P < 0.05$, $**P < 0.01$. Scale bars = 10 μm.

DISCUSSION

Genes encoding RhoBTB proteins exhibit ubiquitous but tissue-differential expression (Berthold et al., 2008b; Ji and Rivero, 2016; Nguyen et al., 2020). RhoBTB1 and RhoBTB2 have been regarded as tumor suppressors. Their expression is significantly decreased in breast cancer tissues as compared to normal tissues (Choi et al., 2017; McKinnon and Mellor, 2017; St-Pierre et al., 2004). McKinnon and Mellor (2017) reported that loss of RhoBTB1 in developing cancer reduces METTL7B expression, thereby promoting the loss of normal epithelial polarity through reduced METTL7B expression and contributes to the switch to an invasive phenotype. RhoBTB2 (also known as deleted in breast cancer, DBC2) was proposed as a candidate tumor suppressor gene, as its expression in breast cancer cells lacking RhoBTB2 transcripts caused growth inhibition (Choi et al., 2017; St-Pierre et al., 2004). The structural differences between RhoBTB1/2 and RhoBTB3 may have led to these contradictory results. The RhoBTB family has a common domain architecture. RhoBTB1 and RhoBTB2 are highly similar, whereas RhoBTB3 is the most divergent member (Aspenström et al., 2004; 2007; Berthold et al., 2008b; Espinosa et al., 2009). Interestingly, the RhoGTPase domain of RhoBTB3 binds and hydrolyses ATP, whereas that of RhoBTB1/2 binds GTP (Aspenström et al., 2004; 2007; Berthold et al., 2008a; Nguyen et al., 2020). Moreover, only RhoBTB3 bears an isoprenylation CAAX motif that is typical of classical RhoGTPases in the C-terminal region (Ji and Rivero, 2016; Junaid et al., 2014; Manolaridis et al., 2013). The CAAX motif is widely involved in global cellular functions, such as proliferation and differentiation (Boudhraa et al., 2020; Burrows et al., 2009; Manolaridis et al., 2013). As an important modulator of biological activity, signal transduction via protein prenylation is a crucial step for most CAAX motif functions, particularly for anchoring these motifs to the cellular membrane system (Junaid et al., 2014; Manolaridis et al., 2013). There are still not many studies on whether RhoBTB3 has a function as oncogene or tumor suppressor on in various cancers. Recently, Zhang et al. (2015) reported that RhoBTB3 as a novel scaffolding protein for a multi-subunit complex that promotes HIFα degradation under both normoxic and hypoxic conditions in human renal carcinomas. In contrast to the tumor suppressive role of RhoBTB3 in human renal carcinomas, our findings demon-

