Regulation of the β-catenin/LEF-1 pathway by the siRNA knockdown of RUVBL1 expression inhibits breast cancer cell proliferation, migration and invasion

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Abstract. RUVBL1 is a protein characterized by its DNA-dependent ATPase activity and DNA deconjugating enzyme function. It is a member of the ATPase (AAA+) protein family associated with various cellular processes. Available research confirms that the expression of RUVBL1 is upregulated in breast cancer (BRCA) cell lines; however, the mechanisms underlying its functional role in BRCA remain unclear. The β-catenin/lymphoid enhancer factor-1 (LEF-1) pathway plays a crucial role in the occurrence and development of BRCA. The aim of the present study was to investigate whether RUVBL1 regulates the proliferation, migration and invasion of BRCA cells by participating in the β-catenin/LEF-1 signaling pathway. Reverse transcription-quantitative PCR (RT-qPCR) and western blot analysis were employed to compare the RUVBL1 expression levels between normal mammary epithelial cells (MCF-10a) and BRCA cell lines (MDA-MB-231 and MCF-7). Scratch, Cell Counting Kit-8 and Transwell assays were utilized to assess the effects of RUVBL1 knockdown on the proliferation, migration and invasion of BRCA cells. Following the downregulation of RUVBL1 expression *in vitro*, western blot analysis and RT-qPCR were conducted to investigate its role in regulating the β-catenin/LEF-1 pathway. The aforementioned experiments proved that the knockdown of RUVBL1 expression inhibited BRCA cell proliferative, migratory and invasive capabilities, modulating the β-catenin/LEF-1 pathway. Collectively, the findings of the present study provide preliminarily confirmation that RUVBL1 participates in the molecular mechanisms of the β-catenin signaling pathway, which may provide a novel target for BRCA treatment.

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Introduction

Breast cancer (BRCA) is the most prevalent malignant tumor affecting the female population worldwide, and its incidence rate continues to increase annually. Additionally, the age of onset is progressively decreasing (1). BRCA treatment encompasses surgery, radiotherapy, chemotherapy, targeted therapy and endocrine therapy. While surgery is a common treatment modality for BRCA, neoadjuvant therapy, comprising of endocrine therapy, combination therapy and targeted therapy, provides improved long-term control over the progression of metastatic BRCA and helps maintain the quality of life of patients (2). Although significant progress has been made in BRCA treatment, tumor resistance and metastasis continue to limit the therapeutic efficacy. Cell signaling pathways play a crucial role in cell proliferation, differentiation and apoptosis. Targeted therapies aimed at these pathways can inhibit the proliferation and differentiation of tumor cells, thereby restraining their growth. Therefore, developing targeted therapeutic strategies against cellular pathways is essential.

The Wnt signaling pathway is an evolutionarily conserved pathway that plays a crucial role in both embryonic development and neoplastic diseases, comprising three main branches: The classical Wnt/β-catenin pathway, the Wnt/PCP pathway (planar cell polarity) and the Wnt/Ca^{2+} pathway. The Wnt pathway has been implicated in various types of human cancer and metastases, including colorectal cancer, hepatocellular carcinoma, oral squamous carcinoma, BRCA, endometrial cancer and hematologic disorders (3-5). In primary oral squamous cell carcinoma, the reduced expression of β-catenin on the cell membrane is associated with a higher likelihood of nuclear metastasis, which is significantly associated with invasion and lymph node metastasis (6). In normal breast tissue, β-catenin is primarily expressed on the cell membrane. However, in BRCA, the activation of the Wnt pathway promotes the nuclear translocation of β-catenin, enhancing signaling. The abnormal expression of β-catenin is associated with Her2 positivity in the Wnt/β-catenin pathway, facilitating invasion and lymph node metastasis (7,8). In summary, the nuclear

translocation of β-catenin enhances tumor invasion and metastasis. Lymphoid enhancer factor-1 (LEF-1), a member of the LEF/TCF family, belongs to the high mobility group. As a key transcription factor, LEF-1 serves as a core component of the Wnt signaling pathway. It has been shown that LEF-1 is highly expressed in BRCA, being associated with the expression of the G1 cell cycle regulator cyclin D1, thus regulating the proliferation of BRCA cells (9). LEF-1 can also bind to estrogen receptor (ER) cis-regulatory elements, inhibiting the binding of ER to chromatin and contributing to BRCA development (10). Furthermore, the abnormal activation of the β-catenin/LEF1 pathway can induce the expression of cyclin D1 and promote tumor transformation in colorectal cancer. The knockdown of LEF-1 effectively blocks the Wnt/β-catenin signaling pathway (11,12). In summary, these studies demonstrate that intervening with the β-catenin/LEF1 signaling pathway may hinder the development of BRCA, establishing it as a key target for targeted therapy. Therefore, upstream and downstream factors of this pathway may represent key targets for intervention therapy and may aid in the development of novel strategies.

RUVBL1 is a protein exhibiting both DNA-dependent ATPase and DNA helicase activities. It belongs to the AAA^+ protein family, which is associated with various cellular activities, including gene regulation, DNA damage repair and chromatin remodeling (13,14). Previous studies have revealed that RUVBL1 is closely linked to the occurrence and progression of cancer. RUVBL1 is overexpressed in several cancers, including colorectal cancer, hepatocellular carcinoma, lung adenocarcinoma, oral squamous cell carcinoma, uveal melanoma, prostate cancer, epithelial ovarian cancer and osteosarcoma. It is involved in several signaling pathways, including the β-catenin/LEF1, NF-κB and PLXNA1-CRAF-MAPK pathways, influencing the occurrence and progression of diseases (15-20). A previous study indicated that the binding reaction between RUVBL1-specific antigens and autoantibodies can serve as a supplement to mammography for diagnosing lymph node-negative early-stage BRCA (21). Additionally, astaxanthin inhibits colony formation, spheroid formation, migration and the invasion of BRCA cells by downregulating RUVBL1 expression (22). However, the mechanism through which the inhibition of RUVBL1 expression affects the occurrence and progression of BRCA remains unclear. Collectively, these findings suggest that RUVBL1 may serve as a novel therapeutic target for BRCA.

The present study identifies RUVBL1 as being positioned at the axis of the β-catenin/LEF-1 pathway through bioinformatics analysis. It is suggested that RUVBL1 may participate in the signal transduction of β-catenin/LEF-1 and is associated with the proliferation, invasion and metastasis of BRCA. Consequently, RUVBL1 may represent a potential therapeutic target within this signaling pathway.

