REC8 inhibits proliferation, migration and invasion of breast cancer cells by targeting CDC20

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Abstract. Breast cancer is one of the most types of common malignant tumor in women. REC8 is a known tumor suppressor in several types of cancer; however, the role of REC8 in breast cancer remains unknown. The purpose of the present study was to investigate the effects and underlying mechanism of REC8 on the proliferation, migration and invasion of breast cancer cells. The expression of REC8 in normal and breast cancer cells was detected by reverse transcription-quantitative PCR and western blotting. Stable REC8-overexpressing breast cancer cells were constructed to modify the expression of REC8. The expression of cell division cycle 20 (CDC20) in breast cancer cells was altered using the CDC20 inhibitor apcin. Cell viability, proliferation, migration, invasion and apoptosis were determined by Cell Counting Kit-8, colony formation, wound healing, Transwell and TUNEL assays, respectively. Western blotting was performed to measure the expression of matrix metalloproteinase-2/9 and apoptosis-associated proteins [Bcl-2, caspase-3, cleaved caspase-3 and cleaved poly (ADP-ribose) polymerase]. Compared with normal breast cells, the expression of REC8 was lower in breast cancer cells. Search Tool for the Retrieval of Interacting Genes/Proteins online database was used to predict the interaction between REC8 and CDC20. Overexpression of REC8 significantly inhibited the proliferation, migration and invasion of breast cancer cells *in vitro*; these changes were reversed by CDC20 overexpression. In conclusion, the present study demonstrated that REC8 decreased proliferation, migration and invasion of breast cancer cells by inhibiting CDC20.

Introduction

Breast cancer is the most common cancer among women and is the second most frequently occurring newly diagnosed cancer worldwide (1). The cause of mortality in patients with breast cancer is often not the primary tumor, but metastasis to distant organs (2). Since 1989, female breast cancer has increased by 41%; the incidence of breast cancer is still increasing at an annual rate of ~0.5% (3). Studies have shown that chemotherapeutic drugs may promote tumor metastasis (4,5). Therefore, there is a need for novel markers to screen patients at high risk of breast cancer metastasis and novel targets for the treatment of breast cancer metastasis should be explored.

REC8 is a member of the structural maintenance of chromosomes protein complex adhesion (6). Adhesin serves an important role in regulating chromosome separation and homologous recombination in mitosis and meiosis, as well as in proliferation of eukaryotic cells (7). It was previously demonstrated that abnormal chromosome segregation is associated with a variety of diseases; in particular, it serves a key role in the occurrence and development of tumors (8). For example, in thyroid cancer, REC8 inhibits tumor cell proliferation and migration by targeting the PI3K pathway (9). REC8 overexpression was shown to significantly suppress metastasis of gastric cancer cells by downregulating early growth response (EGR)1 (10). Although REC8 serves as a tumor suppressor in most types of tumor, to the best of our knowledge, its role in breast cancer has not yet been studied.

Similar to REC8, cell division cycle (CDC)20 serves an important role in chromosome separation and mitosis exit. Activation of CDC20 promotes activation of anaphase-promoting complex/cyclosome, which is a key regulator of mitotic duration (11,12). Previous studies have shown that CDC20 is highly expressed in a variety of malignant tumors and is associated with the development and progression of tumors (13,14). For example, expression of CDC20 is upregulated and associated with poor prognosis in breast cancer (15). CDC20 may also be a potential predictive indicator for prognosis of breast cancer with co-expressed TPX2 gene, indicating that CDC20 inhibition may be of value as a novel strategy for cancer treatment (16). However, whether REC8 affects the progression of breast cancer by regulating CDC20 requires further investigation.

In the present study, the expression of REC8 was examined by reverse transcription-quantitative (RT-q)PCR analysis in breast cancer cells. Furthermore, the role and underlying mechanisms of REC8 on cell proliferation, migration and invasion via targeting CDC20 were investigated by clone

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formation test, wound healing assay and Transwell assay, respectively.

Materials and methods

Cell culture. The breast epithelial cell line MCF-10A and four breast cancer cell lines (T47D, MCF-7, MDA-MB-231 and BT-549) were purchased from The Cell Bank of Type Culture Collection of The Chinese Academy of Sciences. MCF-7 and T47d cell lines represent luminal A condition [estrogen receptor $(ER)^+$, progesterone receptor $(PR)^{+/-}$ and human epidermal growth factor receptor 2 $(HER2^-)$], while MDA-MB-231 and BT-549 cell lines are triple-negative (ER^-, PR^- and $HER2^-$), All cells were cultured in DMEM/F12 Coon's (DMEM/F12) containing 10% FBS (Hyclone; Cytiva), 100 U/ml penicillin and 100 μg/ml streptomycin and maintained in a 5% CO_2 incubator at 37˚C. The cells were passaged once after 3 days. Cells in the logarithmic growth phase were used in the subsequent experiments.

Cell transfection. For overexpression of REC8 and CDC20, pcDNA3.1 vector containing full-length REC8 (OV-REC8) and CDC20 (OV-CDC20) and corresponding empty negative control vectors (OV-NC) were designed and synthesized by Thermo Fisher Scientific, Inc. Untransfected cells were used as the control. Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) was used to transfect plasmids into cells ($1x10^5$ cells/well) at room temperature at 50 ng/ml according to the manufacturer's instructions. After 12 h, the medium was replaced with fresh DMEM and cells were routinely cultured for 72 h at 37˚C.

Bioinformatics. Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; string-db.org/) was used to predict interactions between REC8 and CDC20. The expression of REC8 in breast cancer and normal tissue was predicted by Gene Expression Profiling Interactive Analysis (GEPIA; gepia.cancer-pku.cn/). Kaplan-Meier (KM) Plotter (kmplot.com/analysis/index.php?p=background) was used to determine the prognostic value of REC8 in breast cancer tissue.

RT-qPCR analysis. Total RNA was extracted from MCF-7 cells using TRIzol® reagent (Thermo Fisher Scientific, Inc.) and reverse transcribed to cDNA using a Reverse Transcription System (Promega Corporation) according to the manufacturer's protocol. PCR was performed using SYBR Green Supermix and the ABI 7500 PCR system (both from Applied Biosystems; Thermo Fisher Scientific, Inc.). The qPCR conditions were as follows: 94˚C for 30 sec, 55˚C for 30 sec and 72˚C for 30 sec (22 cycles). The relative expression of target genes was calculated by the $2^{-\Delta\Delta Cq}$ method (17) and normalized to the housekeeping gene GAPDH. The sequences of PCR primers were as follows: REC8 forward, 5'-TACCTG CTCCTGGTGCTCTC-3' and reverse, 5'-AGGTAAGCAGGA CCCAGTGA-3'; CDC20 forward, 5'-GACCACTCCTAG CAAACCTGG-3' and reverse, 5'-GGGCGTCTGGCTGTT TTCA-3' and GAPDH forward, 5'-ACCACAGTCCATGCC ATCAC-3' and reverse, 5'-TCCACCACCCTGTTGCTGTA-3'. The primer sequences were obtained using the PCR sequence design tool provided by National Center for Biotechnology Information (ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome).