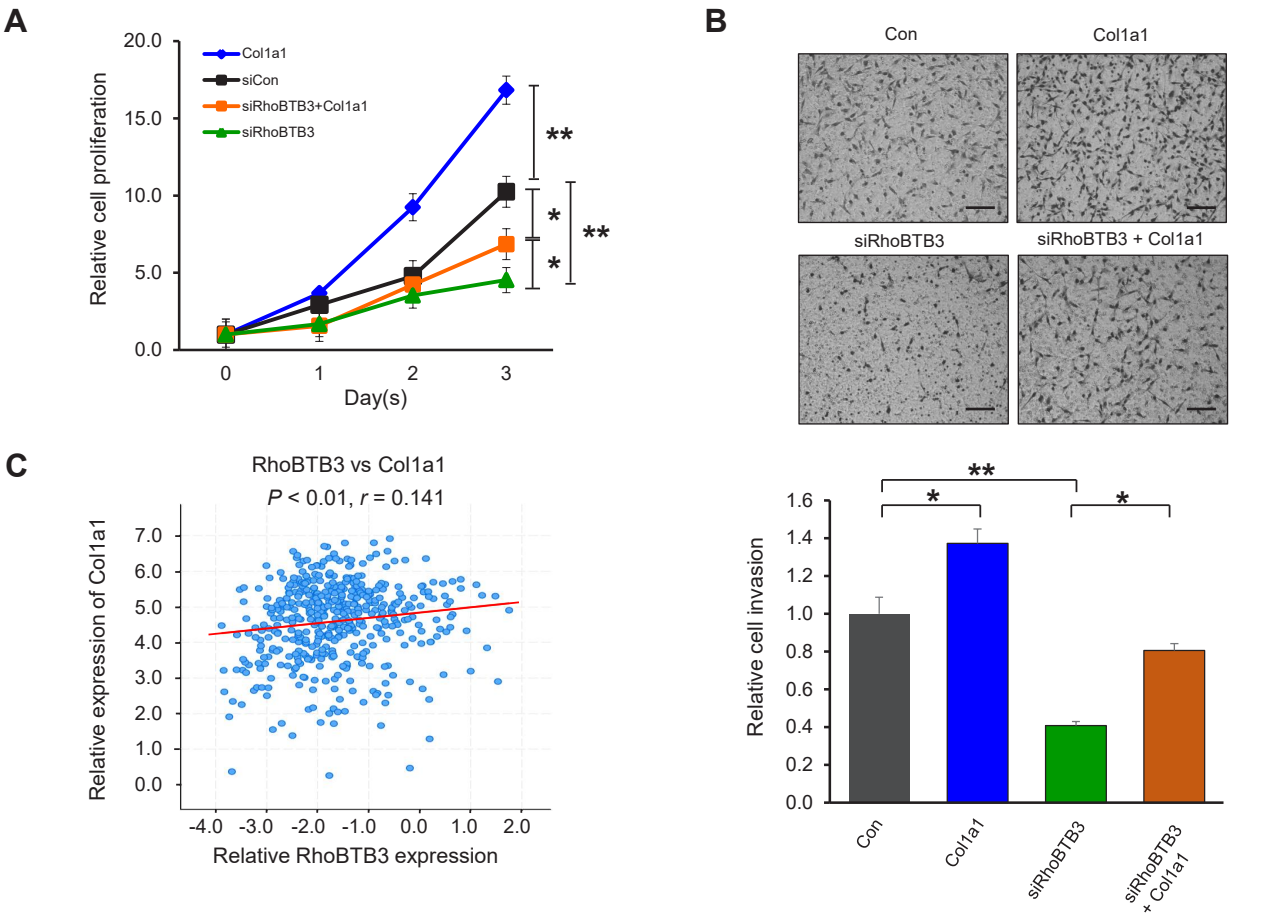

Fig. 5. RhoBTB3 affects proliferation and invasion of MDA-MB-231cells in Col1a1-dependent manner. (A and B) RhoBTB3 knockdown rescued the promotion of proliferation and invasion induced by Col1a1 overexpression in MDA-MB-231cells. Scale bars = 200 μm. (C) Scatterplot of correlated mRNA levels between RhoBTB3 and Col1a1 in normal and malignant breast tissues. Data are representative of three independent experiments. Error bars represent ± SEM. $*P < 0.05$, $**P < 0.01$.

strated an oncogenic role for RhoBTB3 in breast cancers. The function of RhoBTB3 as oncogene or tumor suppressor may appear differently depending on the various cancer types and under different cellular condition.

The cell cycle checkpoints arrest or delay cell cycle progression either in the G1 phase before DNA replication, or in the S phase during DNA replication, or in the G2 phase before mitosis (Akkari et al., 2000; Reichheld et al., 1999). The knockdown of RhoBTB3 cells were arrested in phase S and G2/M because of the deregulation of Cyclin E turnover (Lu and Pfeffer, 2013; Zhang et al., 2015). RhoBTB3 mediates the ubiquitylation of this important cellular cycle regulator, regulating its renewal during the S and G2/M phases an event essential to the progression of the cell cycle.

The Col1a1, a major component of the extracellular matrix in the tumor microenvironment, plays a major role in cancer development and progression (Burns-Cox et al., 2001; Martins Cavaco et al., 2020; Wolf et al., 2009; Xu et al., 2019). It has been reported that Col1a1 is highly expressed in the cytoplasm in breast cancer cells compared to in normal cells (Liu et al., 2018; 2020; Slocum and Germain, 2019). In addition, Liu et al. (2018) demonstrated that downregulation of Col1a1 reduced breast cancer growth and metastasis, whereas its upregulation significantly increased breast cancer proliferation, migration, and invasion. These studies indicate that increased Col1a1 expression promotes breast cancer development and progression by enhancing tumor growth and invasion. However, the regulation of Col1a1 expression in breast cancer cells is not clear.

The Golgi complex plays a central role in processing and sorting of biosynthetic cargo in all eukaryotic cells (Cao et al., 2000; Colanzi et al., 2007). Golgi organization have evolved in part to sense and transduce specific stress signals to the nucleus (Cao et al., 2000; Gurel et al., 2014; Mysior and Simpson, 2021). RhoBTB3 is Golgi associated protein (Mysior and Simpson, 2021). Interestingly, depletion of RhoBTB3 induced strong Golgi fragmentation (Lu and Pfeffer, 2013). Disruption of Golgi architecture and functions, termed as Golgi stress response, alters redox balance and affects cell survivals, contributing to many disorders (Li et al., 2016; Machamer, 2015; Taniguchi and Yoshida, 2017). The synthesis of collagen type I is regulated at the transcriptional and post-translational levels (Laurent, 1987; Siwik et al., 2001). The oxidative stress decreased the levels of mRNA for collagen

type I, indicating that the oxidative stress-stimulated decrease in collagen synthesis was due, at least in part, to a decrease in mRNA transcription or stability (Laurent, 1987; Siwik et al., 2001). Therefore, we speculate that the knockdown of RhoBTB3 constituting the Golgi apparatus causes Golgi fragmentation, which induces oxidative stress and reduces collagen mRNA.

In summary, this study provides *in vitro* evidence of the oncogenic effects of RhoBTB3 mediated by regulating Col1a1 expression in breast cancer. We observed significant upregulation of RhoBTB3 expression in breast cancer tissue specimens compared to that in the corresponding normal tissue specimens. Furthermore, knockdown of RhoBTB3 reduced breast cancer cell proliferative and invasive properties. Additionally, knockdown of Col1a1 in breast cancer cells led to extremely decreased proliferation and invasion, similar to the results obtained for RhoBTB3 downregulation. Therefore, further studies are needed focus on understanding the precise molecular mechanism of RhoBTB3 regulated Col1a1 gene expression in breast cancer cells to explore the potential clinical application of RhoBTB3.

Note: Supplementary information is available on the Molecules and Cells website (www.molcells.org).

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AUTHOR CONTRIBUTIONS

K.K. and Y.-J.K. designed the experiments. K.K. performed the experiments. K.K. and Y.-J.K. wrote the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

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