Materials and methods

Cells and cell culture. The normal breast cell line, MCF-10A, and the BRCA cell lines, MDA-MB-231 and MCF-7, were obtained from Wuhan Procell Life Science & Technology Co., Ltd. The MCF-10A cells were cultured in a specialized medium (CM-0525; Wuhan Procell Life Science & Technology Co., Ltd.). The MDA-MB-231 and MCF-7 cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS; cat. no. 164210; Wuhan Procell Life Science & Technology Co., Ltd) and 1% penicillin-streptomycin (cat. no. PB180120; Wuhan Procell Life Science & Technology Co., Ltd). Following incubation in a cell incubator with 5% CO_2 for 48 h, cell density reached 80 to 90%, and the cells were passaged at a ratio of 1:2.

Cell transfection. The MDA-MB-231 and MCF-7 cells were seeded in six-well plates at a density of $2x10^5$ cells per well. Once the cell density reached 30%, small interfering RNA (siRNA)-RUVBL1 (Table I, Guangzhou RiboBio Co., Ltd.) and siRNA-negative control (NC; siN0000001-1-5; Guangzhou RiboBio Co., Ltd.) transfection complexes were prepared according to the instructions provided with the siRNA transfection reagent (Ribotest; cat. no. R10043.8; Guangzhou RiboBio Co., Ltd.). siRNA was transfected at a concentration of 50 nM. Subsequent experiments were performed 24 h after transfection. The knockdown efficiency was verified by mixing the transfection complex with a complete medium (without antibiotics) in the six-well plates, followed by incubation in a CO_2 culture environment at 37˚C for 48 h.

Cell Counting Kit-8 (CCK-8) assay. Cells in the logarithmic growth phase were seeded in 96-well plates at equal densities and cultured for 0, 12, 24, 48 and 96 h. DMEM and CCK-8 solution (cat. no. GK10001; GlpBio) were added to each well in a 9:1 ratio. Following incubation for 1 h at 37˚C, the optical density (OD) of the cells was measured at 450 nm using a microplate reader (Multiskan SkyHigh; Thermo Fisher Scientific, Inc.). The percentage of cell proliferation inhibition was calculated using the following formula: (absorbance of experimental wells/absorbance of control wells) x100%.

Scratch assay. A scratch was made on the surface of the culture plate containing logarithmic phase cells using a sterile 200-μl pipette tip. The cells were then washed twice with PBS and cultured in a serum-free medium. Cell migration was recorded at 0, 12 and 24 h using a light microscope (LEICA DM6 B; Leica Microsystems GmbH), and the scratch area was measured using ImageJ 1.54d software (National Institutes of Health). The cell migration rate was calculated using the following formula: (initial scratch area-final scratch area)/initial scratch area x100%.

Transwell assay. For proliferation experiments, 200 μl serum-free cell suspension at a density of $2x10^4$ cells was added to the upper chamber, and 600 μl medium containing 20% FBS was added to the lower chamber. For the invasion assay, Matrigel (BD Biocoat; cat. no. 356234; Corning, Inc.) was thawed overnight at 4˚C, transferred to an ice box before the experiment, and diluted with serum-free medium at a ratio of 1:8, was applied to the upper surface of the membrane in the Transwell chamber (8.0-μm pore size; cat. no. 3422; Corning, Inc.). The cells were incubated at 37˚C for 3 h to facilitate membrane formation, after which the cell suspension and medium were added. After 24 h, the cells were fixed with 600 μl 4% paraformaldehyde for 30 min. Subsequently, non-penetrated cells and excess Matrigel were removed using a

Table I. Sequences of primers used for reverse transcription-quantitative PCR.

Gene name	Primer sequence (5'-3')
RUVBL1	F: TGGACATTGAGTGCTTCACCTACC
	R: TGACACAGTTGCCTCGGTTGG
β-catenin	F: ATAGAGGCTCTTGTGCGTACTGTC
	R: TTGGTGTCGGCTGGTCAGATG
lymphoid enhancer factor-1	F: AGTCTTCCTTGGTGAACGAGTCTG
	R: GTAGGGCTCCTGAGAGGTTTGTG
GAPDH	F: CAGGAGGCATTGCTGATGAT
	R: GAAGGCTGGGGCTCATTT
siRNA-RUVBL1 guide strand	TCAAGGTCGAATTCTGTGG
siRNA-RUVBL1 passenger strand	CCACAGAATTCGACCTTGA

F, forward; R, reverse; siRNA, small interfering RNA.

wet cotton swab, and the fixed cells were stained with 1% crystal violet (cat. no. G1063; Beijing Solarbio Science & Technology Co., Ltd.,) for 20 min at room temperature. After washing and drying with PBS, the cells were counted and images were captured under a light microscope (Olympus th4-200; Olympus Corporation).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from the cells using the RNAsimple Total RNA Extraction kit [DP419, RK145, Tiangen Biotech (Beijing) Co., Ltd.] and reverse transcribed using the PrimeScript™ RT Reagent kit with gDNA Eraser (cat. no. RR047A; Takara Bio, Inc.) according to the manufacturer's protocol. qPCR reactions were performed using a Thermal Cycler Dice™ Real-Time System to detect the fluorescence intensity of TB-Green (TB Green Premix Ex Taq™ II; cat. no. RR820A; TaKaRa Bio, Inc.) in the reaction solution and monitor the amplification of PCR products. The thermal cycling conditions for qPCR were as follows: Initial denaturation at 1 cycle of 95˚C for 30 sec; 40 cycles of 60˚C for 30 sec and 95˚C for 5 sec. Relative mRNA expression levels were calculated using the $2^{-\Delta\Delta Cq}$ method (23) with GAPDH as an internal reference. The primer sequences used are listed in Table I.

Western blot analysis. Whole proteins were extracted by lysing the cells with RIPA lysis buffer (cat. no. KGP250; KGI Biotechnology Co., Ltd.). The total amounts of protein (30 μg) were separated by electrophoresis on a 10% SDS-PAGE gel and transferred to PVDF membranes. After blocking with 5% skim milk for 2 h at room temperature and washing three times with 20% TBST, the PVDF membrane was incubated at 4˚C overnight with primary antibodies RUVBL1 (IgG; 1:1,000; cat. no. ab133513; Abcam), β-catenin (IgG; 1:5,000, cat. no. 51067-2-AP; Proteintech Group, Inc.), LEF-1 (IgG; 1:1,000; cat. no. 14972-1-AP; Proteintech Group, Inc.) and cyclinD1 (IgG; 1:5,000; cat. no. 26939-1-AP; Proteintech Group, Inc.), respectively. After re-washing three times with TBST, the membrane was incubated for 2 h with HRP-labeled secondary sheep anti-rabbit IgG (1:10,000; cat. no. S0001; Affinity Biosciences; RRID: AB_2839429.) at room temperature. Following 1 min of incubation with ECL (ECL Western Blotting Substrate; cat. no. KF8005-100; Affinity Biosciences; RRID: AB_2846811) at room temperature, protein bands were visualized using a western blotting exposer. Densitometric analysis was conducted using Image Lab™ software (version 6.0.0 Build 25; Bio-Rad Laboratories, Inc.).