Cell Counting Kit (CCK)-8 assay. Cell viability was detected by CCK-8 assay. Transfected cells were incubated on 96-well plates at a density of $2x10^3$ cells/well for 24, 48 and 72 h at 37˚C. MCF-7 cells were incubated for 24, 48 and 72 h with the specific CDC20 inhibitor apcin (cat. no. 5747/10; R&D Systems, Inc.) dissolved in PBS to concentrations of 25 and 50 μM. CCK-8 solution (10 μl; cat. no. C0037; Beyotime Institute of Biotechnology) was added to each well and incubated for an additional 2 h. The absorbance of each well was measured at a wavelength of 450 nm using the Synergy 2 Multi-Mode Microplate Reader (BioTek Instruments, Inc.).

MTT viability assay. MTT was first prepared as a stock solution of 5 mg/ml in PBS (pH 7.2) and filtered. The cell suspension was added to a 96-well plate with $5x10^3$ cells/well, and then treated with different concentrations of apcin (25 and 50 μM) for 24, 48 and 72 h, followed by the addition of 10 μl MTT solution to each well. After incubation for 4 h at 37˚C, 100 μl dimethyl sulfoxide (Adamas-Beta, Ltd.) was added to each well. After agitation, the 96-well plate was read by microplate reader at 570 nm for absorbance density values in order to determine the cell viability. The viable cells produced a dark blue formazan product, whereas no such staining was formed in the dead cells. The percentage of the viable cells was calculated using the following formula: [100x (sample absorbance)/(control absorbance)].

Colony formation assay. The MCF-7 cells were inoculated in 6-well plates at a density of $4x10^2$ cells/well for 14 days at 37˚C. Subsequently, the cells were fixed with 70% ethanol for 5 min and then stained with 0.05% crystal violet solution for 20 min at room temperature. The number of colonies formed (>50 cells) was counted under an Olympus BX40 light microscope (Olympus Corporation; magnification, x100).

Wound healing assay. Cell migration was detected by wound healing assay. MCF-7 cells were inoculated in 12-well plates at a density of $2x10^4$ cells per well. Serum-free DMEM/F12 was used in place of medium and the cells were incubated overnight at 37˚C under 5% CO_2. When cell confluence reached 90%, a linear wound was created in the cell monolayer using a 200-μl sterile pipette tip. Images were captured at 0 and 48 h under an Olympus BX40 light microscope (Olympus Corporation; magnification, x100).

Transwell assay. Transwell assay was performed to assess cell invasion. The 24-well Transwell plates (Corning, Inc.) with 8-μm pore inserts were coated with Matrigel (BD Biosciences) at 37˚C for 30 min. Cells in logarithmic growth phase ($3x10^4$) were cultured in the top of Matrigel-coated invasion chambers (BD Biosciences) filled with serum-free DMME/F12. DMEM/F12 supplemented with 10% FBS was added to the lower chamber as a chemoattractant. Following 24 h culture at 37˚C with 5% CO_2, cells that had invaded to the bottom chamber were fixed with 80% ethanol for 30 min and stained with 0.1% crystal violet solution for 5 min at room temperature. The stained cells were observed under a Leica DM IL inverted

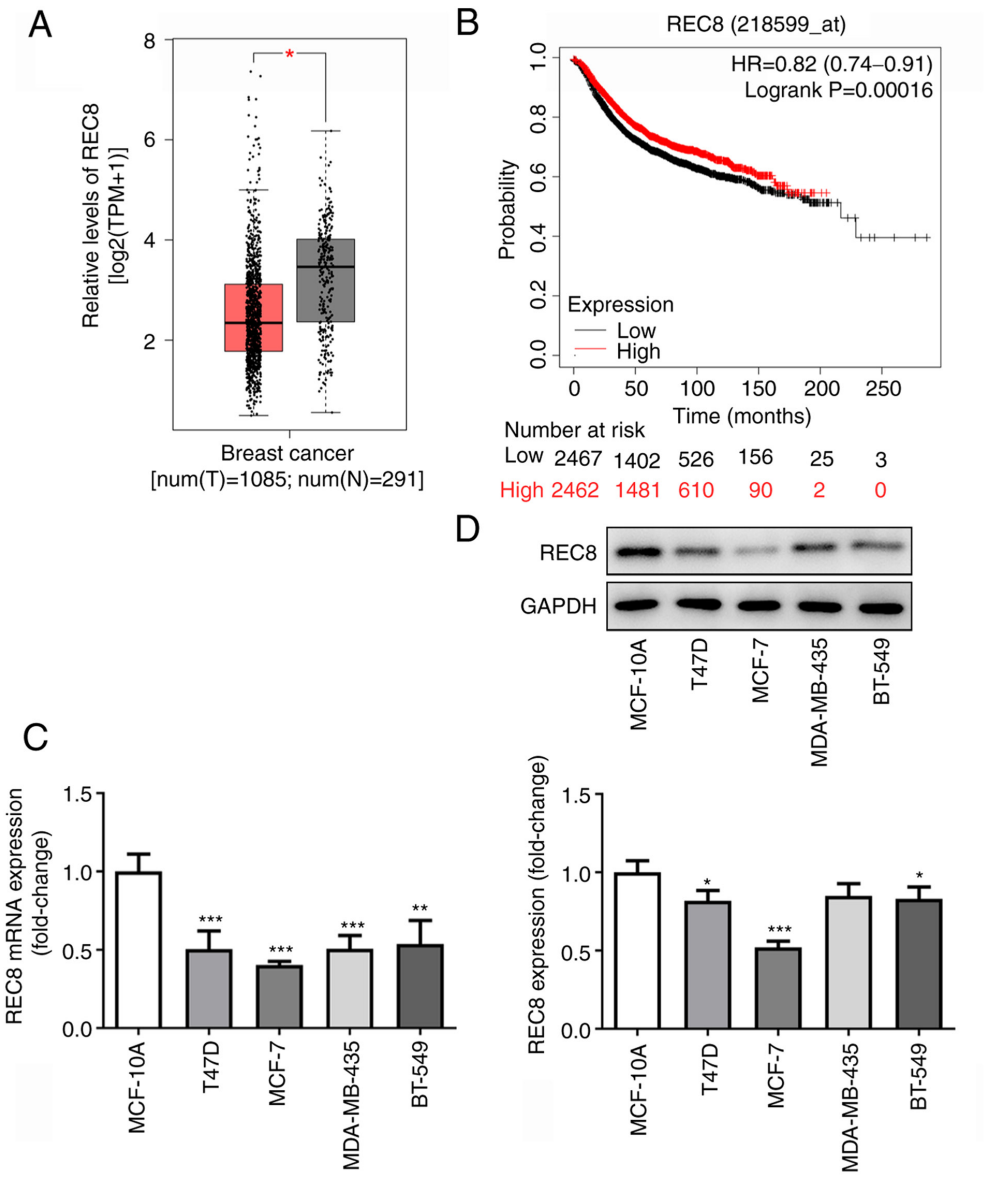

Figure 1. REC8 is downregulated in breast cancer cells and tissue. (A) Prediction of REC8 expression in the serum of patients with breast cancer using Gene Expression Profiling Interactive Analysis. (B) Effect of REC8 levels on relapse-free survival in patients with BRCA. (C) Reverse transcription-quantitative PCR and (D) western blot analysis of REC8 expression in breast cancer cell lines. Data are presented as the mean ± SD and are representative of three independent experiments. *P<0.05, **P<0.01, ***P<0.001 vs. MCF-10A. T, tumor; N, normal.

light microscope (Leica Microsystems, Inc.; magnification, x100).

