Histol Histopathol (2020) 35: 1171-1180

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

LncRNA AY343892 inhibits breast cancer development by positively regulating BRCA1-mediated transcription of PTEN

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Summary. Breast cancer remains a major challenge despite dramatic advances in cancer research. The long non-coding RNA (lncRNA) has been reported to associate with carcinogenesis and progression of various cancers. In this research, we found that lncRNA AY343892 was significantly down-regulated in breast cancer tissues and cells. Besides, breast cancer patients with high AY343892 level exhibited a favorable prognosis. Functional assays indicated that overexpression of AY343892 significantly inhibited proliferation and promoted apoptosis in breast cancer cells. In terms of mechanism, PTEN and BRCA1 were confirmed to be regulated by AY343892 in breast cancer. Luciferase activity and chromatin immunoprecipitation (ChIP) assays indicated that AY343892 can regulate the promoter of PTEN by binding to BCRA1. Further investigation suggested that knockdown of AY343892 significantly promoted MDA-MB-231 cell proliferation and inhibited MDA-MB-231 cell apoptosis. However, these effects were reversed when PTEN was up-regulated. Moreover, PTEN silence can also countervail the inhibitory effect of overexpressed BCRA1 or AY343892 on the expressions of genes related to proliferation and apoptosis in breast cancer. In conclusion, this study illustrated that AY343892 inhibited breast cancer development by positively regulating BRCA1-mediated transcription of PTEN. This finding contributes to a better understanding in the pathogenesis of breast cancer and provides a theoretical basis for the treatment of breast cancer patients.

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DOI: 10.14670/HH-18-245

Key words: LncRNA AY343892, PTEN, BRCA1, Breast cancer

Introduction

Breast cancer is the most commonly-diagnosed cancer and is the second most common cause of cancer-related mortality in women worldwide. The American Cancer Society estimates that breast cancer will account for 30% of new cancer cases and 15% of cancer deaths among females in 2019 (Cancer facts and figures 2019, 2019). Despite the rapid development of new biomarkers for early breast cancer diagnosis and multimodal treatment strategies, the 5-year survival rate of breast cancer patients remains unsatisfying (Beck et al., 2019). Therefore, it is necessary to explore the pathogenesis of breast cancer and find new therapeutic strategies.

Transcription in the human genome is universal. It is worth noting that although less than 2% of the human genome encodes proteins coding exons, about 75% of that is transcribed into RNA under different conditions (Djebali et al., 2012). There are many classes of RNAs that play important roles in cellular processes in this non-coding transcriptome, such as mRNA translation (transfer RNAs and ribosomal RNA), or post-transcriptional regulation of gene expression (microRNAs). In recent years, long noncoding RNAs (lncRNAs), as a relatively new class of non-coding RNAs, have become a major focus of biomedical research. LncRNA is a kind of heterogeneous transcription product that exerts different functions in mammalian cells. LncRNA is a heterologous transcript defined by a sequence length of more than 200

nucleotides and has no ability to encode proteins. These transcriptional products may come from intergenomic regions, enhancers and promoter sequences, or genomic loci that overlap with other genes in the form of sensory or antisense transcriptional products (Derrien et al., 2012). Their molecular functions can be generally divided into two categories: (1) lncRNAs that act in cis regulate the state of chromatin and the expression of adjacent genes in the nucleus, and (2) lncRNAs that leave the transcription site and act in trans, interacting with other nucleic acids or proteins to perform various functions throughout the cell (Ulitsky and Bartel, 2013; Engreitz et al., 2016; Kopp and Mendell, 2018). Although the number of identified lncRNA genes has steadily increased, estimated at tens of thousands (Harrow et al., 2012), the biological function of most lncRNAs remains unclear. In our previous study, we found that lncRNA AY343892 played an important role in the progress of breast cancer, and its underlying mechanism might relate to PTEN and BRCA1.

The occurrence of breast cancer is influenced by complex genetic factors, living and growing environment synergism. Currently, the BRCA1 gene is considered to be an important tumor suppressor gene closely related to the occurrence and development of breast cancer, which plays an important role in DNA damage repair, cell cycle regulation, cell proliferation and differentiation (Jiménez et al., 2012). A study indicated that BRCA1 mutation increased the initiation risk of breast, ovarian and bladder cancers, and increased the lifetime risk by up to 80% (Al-Moghrabi et al., 2014). In addition, BRCA1 gene mutation in breast cancer patients is more likely to occur in high-risk groups such as familial breast cancer, early-onset breast cancer, and triple-negative breast cancer (Shastry and Yardley, 2013).