Public data collection. RUVBL1 expression in BRCA tissues was obtained from The Cancer Genome Atlas (TCGA) and genotype-tissue expression (GTEx) databases via the Gene Expression Profiling Interactive Analysis (GEPIA2) portal (http://gepia2.cancer-pku.cn/#index). RUVBL1 immunohistochemistry (IHC) data were obtained from the Human Protein Atlas (HPA) database (https://www.proteinatlas.org/). Prognostic survival analysis of BRCA sample data was conducted using the Kaplan-Meier Plotter website (https://kmplot.com/analysis/). The Kyoto Encyclopedia of Genes and Genomes (KEGG; https://www.genome.jp/kegg/pathway.html) database was utilized to identify pathways associated with the RUVBL1 gene.

Statistical analysis. GraphPad Prism 9.0 statistical software (Dotmatics) was employed for statistical analysis. One-way ANOVA with Tukey's post hoc test was employed to compare the different treatment groups. Data are presented as the mean ± SD. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Expression of RUVBL1 is upregulated in BRCA tissues and is associated with the poor prognosis of patients. According to the IHC results from the HPA database, RUVBL1 was expressed at higher levels in BRCA tissues than normal breast tissues (Fig. 1A). RNA-seq differential analysis from TCGA and GTEx databases via the GEPIA2 portal revealed that RUVBL1 expression in BRCA tissues was elevated compared with that in normal breast tissues (Fig. 1B). The overall survival analysis of patients with BRCA using the Kaplan-Meier Plotter website indicated that a high mRNA expression of RUVBL1 was associated with a poor prognosis (Fig. 1C). These results

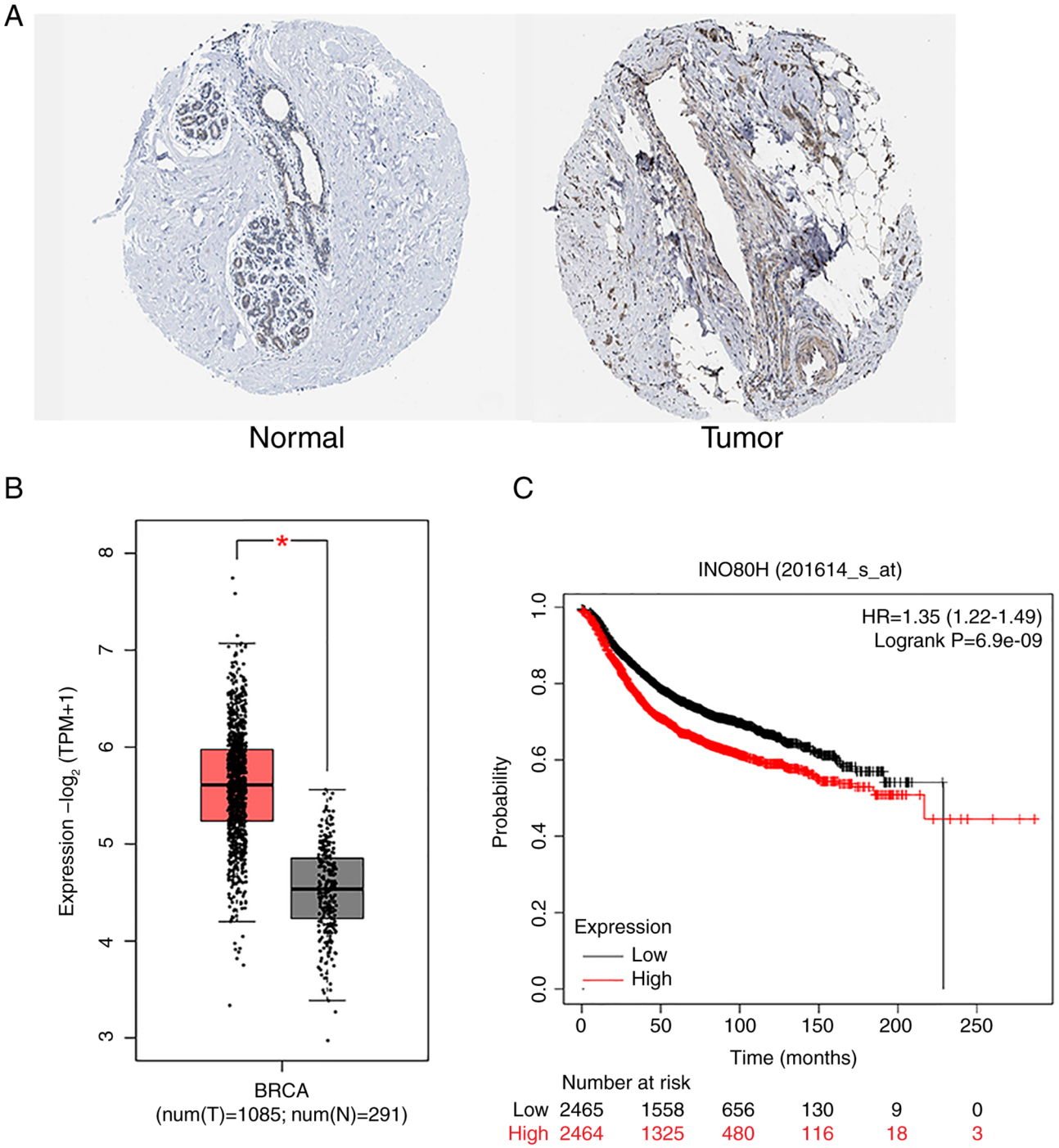

Figure 1. Upregulation of RUVBL1 in BRCA tissues is associated with a poor prognosis of patients with BRCA. (A) Immunohistochemical images of RUVBL1 obtained from the Human Protein Atlas database revealed that the expression level of RUVBL1 protein was higher in BRCA tissues than in normal breast tissues. (B) RUVBL1 expression in BRCA tissues was higher than that in normal breast tissues in The Cancer Genome Atlas and GTEx databases (red, breast cancer tissues; gray, normal breast tissue). (C) Kaplan-Meier analysis was used to analyze the effects of RUVBL1 expression on the prognosis of patients with BRCA (red line, high RUVBL1 expression; black line, low expression of RUVBL1). *P<0.05. BRCA, breast cancer.

(Fig. 1) confirmed that RUVBL1 expression is upregulated in BRCA tissues and may be linked to patient prognosis.