TUNEL assay. MCF-7 cells (2x10^5 cells/well) were collected and washed three times with PBS. Following fixing with 4% paraformaldehyde at room temperature for 20 min, cells were washed twice with PBS. Subsequently, a small amount of DAPI staining solution (cat. no. C1005; Beyotime Institute of Biotechnology) was added to cover the cells and incubated at room temperature for 3-5 min. Triton X-100 (0.2%) was then added to cells at room temperature for 5 min. Subsequently, 50 μl TUNEL assay solution (Roche Diagnostics GmbH) was added to the cells and incubated at 37˚C in the dark for 60 min. The detection solution was discarded and cells were washed three times with PBS. Cells were sealed with anti-fluorescence quenched sealing solution (cat. no. P0126; Beyotime Institute of Biotechnology) and three fields of view were randomly selected for observation under a fluorescence microscope (Zeiss GmbH; magnification, x200).

Western blot analysis. Total RNA was extracted from MCF-7 cells using RIPA buffer (cat. no. P0013B; Beyotime Institute of Biotechnology). The protein concentration was detected by BCA protein quantitative kit (cat. no. P0012; Beyotime Institute of Biotechnology). Protein (20 μg/lane) was separated by 10% SDS-PAGE and transferred to PVDF membranes (cat. no. FFP30; Beyotime Institute of Biotechnology). The membranes were blocked with 5% skimmed milk at room temperature for 30 min and incubated with primary antibodies (all 1:1,000; all Abcam) against REC8 (cat. no. ab192241), CDC20 (cat. no. ab215908), MMP-2 (cat. no. ab92536), MMP-9 (ab76003), Bcl-2 (cat. no. ab182858), caspase-3 (cat. no. ab32351), cleaved caspase-3 (cat. no. ab2302), cleaved poly(ADP-ribose) polymerase (PARP; cat. no. ab32064) and

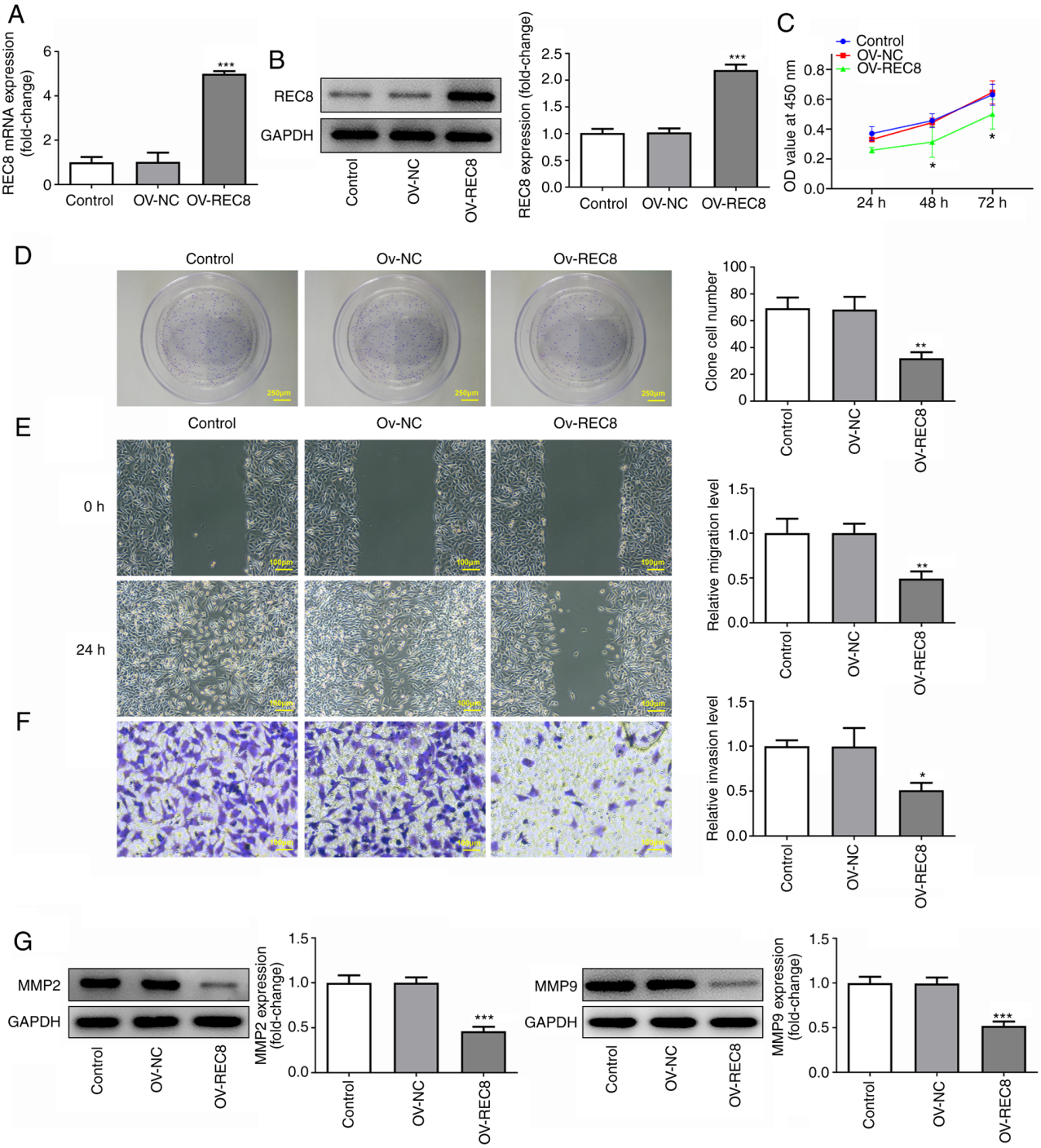

Figure 2. Overexpression of REC8 inhibits proliferation, migration and invasion of MCF-7 cells. (A) Reverse transcription-quantitative PCR and (B) western blot analysis of REC8 expression in MCF-7 cells. (C) OV-NC, OV-REC8 and Control cells were seeded into a 96-well plate. Cells were treated with Cell Counting Kit-8 and OD was measured 24, 48 and 72 h after cell seeding. (D) Effect of OV-REC8 on MCF-7 cell proliferation was measured by colony formation assay. Effects of OV-REC8 on MCF-7 cell (E) migration and (F) invasion were determined by wound healing and Transwell assay. (G) Western blot analysis of MMP2 and MMP9 expression in MCF-7 cells. Data are presented as the mean ± SD and are representative of three independent experiments. *P<0.05, **P<0.01, ***P<0.001 vs. OV-NC. OV, overexpression; NC, negative control; OD, optical density; MMP, matrix metalloproteinase.

GAPDH (cat. no. ab9485) overnight at 4˚C. Subsequently, membranes were incubated with goat anti-rabbit horseradish peroxidase binding IgG (1:5,000; cat. no. ab6721; Abcam) at 37˚C for 2 h. The ECL Plus kit (cat. no. P0018; Beyotime Institute of Biotechnology) was used to visualize the protein bands and protein expression levels were semi-quantified using ImageJ software (version 1.46; National Institutes of Health) with GAPDH as the loading control.