PTEN, also known as TEP1 (TGF-β-regulated and epithelial cell-enriched phosphatase), is localized on chromosome 10q23 and is a well-known tumor suppressor gene. In breast tumors, PTEN is an important factor, and the decrease of PTEN expression is associated with tumor cell invasiveness and poor prognosis of patients (Depowski et al., 2001; Heikkinen et al., 2011). Expression of PTEN protein is precisely regulated by transcriptional and post-transcriptional modifications, including epigenetic silencing, microRNA (miRNA) regulation, abnormal localization of PTEN and regulation of proteins interacting with PTEN. In a mouse model, it was found that the expression of PTEN affects the occurrence of breast cancer (Alimonti et al., 2010). The loss of PTEN is closely related to death and lymph node metastasis in breast cancer patients (Depowski et al., 2001). In addition, PTEN is also involved in breast cancer target therapies, including endocrine therapy and humane epidermal growth factor receptor-2 (HER2) target therapy (Fu et al., 2014; Nagata et al., 2004).

In a recent study, we identified a potential tumor suppressor, lncRNA AY343892, in breast cancer, which can regulate the expression of PTEN via BRCA1 to inhibit the proliferation of breast cancer.

Material and methods

Tissue samples

Human breast cancer tissues and adjacent normal breast tissues were obtained from breast cancer patients who received surgical resection at Anqing Municipal Hospital (Anhui, China). None of the patients received treatment prior to the surgery. The collected tissues were snap-frozen immediately in liquid nitrogen and stored at -80°C. All patients have signed written informed consent and this study was approved by the Ethics Committee of Anqing Medical College (Anhui, China).

Cell culture

The human breast cancer cell lines MDA-MB-231, MDA-MB-468, MCF-7, BT-474, SK-BR-3, UACC-3199 and human normal breast epithelial cell MCF10A were used in this study. All cells were purchased from Cell Bank of Shanghai Institute of Biochemistry and Cell Biology (China). MDA-MB-231, MDA-MB-468 and UACC-3199 were cultured in Leibovitz's L-15 Medium (Thermo Fisher Scientific, Waltham, MA, USA), supplemented with 10% fetal bovine serum (FBS, Gibco-BRL, Carlsbad, CA, USA). SK-BR-3, MCF7 and MCF10A were cultured in Dulbecco's modified Eagle's medium (DMEM, Thermo Fisher Scientific, Waltham, MA, USA), supplemented with 10% FBS (Gibco-BRL, Carlsbad, CA, USA). BT-474 was cultured in RPMI-1640 medium (Thermo Fisher Scientific, Waltham, MA, USA), supplemented with 10% heat-inactivated FBS (Gibco-BRL, Carlsbad, CA, USA). All cells were maintained in a humidified atmosphere of 5% CO_2 at 37°C.

Lentiviral vector production and infection

AY343892, PTEN and BRCA1 lentivirus vectors and empty vectors were obtained from Sangon Biotech (Shanghai, China). Lentivirus infection was performed according to the manufacturer's instructions. Cells were treated with 5 μg/mL polybrene (GenePharma Biotechnology) at 70% confluence before lentivirus infection, and the cells were transferred to fresh medium after 24 h. Cells with stable expression were selected using puromycin (Sigma, USA).

RNA isolation and quantitative reverse transcription-polymerase chain reaction (RT-qPCR)

Total RNA was extracted from cells using RNAiso Plus (TAKARA Biotechnology, Japan) according to the manufacturer's instructions. To measure AY343892,

phosphatase and tensin homolog (PTEN), BRCA1, Cyclin A1, CDK2, Bax and Bcl-2 mRNA expression, RT-qPCR was performed using a SYBR Green-based PCR kit (TAKARA Biotechnology, Japan). An ABI Step-One Plus Real Time PCR System (Applied Biosystems, Foster City, CA, USA) was used for the quantitative detection of lncRNA and mRNA. The expression of each gene was quantified by measuring the cycle threshold (Ct) values and normalized using the $2^{-\Delta\Delta Ct}$ method compared with GAPDH or 18S RNA levels. All the primers used are listed as follow:

AY343892: F: 5'-TGCGAAAGAAGTTTGTGTG G-3', R: 5'-GGGGAGGGGCAATACATAAT-3'; 18S RNA: F: 5'-CGTTCTTAGTTGGTGGAGCG-3', R: 5 '-CCGGACATCTAAGGGCATCA-3'; PTEN: F: 5'-CTGTAAAGCTGGAAAGGGACG-3', R: 5'-GGAA TAGTTACTCCCTTTTTGTCTC-3'; Cyclin A1: F: 5'-CTTAACCGCGATCCTCCAGTG-3', R: 5'-AGGG TACATGATTGCGGGAA-3'; CDK2: F: 5'-GACAC GCTGCTGGATGTCA-3', R: 5'-CAGAAAGCTA GGCCCTGGAG-3'; Bax: F: 5'-AGCGACTGATG TCCCTGTCT-3', R: 5'-CTTCCAGATGGTGAGT GAGGC-3'; Bcl-2: F: 5'-CTCTGCTCAGTTTG GCCCTG-3', R: 5'-CCTGCAGCTTTGTTTCATGGT-3'; GAPDH: F: 5'-ATGGGCAGCCGTTAGGAAAG-3', R: 5'-ATCACCCGGAGGAGAAATCG-3'.

Western blotting assay

Total proteins were extracted with Radio-Immunoprecipitation Assay (RIPA) buffer. Proteins extracted from cells were separated by sodium dodecyl sulfate-polyacrylamide gel electroph-oresis (SDS-PAGE; Beyotime Institute of Biotechnology, Shanghai, China) and transferred onto polyvinylidene fluoride (PVDF, Beyotime Institute of Biotechnology) membranes. The PVDF membranes were incubated at 4°C overnight with the following primary antibodies: anti-Cyclin A1 (1:1000), anti-CDK2 (1:1000), Bax (1:1000) and Cleaved caspase-3 (1:1000), anti-phosphorylated PI3K (anti-p-PI3K), anti-PI3K, anti-phosphorylated AKT (anti-p-AKT), anti-AKT, anti-phosphorylated NF-κB p65 (anti-p-NF-κB p65), anti-NF-κB p65, anti-PTEN (Proteintech Group, Rosemont, IL, USA). The membranes were washed with PBST and then incubated with a horseradish peroxidase (HRP)-linked secondary antibody (anti-rabbit IgG, 1:1000, Proteintech). Signals were detected by incubating the membranes with SuperSignal West Pico Chemiluminescent substrate (Thermo Fisher Scientific, MA, USA) followed by exposure to film.

Colony formation assay

Cells were plated in 6-well plates (0.5×10^3 cells/well) in the medium for about 2 weeks followed by the treatment with 10% formaldehyde for 5 min. Cells were stained with 1% crystal violet for 1 min before calculating the number of colonies.

Cell viability assay

Cell viability was assessed with CCK8 assay (KeyGen Biotech, Nanjing, China). Briefly, cells (2×10^3 cells/well) were cultured overnight in 96-well plates. Then, the medium was replaced with fresh medium. After incubation for 24 h, 48 h, 72 h, 10 μL CCK8 reagent was added to each well and the cells were incubated for 4 h at 37°C. The absorbance was recorded at 450 nm wavelength using a microplate reader (Multiskan™ GO microplate spectrophotometer; Thermo Fisher Scientific, Inc.). Cell viability was calculated according to the following formula:

Cell viability (%) = (OD treatment - OD blank)/(OD control - OD blank).

Apoptosis assay

Breast cancer cells were seeded in 6-well plates (Corning, USA). After incubation for 24 h, cells (5×10^5 cells/mL) were resuspended and mixed in 500 μL of a binding buffer with 5 μL of Annexin V-FITC and 5 μL of PI using Annexin V/PI Cell Apoptosis Detection Kit (KeyGen Biotech, Nanjing, China). After incubation for 15 min, data acquisition and analysis were performed with a Becton Dickinson FACS Calibur flow cytometer using Flowjo 7 software at Ex./Em. 488/530 nm.