RUVBL1 is highly expressed in human breast carcinoma cells. RT-qPCR and western blot analysis were performed on normal BRCA epithelial cell lines and BRCA cell lines, and the results are presented in Fig. 2. Compared with the normal BRCA cell line, MCF-10a, the mRNA and protein expression of RUVBL1 in the BRCA cell lines (MDA-MB-231 and MCF7) was upregulated (P<0.01). The mRNA and protein expression of RUVBL1 in the MDA-MB-231 cell line was higher than that in the MCF-7 cell line (P<0.01). This may be due to the different regulation mode of RUVBL1 in invasive and non-invasive BRCA.

Knockdown of RUVBL1 inhibits the proliferation of BRCA cell lines. To evaluate whether RUVBL1 affects the proliferation of BRCA cells, a CCK-8 assay was conducted

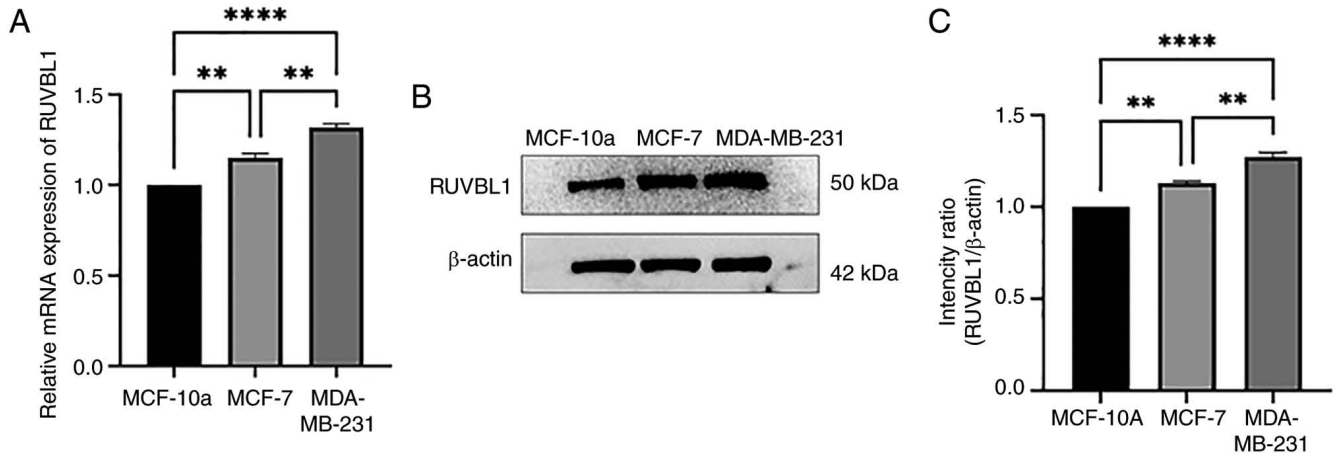

Figure 2. Expression of RUVBL1 in normal mammary epithelial cells and breast cancer cells. The relative (A) mRNA, and (B and C) protein expression levels of RUVBL1 were detected in MCF-10a, MDA-MB-231 and MCF-7 cell lines using reverse transcription-quantitative PCR and western blot analysis, respectively. **P<0.01 and ****P<0.0001 vs. MCF-10a group or MCF-7 group or MDA-MB-231group.

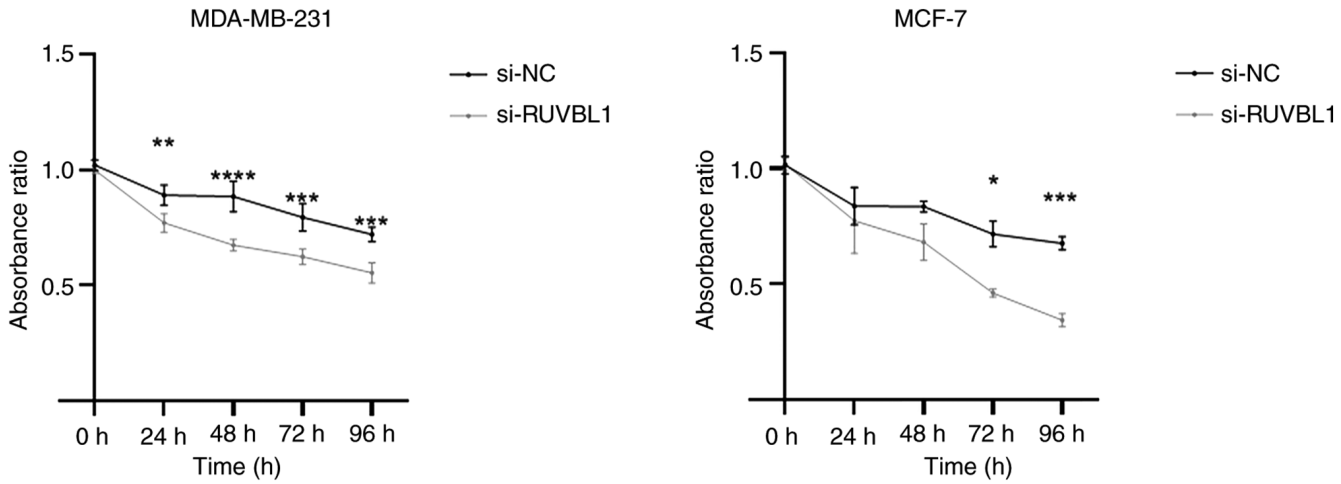

Figure 3. Effect of si-RUVBL1 on the proliferation of breast cancer cell lines. Cell Counting Kit-8 assay was used to detect the proliferation of cells in the vehicle control group, si-NC group, and si-RUVBL1 group at different time points, and the absorbance ratio (absorbance value of detection wells/absorbance value of control wells) was calculated using the formula. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001 vs. si-NC group. si-, small interfering; NC, negative control.

following the siRNA-mediated knockdown of RUVBL1. The proliferation of the BRCA cell lines in the vehicle control, si-NC and si-RUVBL1 groups was assessed at various time points: 24, 48, 72 and 96 h. Compared with the si-NC group, the si-RUVBL1 group of BRCA cell lines exhibited a significant inhibition of proliferation at 48, 72 and 96 h (Fig. 3).

Knockdown of RUVBL1 inhibits BRCA cell migration and invasion. The scratch test was performed to assess the cell migration ability (Fig. 4A and B). The results indicated that the migration rate of the BRCA cell lines, MDA-MB-231 and MCF-7, decreased at 12 and 24 h in the si-RUVBL1 group compared with the vehicle control and si-NC groups. Transwell migration (Fig. 4C and D) and invasion assays (Fig. 4E and F) revealed that the migration and invasion of the MDA-MB-231 and MCF-7 cells in the si-RUVBL1 group were reduced compared with the vehicle control and si-NC groups. In summary, si-RUVBL1 significantly inhibited the migration and invasion of both MDA-MB-231 and MCF-7 BRCA cell lines.