Statistical analysis. Data were analyzed using GraphPad Prism 7 (GraphPad Software, Inc.). One-way ANOVA followed by Tukey's post hoc test was used to compare differences between multiple groups and unpaired Student's t-test was used to analyze differences between two groups. Data are presented as the mean ± standard deviation from ≥3 independent experiments. P<0.05 was considered to indicate a statistically significant difference.

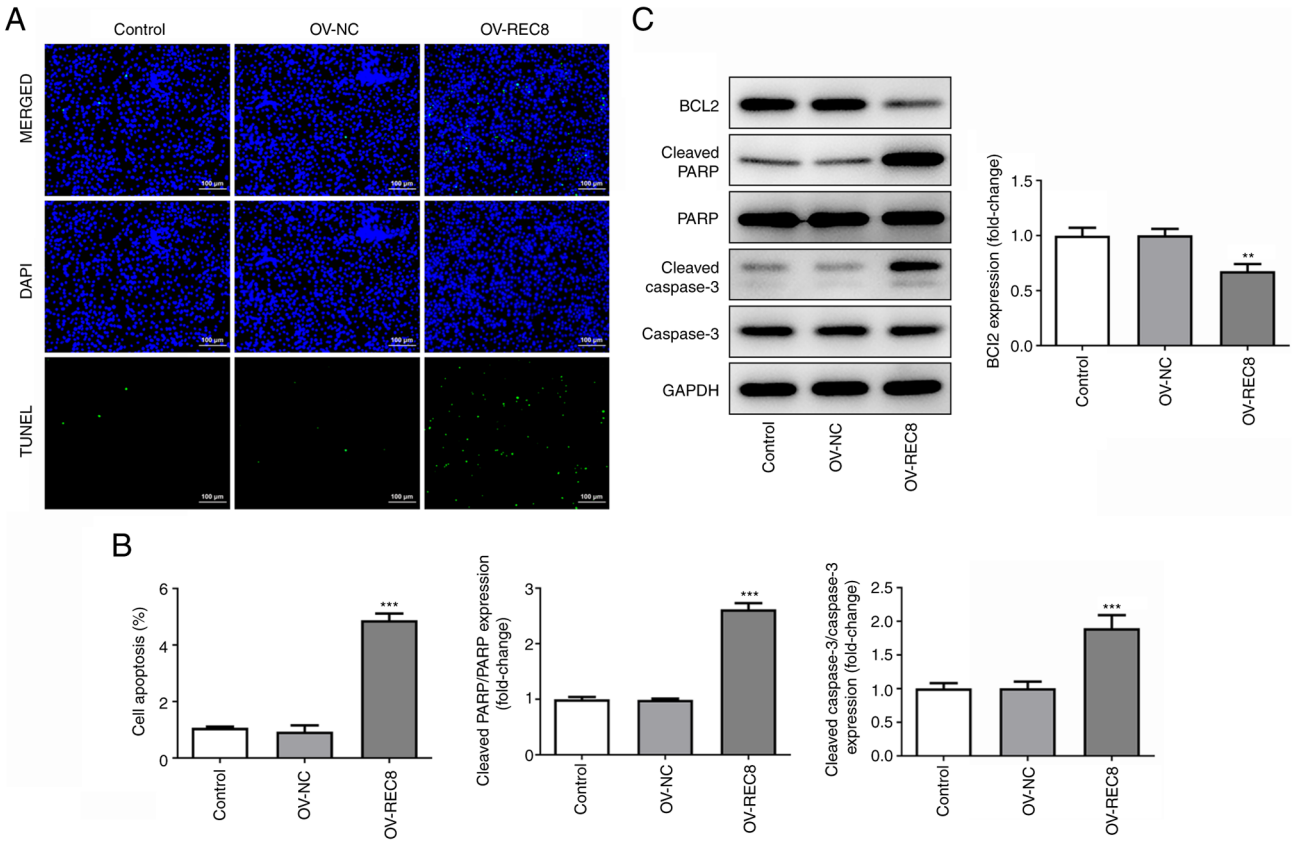

Figure 3. Overexpression of REC8 induces apoptosis in MCF-7 cells. (A) Effect of OV-REC8 on MCF-7 cell apoptosis were measured by TUNEL staining assay. (B) Statistical analysis of apoptotic rate. (C) Western blot analysis of Bcl2, cleaved PARP, PARP, cleaved caspase-3 and caspase-3 expression in MCF-7 cells. Data are presented as the mean ± SD and are representative of three independent experiments. **P<0.01, ***P<0.001 vs. OV-NC. OV, overexpression; PARP, poly(ADP-ribose) polymerase; NC, negative control.

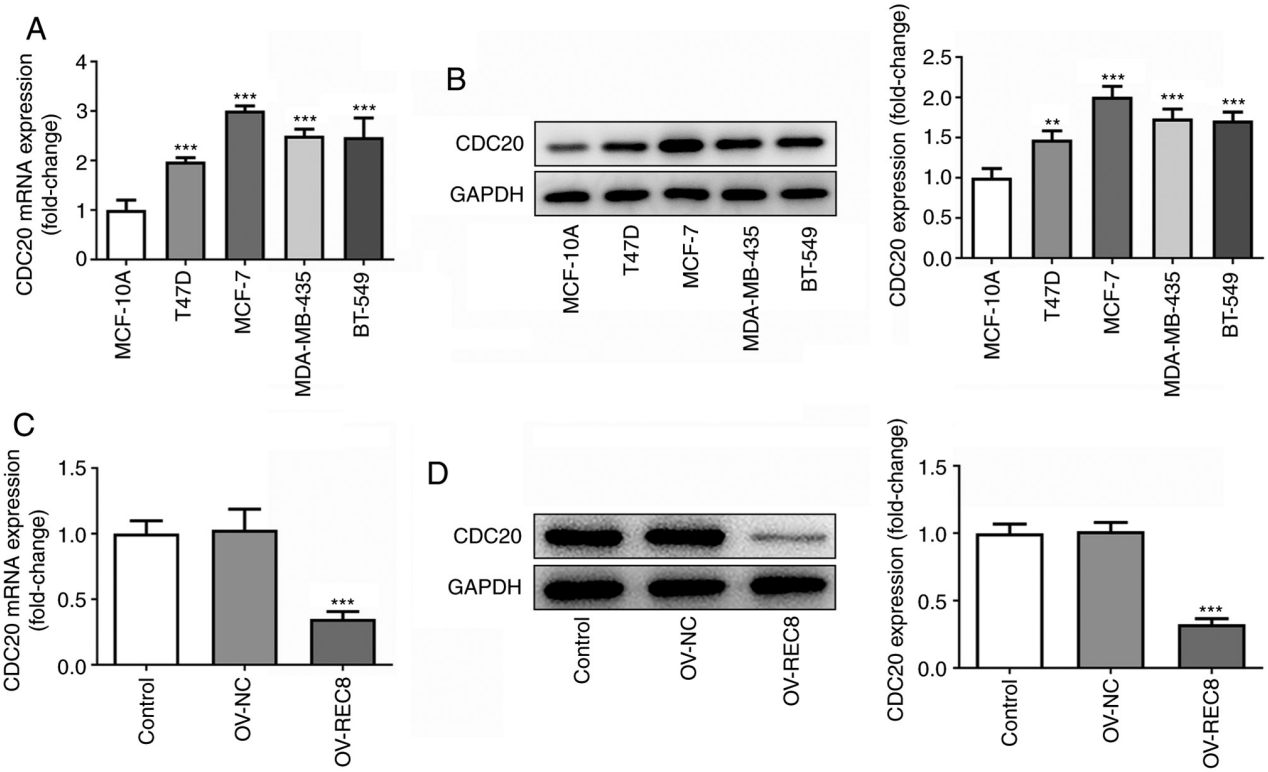

Figure 4. REC8 negatively regulates CDC20. (A and B) RT-PCR and western blot analysis of CDC20 expression in various breast cancer cell lines and (C and D) MCF-7 cells transfected with OV-NC and OV-REC8. Data are presented as mean ± SD. Results are representative of three independent experiments. **P<0.01, ***P<0.001 vs. OV-NC. CDC20, cell division cycle 20.