Subcellular fractionation assay

According to the supplier's suggestions, a PARIS™ Kit (Invitrogen) was utilized to conduct subcellular fractionation assay. RNAs in the cytoplasm or nucleus of MDA-MB-231 cells were separated, extracted, and purified. Then, the expression of AY343892 was evaluated by RT-qPCR, normalized to U6 (nucleus control) and GAPDH (cytoplasm control).

Luciferase reporter assay

The promoter binding sites in the PTEN sequences were predicted by hTF-target. The pcDNA, pcDNA-BRCA1, pcDNA-AY343892, pcDNA-sh-NC and pcDNA-shAY343892 were transfected into the MDA-MB-231 cells (Sangon Biotech, Shanghai, China). For the luciferase reporter assay, MDA-MB-231 cells were plated onto 96-well plates and transfected with PTEN promoter and indicated plasmids using Lipofectamine 2000. The cells were harvested and analyzed by a luciferase reporter assay system (Promega, Madison, WI, USA) after transfection for 48 h, according to the manufacturer's instructions. The experiment was performed in triplicate, and the data are expressed as the means ± SD.

RNA binding protein immunoprecipitation (RIP) assay

MDA-MB-231 cells were harvested by trypsinization, resuspended in PBS and nuclear isolation

buffer, and kept on ice (20 min, frequent mixing). Then, the nuclei were isolated by centrifugation (2,500 rpm, 15 min), and resuspend in RIP buffer (1 mL). The resuspended nuclei were divided into two parts, and each part was 500 mL (for Mock and IP). The cells were gently homogenized (15-20 strokes) using a motor pestle homogenizer (Kimble-Kontes). The nuclear membranes and debris were precipitated by centrifugation (13,000 rpm, 10 min). Then, antibody of protein (2 to 10 μg) was added to the supernatant (6 mg to 10 mg). Incubated at 4°C overnight with gentle rotation, unbound antibody was washed off. Pellet beads (2,500 rpm, 30s) were removed from the supernatant, and then were resuspended in RIP buffer. Later, the beads were given a total of 3 RIP washes followed by 1 wash in PBS. The coprecipitated RNA was isolated by resuspending beads in TRIzol RNA extraction reagent (1 mL) according to the manufacturer's instructions, and eluting RNA with nuclease-free water. The protein isolated by the beads was detected by western blot. Finally, the DNAse-treated RNA was reverse transcribed according to the manufacturer's instructions.

Chromatin immunoprecipitation (ChIP) assay

The ChIP assay was carried out by use of the ChIP Assay Kit (Beyotime, China). Briefly, MDA-MB-231 cells were cross-linked with 1% formaldehyde solution for 10 min at room temperature and sonicated to create 200-500 bp fragments. Then, the lysate was immunoprecipitated with anti-BRCA1 or IgG antibodies. Finally, immunoprecipitated DNAs were subjected to RT-qPCR.

Statistical analysis

All data shown represent mean ± SD from triplicate experiments performed in a parallel manner unless otherwise indicated. All comparisons of data were made using Student's t-test or ANOVA. The survival curves were calculated and statistically compared using the Kaplan-Meier method and a log-rank test, relatively. $P<0.05$ was considered to be statistically significant.

Results

LncRNA AY343892 was down-regulated in breast cancer

According to the Oncomine database, an online tool which displayed the expression profiles of breast cancer and adjacent normal tissues at stage IV in breast cancer patients, the expression of lncRNA AY343892 was markedly down-regulated in breast cancer tissues (Fig. 1A). Later, we used the TCGA database to analyze AY343892 expression in patients with different types of breast cancer. The results indicated that the expression of AY343892 was significantly down-regulated in the type of Luminal A (LumA), Luminal B (LumB), Basal-like breast cancer and HER2 positive breast cancer compared with that in normal-like tissues (Fig. 1B). To determine the clinical relevance of AY343892 expression in patients with breast cancer, we analyzed the expression of AY343892 in patients' clinical samples. It was found that most samples showed a lower level of AY343892 than corresponding adjacent normal tissues (Fig. 1C). Importantly, we found that breast cancer patients with high AY343892 expression displayed a longer survival compared with patients with low AY343892 expression (Fig. 1D). In addition, most breast cancer cells (MDA-MB-231, MDA-MB-468, MCF-7, and SK-BR-3) also exhibited lower expression of AY343892 in comparison with normal mammary cells (MCF-10A) (Fig. 1E). Collectively, these findings suggested that AY343892 was lowly expressed in breast cancer tissues and cells and closely correlated with the prognosis of breast cancer.