Knockdown of RUVBL1 downregulates the mRNA and protein levels of β-catenin and LEF. To investigate whether RUVBL1 regulates the β-catenin/LEF1 pathway and to identify its downstream genes, a signaling pathway map of RUVBL1 was accessed using the KEGG database (Fig. 5A). RT-qPCR (Fig. 5B and C) and western blot analysis (Fig. 5D and F) were utilized to measure the mRNA and protein expression levels in the MDA-MB-231 and MCF-7 BRCA cell lines following siRNA transfection. The results indicated that, compared with the vehicle control and si-NC groups, the mRNA and protein levels of β-catenin and LEF-1 were decreased following RUVBL1 knockdown. Additionally, the protein level of cyclin D1, a downstream target of LEF-1, was significantly reduced. These results suggested that the knockdown of RUVBL1 effectively blocks the β-catenin/LEF1 pathway, reduces cyclin D1 levels and affects the cell cycle.

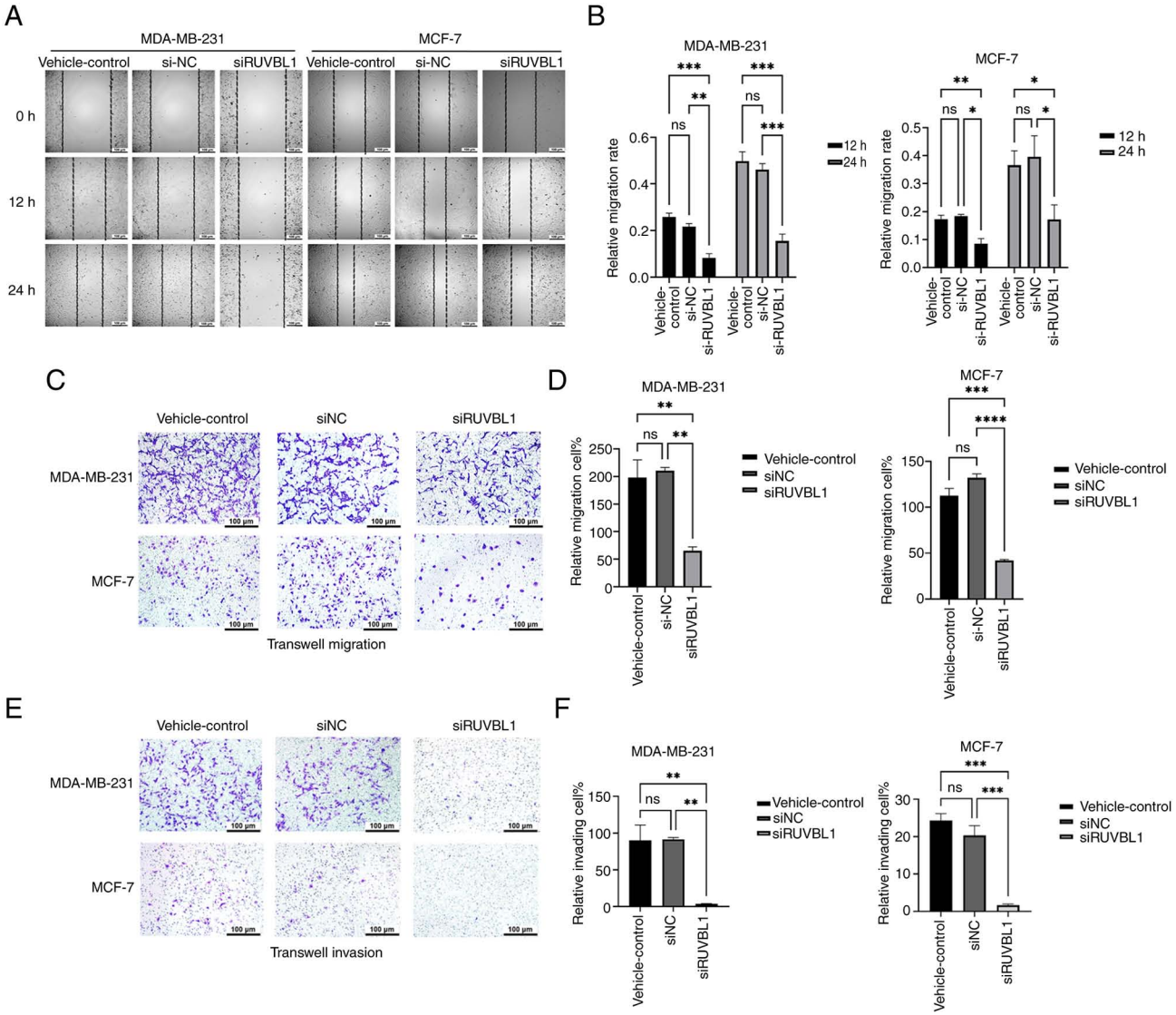

Figure 4. Role of RUVBL1 in the migration and invasion of BRCA cells. (A and B) Scratch assay was used to detect the migration area of the cell scratch at 0, 12 and 24 h, and the results revealed that the migratory ability of the si-RUVBL1 group was decreased compared with that in the vehicle control and si-NC group. (C-F) Transwell assay was used to detect the migratory and invasive ability of the three groups of BRCA cells (vehicle control, si-NC and si-RUVBL1). (C and D) Cell staining at 0 and 48 h revealed that the migratory ability of the cells in the si-RUVBL1 group was decreased. (E and F) The results of cell staining at 0 and 48 h revealed that the invasive ability of cells in the si-RUVBL1 group was significantly reduced. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001 vs. si-NC group. BRCA, breast cancer; si-, small interfering; NC, negative control; ns, not significant.

Discussion

Previous studies have shown that RUVBL1 promotes tumorigenesis and progression by regulating chromatin remodeling and transcriptional activity (15,17,24), and interacts with the β-catenin pathway to facilitate tumor progression. However, the molecular mechanisms underlying RUVBL1-associated proliferation, invasion and metastasis in BRCA cells remain poorly understood. A previous study of highly metastatic BRCA cells confirmed that the ectopic expression of RUVBL1 in the cytoplasm and cell membrane and the formation of RUVBL1-ITFGI complex jointly promoted the collective invasion of tumor cells (25). In the present study, it was found that RUVBL1 knockdown not only significantly inhibited BRCA cell proliferation, invasion and migration, but was also associated with the β-catenin/LEF-1 pathway. This pathway can be regulated by the knockdown of RUVBL1, thereby affecting the occurrence and development of BRCA cells (Fig. 6). The involvement of RUVBL1 in the Wnt/β-catenin pathway has also been confirmed in oral squamous cell carcinoma (18). In the present study, it was found that RUVBL1 knockdown significantly inhibited the proliferation, invasion and migration of BRCA cells.