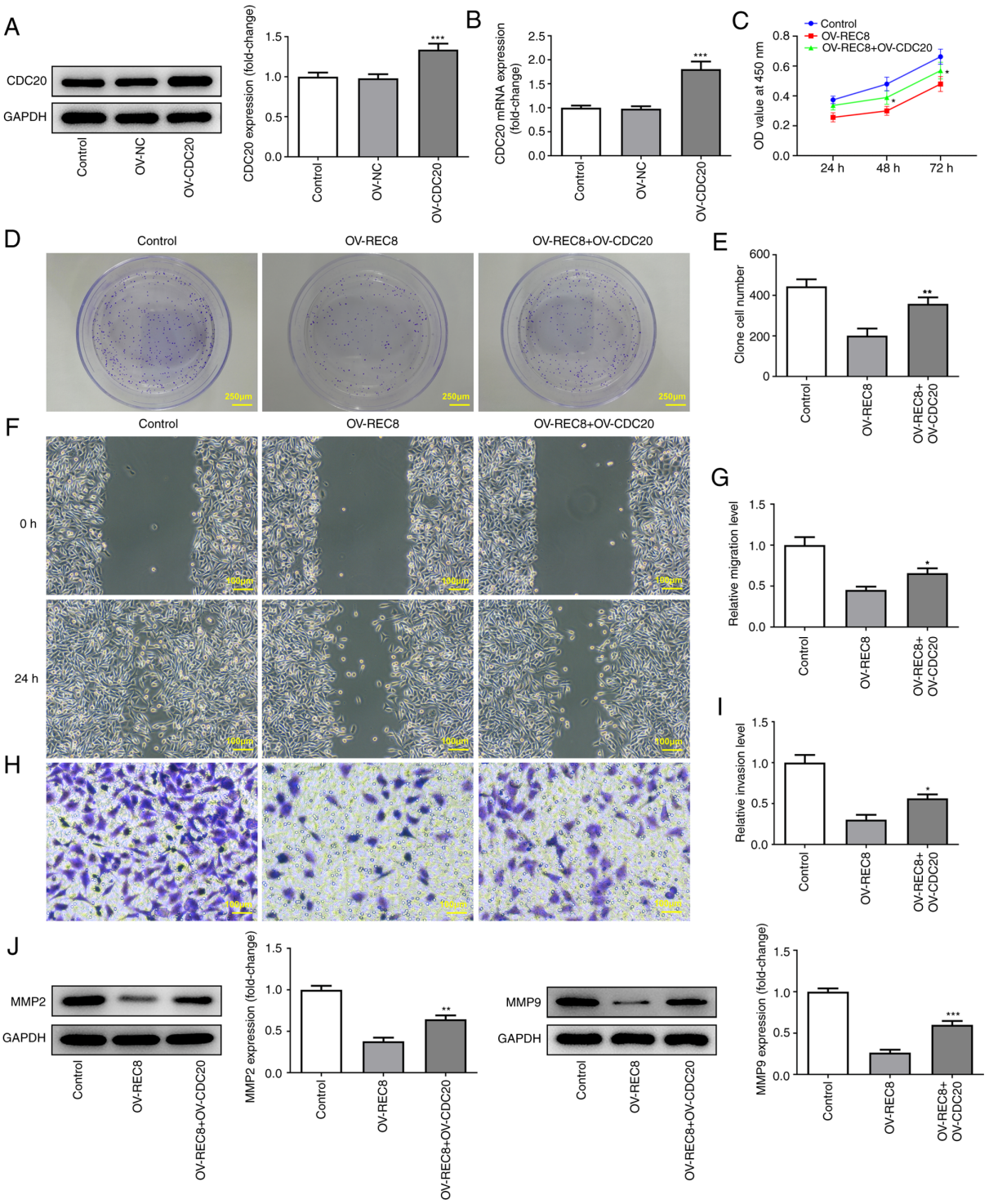

Figure 5. Overexpression of CDC20 reverses the inhibitory effect of REC8 overexpression on proliferation, migration and invasion of MCF-7 cells. (A) Western blot and (B) Reverse transcription-quantitative PCR analysis of CDC20 expression in MCF-7 cells. Cell (C) viability and (D) proliferation were measured by Cell Counting Kit-8 and (E) colony formation assay, respectively. Effect of OV-CDC20 on MCF-7 cell (F and G) migration and (H) invasion was determined by wound healing and (I) Transwell assay. (J) Western blot analysis of MMP2 and MMP9 expression in MCF-7 cells. Data are presented as the mean ± SD and are representative of three independent experiments. *P<0.05, **P<0.01, ***P<0.001 vs. OV-REC8. OV, overexpression; MMP, matrix metalloproteinase; NC, negative control; OD, optical density; CDC20, cell division cycle 20.

Results

REC8 expression is downregulated in breast cancer cells and tissue. Information on expression of REC8 in the serum of patients with breast cancer was obtained via GEPIA (http://gepia.cancer-pku.cn/) and KM Plotter (kmplot.com/analysis/index.php?p=service); low expression of REC8 was found to be positively associated with poor prognosis of patients with breast cancer (Fig. 1A and B). Therefore, the expression of REC8 in breast cancer cells was measured by RT-qPCR and western blotting. REC8 was downregulated in T47D, MCF-7, MDA-MB-231 and BT-549 breast cancer