Numerous studies have shown that the dysregulation of the Wnt signaling pathway is a major contributor to BRCA development and is implicated in BRCA proliferation (26), metastasis (27), immune microenvironment regulation (28), stem cell maintenance (29,30) and treatment resistance (31). Inhibiting the β-catenin/LEF-1 signaling pathway is an emerging therapeutic strategy. Targeted therapy combined with radiotherapy and chemotherapy has shown a favorable prospect in the field of cancer treatment. By identifying specific molecular markers of tumors, precision targeted drugs can effectively inhibit the proliferation of cancer cells

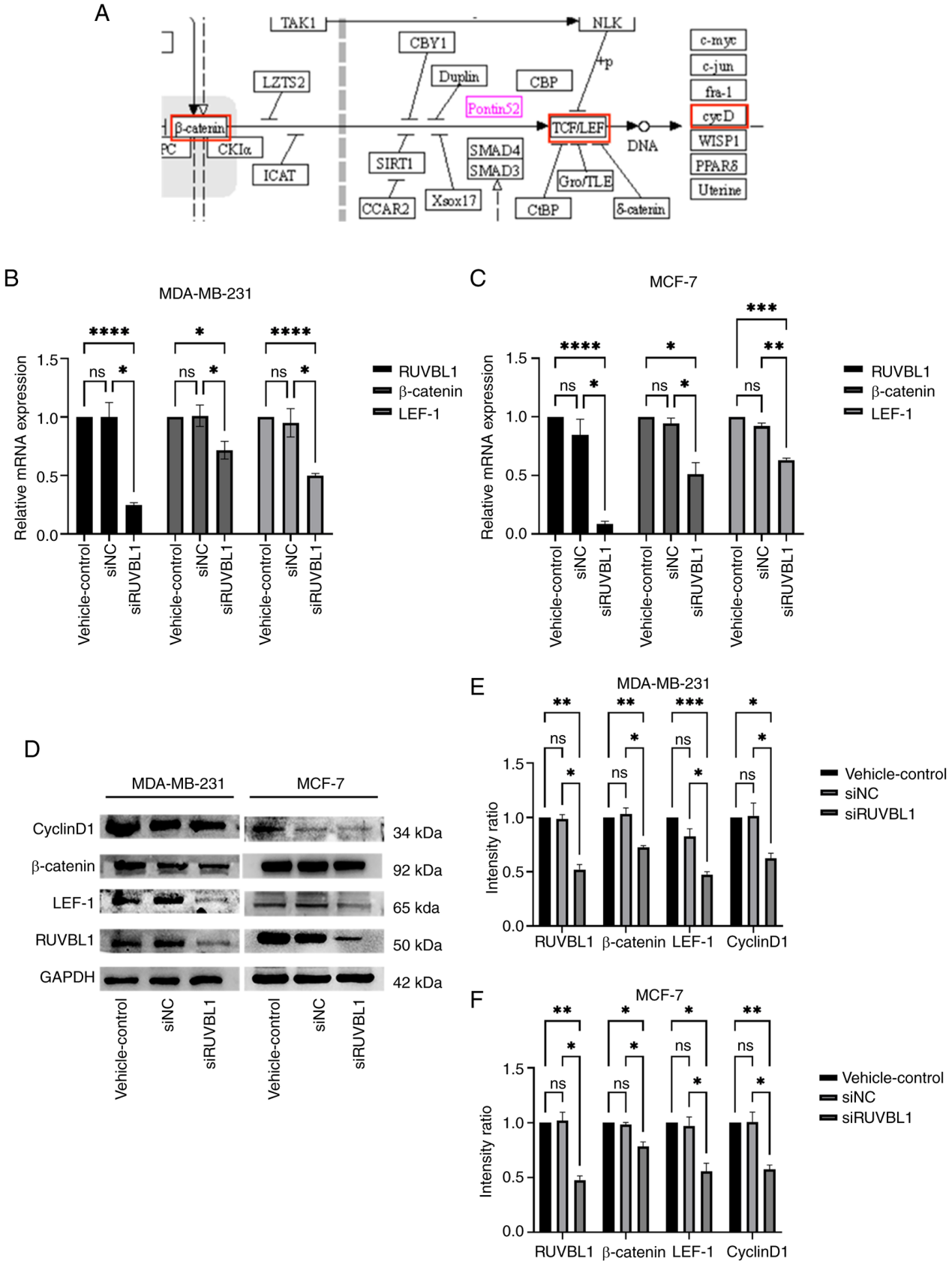

Figure 5. Expression of β-catenin pathway-related proteins was detected using western blot analysis. The intensity of the gray band was calculated using ImageJ software and shown in the figure. (A) The Kyoto Encyclopedia of Genes and Genomes database was used to identify the signaling pathway map of RUVBL1 (Pontin52 is the alias of the RUVBL1 gene; red box indicates the pathway-related protein). The results revealed that RUVBL1 was involved in the β-catenin/LEF-1 pathway, and its downstream genes were related to the cell cycle. (B and C) Reverse transcription-quantitative PCR and (D-F) western blot analysis were used to detect the mRNA and protein levels of β-catenin and LEF-1 in breast cancer cells of the three groups of vehicle control, si-NC and si-RUVBL1, respectively. The expression levels of β-catenin, LEF-1 and cyclinD1 in the si-RUVBL1 group were decreased in the MDA-MB-231 and MCF-7 cells. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001 vs. si-NC. LEF-1, lymphoid enhancer factor-1; si-, small interfering; NC, negative control; ns, not significant.

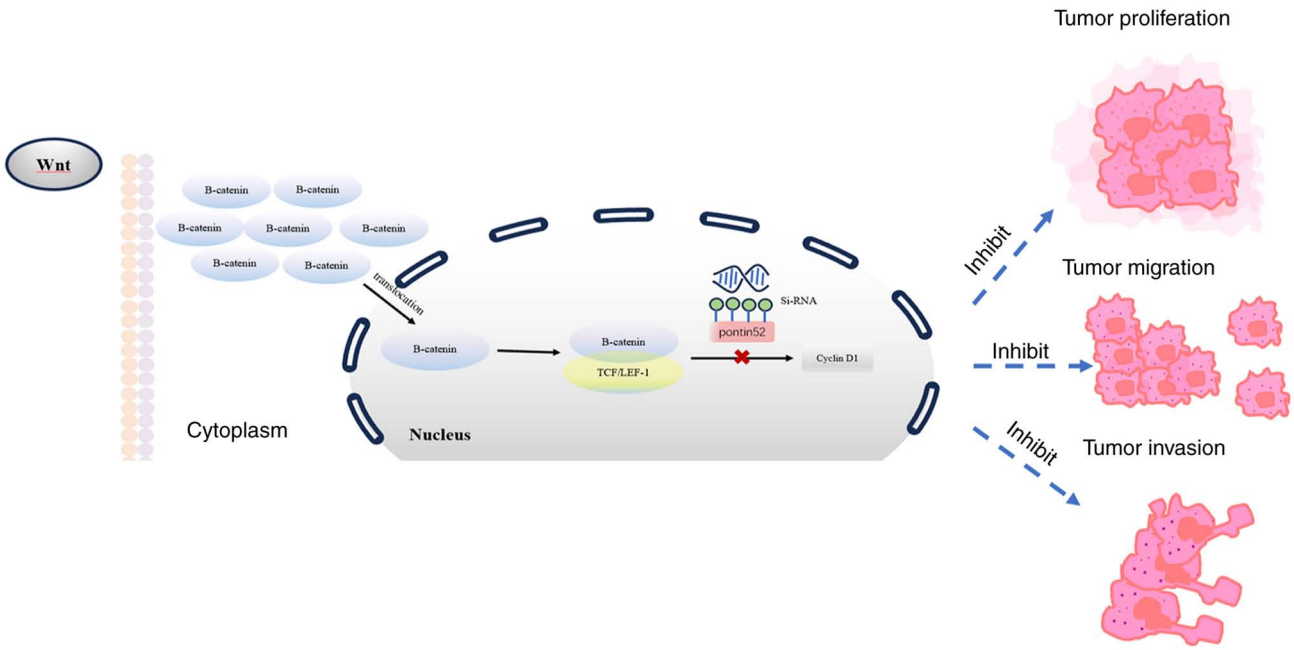

Figure 6. RUVBL1 knockdown inhibits breast cancer cell proliferation, invasion and migration by affecting the β-catenin/LEF-1 pathway and cell cycle. LEF-1, lymphoid enhancer factor-1; siRNA, small interfering RNA.

and reduce side effects. RUVBL1 is expected to become a new therapeutic target by inhibiting β-catenin/LEF-1 pathway. In the future, if RUVBL1 is combined with radiotherapy and chemotherapy, it can enhance the synergistic effect of treatment and improve the cure rate of tumors, which will promote the progress of precision medicine and the expansion of clinical application. Thus, investigating specific molecular targets is crucial for developing novel cancer therapeutic strategies. In the present study, the knockdown of RUVBL1 expression in the BRCA cell lines, MDA-MB-231 and MCF-7, not only decreased β-catenin and LEF-1 expression, but also affected the regulation of cyclin D1, which is associated with the proliferation of BRCA cells. The upregulation of RUVBL1 in invasive BRCA cells is higher than that in non-invasive BRCA cells, suggesting that RUVBL1 may be closely related to the proliferation, spread and invasion of invasive BRCA cells. It may also be involved in mechanisms such as promoting changes in the tumor microenvironment by participating in signal transduction and regulating cell cycle. This finding also suggests that RUVBL1 may be more effective in the treatment of invasive BRCA than non-invasive BRCA, which will be the direction of our future research. A high expression of RUVBL1 in BRCA tissues is associated with the patient survival time. Bioinformatics analysis revealed that RUVBL1 was involved in the β-catenin/LEF-1 signaling pathway, which was initially verified by experiments. Therefore, RUVBL1 may be one of the markers for predicting the survival and prognosis of patients with BRCA, and its overexpression may become a potential biological target for the diagnosis, prognosis and treatment of BRCA. The present study was only a preliminary study on the mechanism of the RUVBL1 gene in the proliferation, migration and invasion of BRCA cells at the cellular level. Thus, further studies are required in the future to determine the drug intervention effects of the RUVBL1 gene in BRCA cells. In addition, further studies with a large number of clinical samples are required for in-depth research and verification to explore the mechanisms through which RUVBL1 interacts and regulates cellular pathways in BRCA. This will be the focus of future research.

In conclusion, the present study demonstrates that RUVBL1 knockdown in BRCA cells regulates the β-catenin/LEF-1 signaling pathway and the expression levels of specific cell cycle-related genes. These findings suggest that targeting RUVBL1 may serve as a potential therapeutic strategy within the Wnt pathway, contributing to the development of novel molecular approaches against BRCA. In further studies, direct binding experiments such as luciferase assay will be performed to elucidate the mechanism, and the effect of this factor on the cell cycle will be examined by flow cytometry. In addition, cell and animal experiments with combined drugs will be conducted, and a large number of clinical samples for further research and analysis shall be collected.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