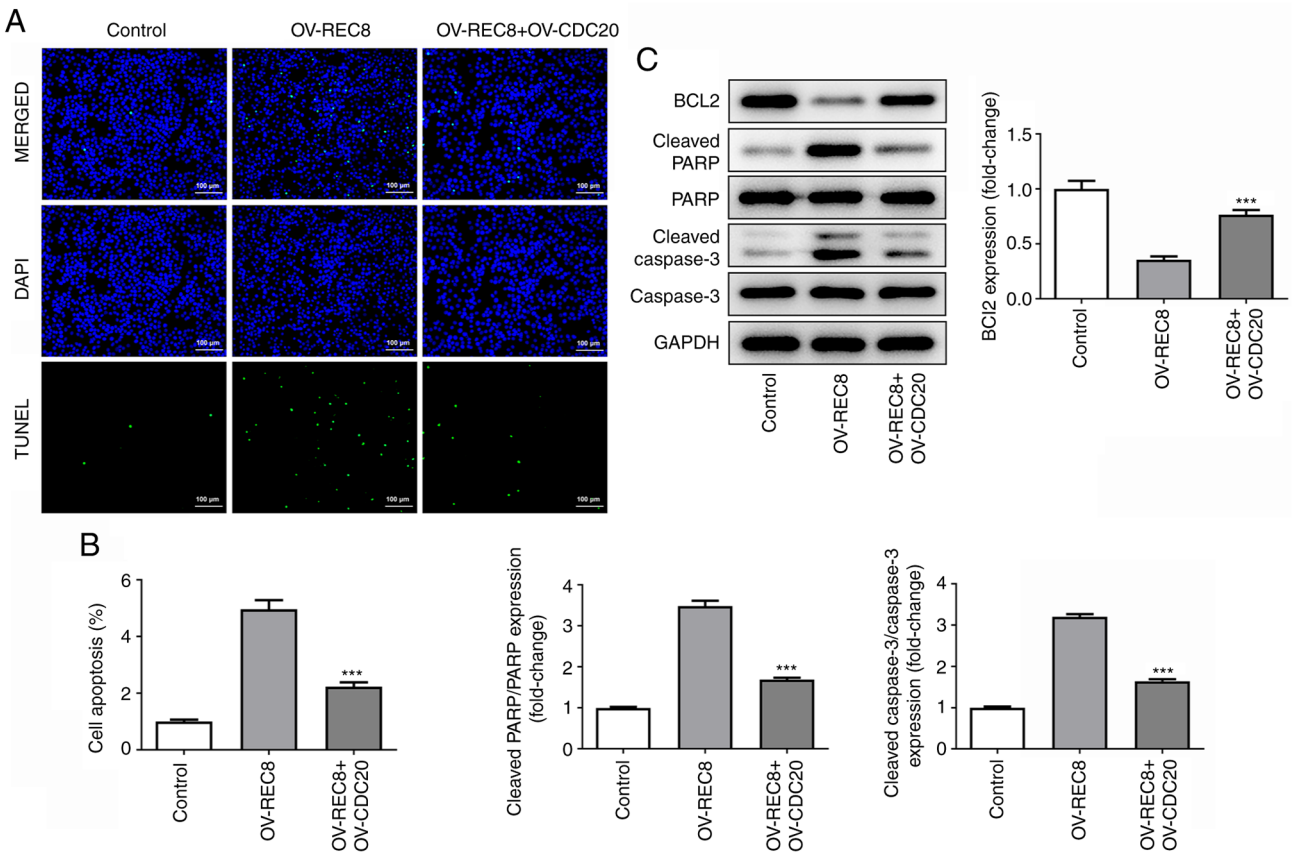

Figure 6. Overexpression of CDC20 reverses the inhibitory effect of REC8 overexpression on MCF-7 cell apoptosis. (A and B) Effect of OV-CDC20 on MCF-7 cell apoptosis was measured by TUNEL staining assay. (C) Western blot analysis of Bcl2, cleaved PARP, PARP, cleaved caspase-3 and caspase-3 expression in MCF-7 cells. Data are presented as the mean ± SD and are representative of three independent experiments. ***P<0.001 vs. OV-REC8. OV, overexpression; PARP, poly(ADP-ribose) polymerase; NC, negative control; CDC20, cell division cycle 20.

cell lines compared with the MCF-10A breast epithelial cell line (Fig. 1C and D). The expression of REC8 was lowest in MCF-7 cells. Thus, MCF-7 cells were selected for subsequent experiments. These results indicated that REC8 was down-regulated in breast cancer cells and tissue.

Overexpression of REC8 inhibits proliferation, migration and invasion of MCF-7 cells. To study the specific role of REC8 in breast cancer, REC8 was overexpressed in MCF-7 cells by transfection with pcDNA-REC8. Protein and mRNA expression levels of REC8 in the pcDNA-REC8 group were significantly higher compared with the control (Fig. 2A and B). CCK-8 assay showed that overexpression of REC8 significantly inhibited cell viability compared with the control at 48 and 72, but not 24, h (Fig. 2C). Similar results were obtained from the colony formation assay. Overexpression of REC8 significantly decreased the number of colonies compared with the control (Fig. 2D). In addition, cell migration and invasion were determined by wound healing and Transwell assay, respectively. The results demonstrated that overexpression of REC8 significantly decreased migration and invasion of MCF-7 cells compared with the control (Fig. 2E and F). Furthermore, expression levels of the invasion-associated factors MMP-2 and MMP-9 were detected by western blotting. Overexpression of REC8 significantly decreased expression of MMP-2 and MMP-9 compared with the control (Fig. 2G). Taken together, these findings indicated that overexpression of REC8 exerted an inhibitory effect on proliferation, migration and invasion of MCF-7 cells.

Overexpression of REC8 induces apoptosis in MCF-7 cells. The effect of REC8 on apoptosis of MCF-7 cells was detected by TUNEL assay and western blotting. TUNEL staining assay showed that overexpression of REC8 significantly enhanced apoptosis of MCF-7 cells (Fig. 3A and B). Compared with the control, overexpression of REC8 significantly decreased the expression levels of the anti-apoptotic protein Bcl-2 but promoted expression of the pro-apoptotic proteins cleaved caspase-3 and cleaved PARP (Fig. 3C). Taken together, these findings indicated that overexpression of REC8 induced apoptosis in MCF-7 cells.

REC8 negatively regulates CDC20 expression. To determine the mechanism underlying the function of REC8 in breast cancer, STRING was used to predict that CDC20 may interact with REC8 (data not shown). Analysis of the association between expression levels of REC8 and CDC20 in breast cancer cells demonstrated that CDC20 was highly expressed in MCF-7 cells (Fig. 4A and B) and overexpression of REC8 significantly inhibited the expression of CDC20 (Fig. 4C and D). Taken together, these findings indicated that REC8 may negatively regulate expression of CDC20 in MCF-7 cells.

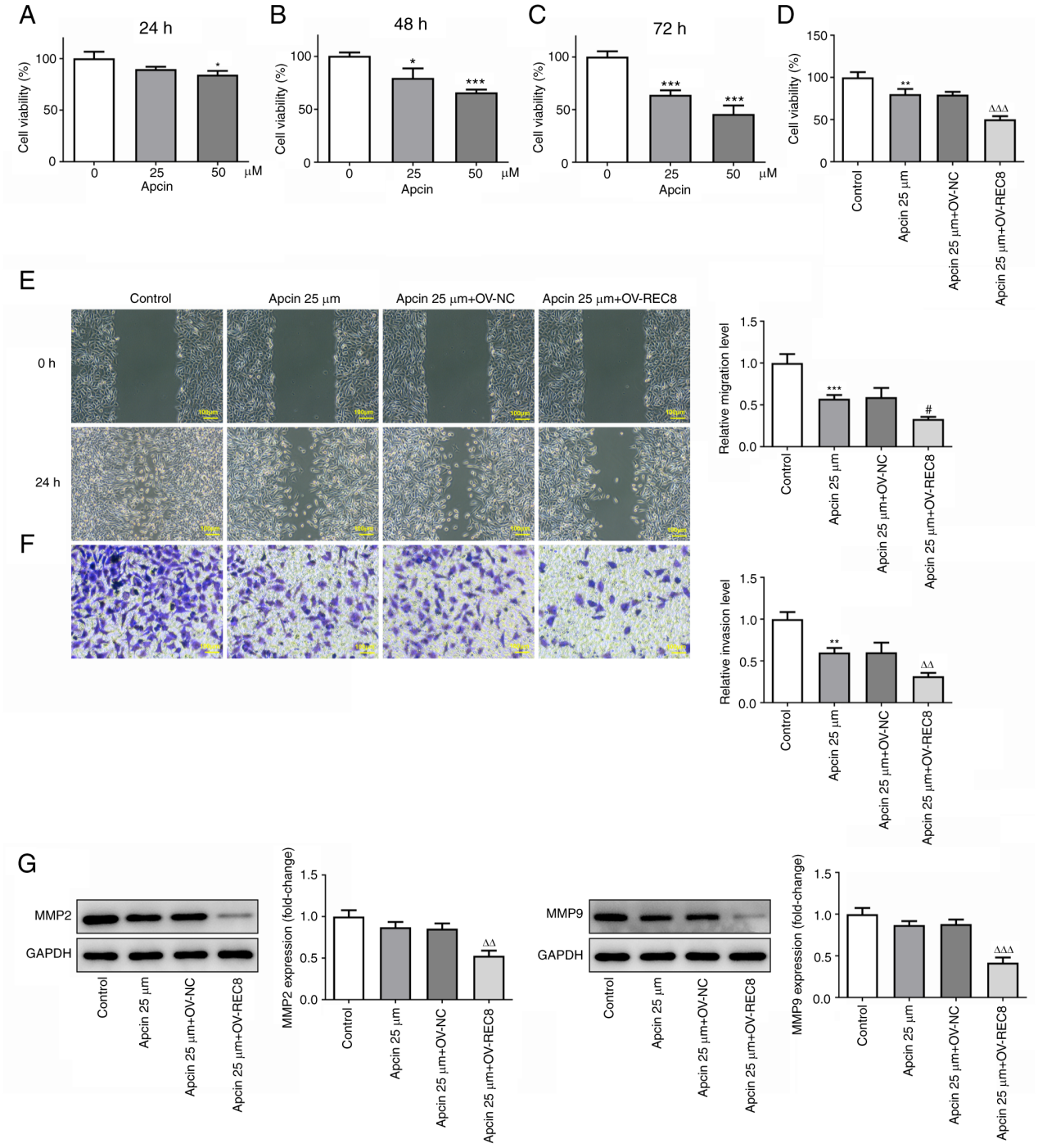

Figure 7. OV-REC8 and apcin inhibits MCF-7 cell proliferation, migration and invasion. MTT assay was used to detect MCF-7 cell viability after (A) 24, (B) 48 and (C) 72 h of treatment with apcin. (D) Effect of apcin + OV-REC8 on the viability of MCF-7 cells. Effect of apcin + OV-REC8 on MCF-7 cell (E) migration and (F) invasion were determined by wound healing and Transwell assays, respectively. (G) Western blot analysis of MMP2 and MMP9 expression in MCF-7 cells. Data are presented as the mean ± SD and are representative of three independent experiments. *P<0.05, **P<0.01, ***P<0.005 vs. Control; $^{\#}$P<0.05 vs. apcin + OV-NC; $^{\Delta\Delta}$P<0.01, $^{\Delta\Delta\Delta}$P<0.001 vs. apcin + OV-NC. OV, overexpression; MMP, matrix metalloproteinase; NC, negative.

REC8 suppresses proliferation, migration and invasion and induces apoptosis of MCF-7 cells by downregulating CDC20. To confirm whether REC8 serves an anticancer role by inhibiting CDC20, an overexpression plasmid of CDC20 was constructed; RT-qPCR and western blotting showed that expression levels of CDC20 increased in MCF-7 cells (Fig. 5A and B). CCK-8, clone formation assay, wound healing and Transwell experiments were performed to test the effect of overexpressed CDC20 on proliferation, invasion and migration of REC8-overexpressed cells. The results indicated that overexpressed CDC20 significantly reversed the inhibitory effect of overexpressed REC8 on cell proliferation, migration and invasion (Fig. 5C-J).

As determined by TUNEL staining, the green fluorescence of the OV-REC8 + OV-CDC20 group was significantly reduced compared with that in the OV-REC8 group, indicating that overexpression of CDC20 significantly reversed the pro-apoptotic effects of OV-REC8 (Fig. 6A and B). In