XZ, DC, WS, GY and WW significantly contributed to the conception and design of the study, as well as to data collation and statistical analysis, and data analysis and interpretation. CM contributed to the design of the study, ensured the quality and completeness of the data, revised the manuscript several times to ensure its quality, and provided financial support for the experiment. XZ and DC confirm the authenticity of all the raw data. All authors read and the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Sancho-Garnier H and Colonna M: Breast cancer epidemiology. Presse Med 48: 1076-1084, 2019.
2. Harbeck N and Gnant M: Breast cancer. Lancet 389: 1134-1150, 2017.
3. Kal S, Chakraborty S, Karmakar S and Ghosh MK: Wnt/β-catenin signaling and p68 conjointly regulate CHIP in colorectal carcinoma. Biochim Biophys Acta Mol Cell Res 1869: 119185, 2022.
4. Mani SKK, Yan B, Cui Z, Sun J, Utturkar S, Foca A, Fares N, Durantel D, Lanman N, Merle P, *et al*: Restoration of RNA helicase DDX5 suppresses hepatitis B virus (HBV) biosynthesis and Wnt signaling in HBV-related hepatocellular carcinoma. Theranostics 10: 10957-10972, 2020.
5. Liu C, Wang L, Jiang Q, Zhang J, Zhu L, Lin L, Jiang H, Lin D, Xiao Y, Fang W and Guo S: Hepatoma-derived growth factor and DDX5 promote carcinogenesis and progression of endometrial cancer by activating β-catenin. Front Oncol 9: 211, 2019.
6. Cai ZG, Shi XJ, Gao Y, Wei MJ, Wang CY and Yu GY: Beta-catenin expression pattern in primary oral squamous cell carcinoma. Chin Med J (Engl) 121: 1866-1870, 2008.
7. Zhu L, Tian Q, Gao H, Wu K, Wang B, Ge G, Jiang S, Wang K, Zhou C, He J, *et al*: PROX1 promotes breast cancer invasion and metastasis through WNT/β-catenin pathway via interacting with hnRNPK. Int J Biol Sci 18: 2032-2046, 2022.
8. Han S, Hao H, Han H, Xue D, Jiao Y, Xie Y, Xu Y, Huangfu L, Fu J, Wang S, *et al*: CUEDC2 drives β-catenin nuclear translocation and promotes triple-negative breast cancer tumorigenesis. Cells 11: 3067, 2022.
9. Bucan V, Mandel K, Bertram C, Lazaridis A, Reimers K, Park-Simon TW, Vogt PM and Hass R: LEF-1 regulates proliferation and MMP-7 transcription in breast cancer cells. Genes Cells 17: 559-567, 2012.
10. Holmes KA, Song JS, Liu XS, Brown M and Carroll JS: Nkx3-1 and LEF-1 function as transcriptional inhibitors of estrogen receptor activity. Cancer Res 68: 7380-7385, 2008.
11. Shtutman M, Zhurinsky J, Simcha I, Albanese C, D'Amico M, Pestell R and Ben-Ze'ev A: The cyclin D1 gene is a target of the beta-catenin/LEF-1 pathway. Proc Natl Acad Sci USA 96: 5522-5527, 1999.
12. Gu S, Liu F, Xie X, Ding M, Wang Z, Xing X, Xiao T and Sun X: β-Sitosterol blocks the LEF-1-mediated Wnt/β-catenin pathway to inhibit proliferation of human colon cancer cells. Cell Signal 104: 110585, 2023.
13. Tucker PA and Sallai L: The AAA+ superfamily-a myriad of motions. Curr Opin Struct Biol 17: 641-652, 2007.
14. Qiu XB, Lin YL, Thome KC, Pian P, Schlegel BP, Weremowicz S, Parvin JD and Dutta A: An eukaryotic RuvB-like protein (RUVBL1) essential for growth. J Biol Chem 273: 27786-27793, 1998.
15. Chen J, Liu G, Wu Y, Ma J, Wu H, Xie Z, Chen S, Yang Y, Wang S, Shen P, *et al*: CircMYO10 promotes osteosarcoma progression by regulating miR-370-3p/RUVBL1 axis to enhance the transcriptional activity of β-catenin/LEF1 complex via effects on chromatin remodeling. Mol Cancer 18: 150, 2019.
16. Mello T, Materozzi M, Zanieri F, Simeone I, Ceni E, Bereshchenko O, Polvani S, Tarocchi M, Marroncini G, Nerlov C, *et al*: Liver haploinsufficiency of RuvBL1 causes hepatic insulin resistance and enhances hepatocellular carcinoma progression. Int J Cancer 146: 3410-3422, 2020.
17. Zhang C and Wu S: RUVBL1-modulated chromatin remodeling alters the transcriptional activity of oncogenic CTNNB1 in uveal melanoma. Cell Death Discov 9: 132, 2023.
18. Zeng Y, Kong Y, Liao L and Zhu H: Involvement of RUVBL1 in WNT/β-catenin signaling in oral squamous cell carcinoma. Dis Markers 2022: 3398492, 2022.
19. Dauden MI, López-Perrote A and Llorca O: RUVBL1-RUVBL2 AAA-ATPase: A versatile scaffold for multiple complexes and functions. Curr Opin Struct Biol 67: 78-85, 2021.
20. Li X, Li H, Shao MM, Miao J, Fu Y and Hu B: Downregulation of AHNAK2 inhibits cell cycle of lung adenocarcinoma cells by interacting with RUVBL1. Thorac Cancer 14: 2093-2104, 2023.
21. Lacombe J, Mangé A, Jarlier M, Bascoul-Mollevi C, Rouanet P, Lamy PJ, Maudelonde T and Solassol J: Identification and validation of new autoantibodies for the diagnosis of DCIS and node negative early-stage breast cancers. Int J Cancer 132: 1105-1113, 2013.
22. Ahn YT, Kim MS, Kim YS and An WG: Astaxanthin reduces stemness markers in BT20 and T47D breast cancer stem cells by inhibiting expression of pontin and mutant p53. Mar Drugs 18: 577, 2020.
23. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
24. Li M, Yang L, Chan AKN, Pokharel SP, Liu Q, Mattson N, Xu X, Chang WH, Miyashita K, Singh P, *et al*: Epigenetic control of translation checkpoint and tumor progression via RUVBL1-EEF1A1 axis. Adv Sci (Weinh) 10: e2206584, 2023.
25. Fan W, Xie J, Xia J, Zhang Y, Yang M, Wang H, Pan Y, Zhang M, Han B, Wu B, *et al*: RUVBL1-ITFG1 Interaction is required for collective invasion in breast cancer. Biochim Biophys Acta Gen Subj 1861: 1788-1800, 2017.
26. Liu X, Xie P, Hao N, Zhang M, Liu Y, Liu P, Semenza GL, He J and Zhang H: HIF-1-regulated expression of calreticulin promotes breast tumorigenesis and progression through Wnt/β-catenin pathway activation. Proc Natl Acad Sci USA 118: e2109144118, 2021.
27. El Ayachi I, Fatima I, Wend P, Alva-Ornelas JA, Runke S, Kuenzinger WL, Silva J, Silva W, Gray JK, Lehr S, *et al*: The WNT10B network is associated with survival and metastases in chemoresistant triple-negative breast cancer. Cancer Res 79: 982-993, 2019.
28. Malladi S, Macalinao DG, Jin X, He L, Basnet H, Zou Y, de Stanchina E and Massagué J: Metastatic latency and immune evasion through autocrine inhibition of WNT. Cell 165: 45-60, 2016.
29. Wang X, Jung YS, Jun S, Lee S, Wang W, Schneider A, Sun Oh Y, Lin SH, Park BJ, Chen J, *et al*: PAF-Wnt signaling-induced cell plasticity is required for maintenance of breast cancer cell stemness. Nat Commun 7: 10633, 2016.
30. Won HY, Lee JY, Shin DH, Park JH, Nam JS, Kim HC and Kong G: Loss of Mel-18 enhances breast cancer stem cell activity and tumorigenicity through activating Notch signaling mediated by the Wnt/TCF pathway. FASEB J 26: 5002-5013, 2012.
31. Shen H, Yan W, Yuan J, Wang Z and Wang C: Nek2B activates the wnt pathway and promotes triple-negative breast cancer chemothezrapy-resistance by stabilizing β-catenin. J Exp Clin Cancer Res 38: 243, 2019.