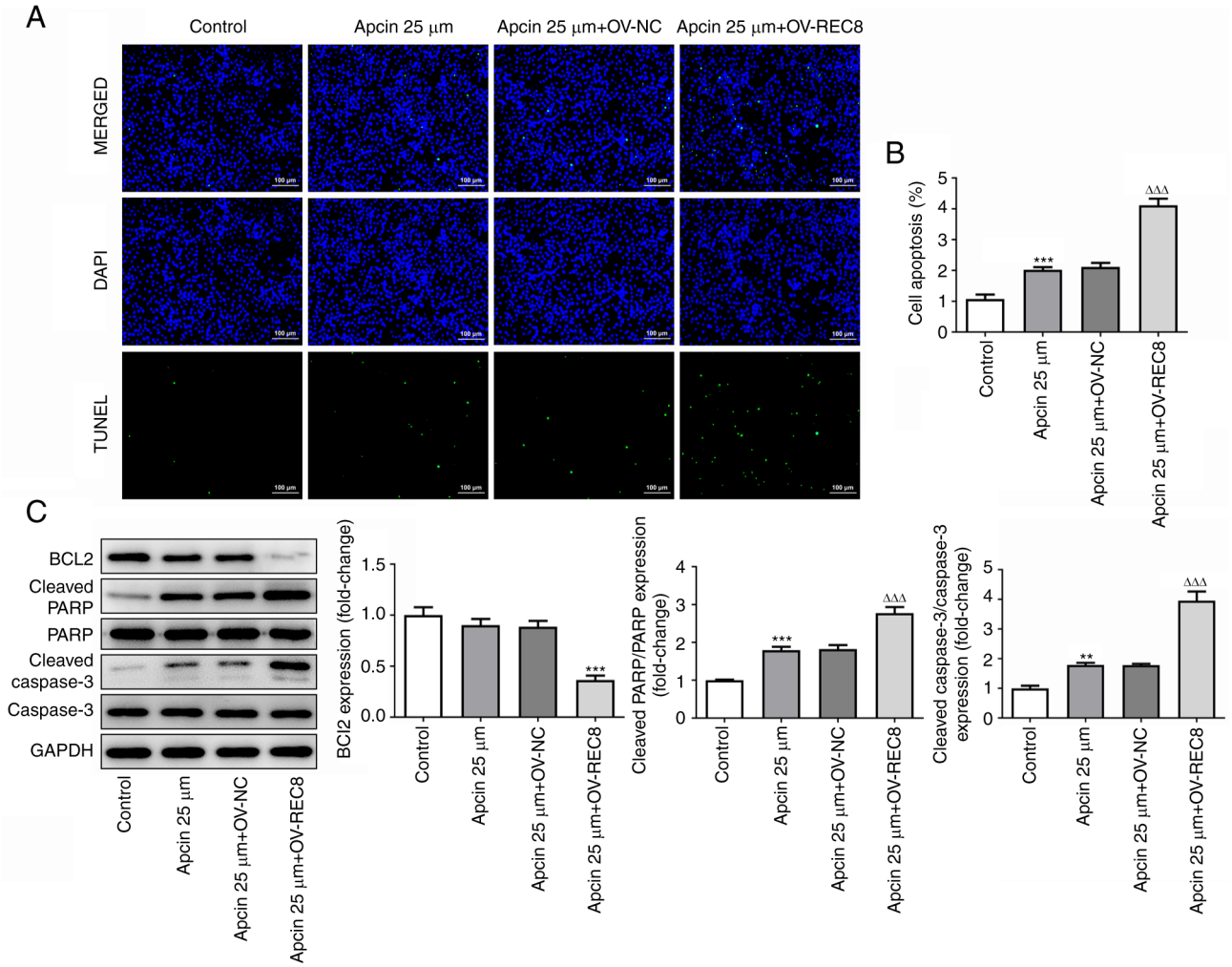

Figure 8. OV-REC8 and apcin promotes MCF-7 cell apoptosis. (A) Effect of apcin + OV-REC8 on MCF-7 cell apoptosis was measured by TUNEL staining assay. (B) Statistical analysis of apoptotic rate. (C) Western blot analysis of Bcl2, cleaved PARP, PARP, cleaved caspase-3 and caspase-3 expression in MCF-7 cells. Data are presented as the mean ± SD and are representative of three independent experiments. **P<0.01, ***P<0.001 vs. Control; $^{\Delta\Delta\Delta}$P<0.001 vs. apcin + OV-NC. CDC20, cell division cycle 20; NC, negative control; OV, overexpression; PARP, poly(ADP-ribose) polymerase.

addition, similar results were obtained when the expression levels of apoptosis-related proteins (cleaved-PARP, cleaved caspase-3 and Bcl-2) were further detected by western blot analysis (Fig. 6C). These results indicated that REC8 served an antitumor role by inhibiting CDC20.

Apcin + OV-REC8 exerts an antitumor effect. The effects of REC8 on proliferation, migration, invasion and apoptosis of MCF-7 cells were detected following treatment with CDC20 inhibitor apcin. The effect of apcin on cell viability after treatment for 24, 48 and 72 h was detected by MTT assay; the results showed that Apcin significantly inhibited the viability of MCF-7 cells in a concentration-dependent manner (Fig. 7A-C). Therefore, in subsequent experiments, 25 μM apcin was selected to treat cells for 48 h. Compared with apcin alone, apcin + OV-REC8 showed a significant inhibitory effect on MCF-7 cell viability (Fig. 7D). In addition, apcin + OV-REC8 effectively inhibited migration (Fig. 7E) and invasion (Fig. 7F) of MCF-7 cells. Futhermore, western blot analysis showed that compared with the Apcin 25 μM + OV-REC8 group, further overexpression of REC8 decreased the expression of migration-related proteins MMP2 and MMP9. TUNEL staining (Fig. 8A and B) and western blot (Fig. 8C) detection of apoptosis-associated proteins (Bcl-2, cleaved PARP and cleaved caspase-3) showed that apcin 25 μM + OV-REC8 significantly promoted MCF-7 cell apoptosis compared with apcin 25 μM + OV-NC. Taken together, these findings indicated that apcin + OV-REC8 exerted an antitumor effect.

Discussion

Breast cancer is the most common type of female hormone-dependent malignancy. There are ~1.05 million new cases of breast cancer annually, accounting for 23% of all female malignancies; this incidence continues to rise at a rate of 3.1% per year (18). Surgery, chemotherapy, radiotherapy, endocrine therapy and targeted therapy are the primary methods presently used in the clinical treatment of breast cancer, but ~500,000 cases of breast cancer-associated mortality are reported annually, accounting for 25% of all malignant tumor mortality (19). Therefore, the search for key molecules and genes involved in the progression of breast cancer is crucial for its treatment.

REC8 is a component of mucin specifically expressed during meiosis, which serves a key role in maintaining

the centromere and ensuring correct separation of chromosomes (6). There is a negative association between the expression of REC8 and tumor occurrence, development, deterioration and metastasis. Upregulation of REC8 is reported to inhibit the proliferation and colony formation of thyroid cancer cells (9). Among all types of thyroid cancer, the greatest hypermethylation of REC8 occurs in the most invasive anaplastic thyroid cancer, suggesting that REC8 may be a novel tumor suppressor gene in thyroid cancer (9). REC8 is also hypermethylated in Epstein-Barr virus-associated gastric cancer and overexpression of REC8 significantly decreases cell viability, proliferation and migration and induces early apoptosis to inhibit the occurrence and development of gastric cancer (10,20). In addition, recent findings demonstrated that REC8 inhibits proliferation and metastasis of gastric cancer cells by downregulating EGR1 to inhibit epithelial-to-mesenchymal transition in gastric cancer cells (10). As a novel tumor suppressor gene, REC8 has shown a good application prospect in several types of tumors (9,21). To the best of our knowledge, however, the role and mechanism of action of REC8 in breast cancer has not been studied to date. In the present study, REC8 expression was low in breast cancer cells and overexpression of REC8 significantly inhibited the proliferation, migration and invasion of MCF-7 cells and induced apoptosis. These results demonstrated the inhibitory effect of REC8 on breast cancer.

To study the specific mechanism of REC8 in breast cancer, STRING was used to show that CDC20 was associated with REC8. CDC20 is a key cell cycle regulatory molecule in mitosis and promotes mitosis by regulating ubiquitination and degradation of isolated inhibitor protein and cyclin B (22). In addition, CDC20 is upregulated in pancreatic cancer (23), lung cancer (24) and hepatocellular carcinoma (25), and predicts a poor prognosis. CDC20 not only affects proliferation of tumor cells, but expression of CDC20 is also positively associated with the invasive ability of breast cancer cells (15). To clarify the effect of the interaction between CDC20 and REC8 on the progression of breast cancer, the effect of CDC20 overexpression on proliferation, migration, invasion and apoptosis of MCF-7 cells overexpressing REC8 was assessed. The results showed that overexpression of CDC20 significantly reversed the inhibitory effect of REC8 overexpression on proliferation, migration and invasion of MCF-7 cells and inhibited apoptosis of MCF-7 cells, indicating that REC8 exerted an antitumor effect by inhibiting CDC20. Furthermore, the present study demonstrated that the combination of apcin and OV-REC8 effectively inhibited the viability, proliferation, migration and invasion of MCF-7 cells and promoted cell apoptosis.

In conclusion, the present study demonstrated that REC8 inhibited proliferation, migration and invasion and induced apoptosis of breast cancer cells by downregulating the expression of CDC20. To the best of our knowledge, the present study is the first to propose a mechanism by which REC inhibits breast cancer by regulating CDC20. However, the present study did not determine the molecular mechanism underlying REC8 inhibition of CDC20 expression. It is also necessary to verify the present results using other breast cancer cell lines as well as *in vivo* animal models. In addition, the role of apcin in REC8 knockdown cells will be investigated in future.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ZHC and SDH conceptualized and designed the current study. DPL, ZHC and SDH acquired, analyzed and interpreted data. ZHC and SDH drafted the manuscript and revised it critically for important intellectual content. All authors agreed to be held accountable for the current study in ensuring questions related to the integrity of any part of the work are appropriately investigated and resolved. ZHC and SDH confirm the authenticity of all the raw data. All authors read approved the final 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.

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