AY343892 inhibited cell proliferation and induced apoptosis in breast cancer cells

Subsequently, we aimed to explore the biological function of AY343892 in breast cancer. To confirm this, an MDA-MB-231 cell line exhibiting the lowest expression of AY343892 was chosen to transfect pcDNA and pcDNA-AY343892. As a result, AY343829 expression was increased in pcDNA-AY343892 transfected cells (Fig. 2A). Then, a colony formation assay was performed to evaluate the colony-formation ability upon pcDNA-AY343892 transfection. The results indicated that overexpressed AY343892 inhibited the ability of colony-formation in MDA-MB-231 cells (Fig. 2B,C). Later, a CCK-8 assay was conducted to determine the proliferation of MDA-MB-231 cells transfected with pcDNA-AY343892 or pcDNA. The results revealed that pcDNA-AY343892 transfection significantly suppressed the proliferation of MDA-MB-231 cells (Fig. 2D). Furthermore, flow cytometry was performed by Annexin V-FITC/PI staining to evaluate the effect of AY343892 on cell apoptosis. As we observed, pcDNA-AY343892 transfected cells showed higher apoptosis rate than pcDNA transfected cells (Fig. 2E,F). Finally, expressions of apoptosis-relevant proteins (Bax and cleaved caspase-3) and proliferation-related proteins (Cyclin A1 and CDK2) in pcDNA-AY343892 group and pcDNA group were tested by western blot analysis. The results showed that levels of Cyclin A1 and CDK2 were decreased, while expressions of Bax and cleaved caspase-3 were upregulated in pcDNA-AY343892 transfected cells (Fig. 2G,H). These results indicated that the up-regulation of AY343892 inhibited proliferation and induced apoptosis in MDA-MB-231 cells.

AY343892 was involved in the BRCA1-PTEN signaling pathway to regulate PTEN transcription in breast cancer

Next, we explored the underlying mechanism of lncRNA AY343892 in breast cancer. It has been known that abnormal expression of PTEN gene was closely

related to breast cancer, and PTEN inhibited cell proliferation in breast cancer. Through RT-qPCR analysis, we found that AY343892 overexpression elevated the mRNA level of PTEN (Fig. 3A). Further analysis of subcellular localization revealed that AY343892 was mainly distributed in the nucleus (Fig. 3B). Thus, we speculated that AY343892 could regulate PTEN expression at a transcriptional level. To explore whether AY343892 regulates the activity of PTEN promoter, we performed luciferase activity assay. The results indicated that the activity of PTEN promoter was increased when AY343892 was overexpressed (Fig. 3C). Based on the above results, we concluded that AY343892 is able to increase PTEN transcription.

BRCA1 was previously reported to be an anticancer gene and negatively regulate tumor growth (Zhang et al., 2015). It plays an important role in DNA damage repair, transcriptional activation and inhibition, and cell cycle regulation. The polymorphism of this gene can lead to changes in the hyperplasia of breast epithelial cells, and then promotes the progress and development of breast cancer. In our study, we found that BRCA1 was able to bind to PTEN promoter and AY343892, which was predicted by hTF-target. To verify this prediction, anti-BRCA1 was used for RIP assay to capture RNA sequences which bound to BRCA1. The results indicated that the expression of AY343892 was higher in the anti-BRCA1 group than in the anti-IgG group (Fig. 3D). Moreover, ChIP assay disclosed that the level of PTEN promoter was increased in anti-BRCA1 group instead of anti-IgG group, indicating BRCA1 is able to bind to PTEN promoter in breast cancer (Fig. 3E). Then, we transfected pcDNA-BRCA1, pcDNA-AY343892, or shAY343892 into breast cancer cells to determine the mRNA level of PTEN. The results showed that BRCA1 is able to increase PTEN mRNA level, and AY343892 overexpression further strengthened the effect of BRCA1 on PTEN mRNA level, while silencing AY343892 weakened the effect of BRCA1 on mRNA level of PTEN (Fig. 3F). We also detected PTEN promoter activity by luciferase activity assay. The results indicated that BRCA1 is able to increase the activity of

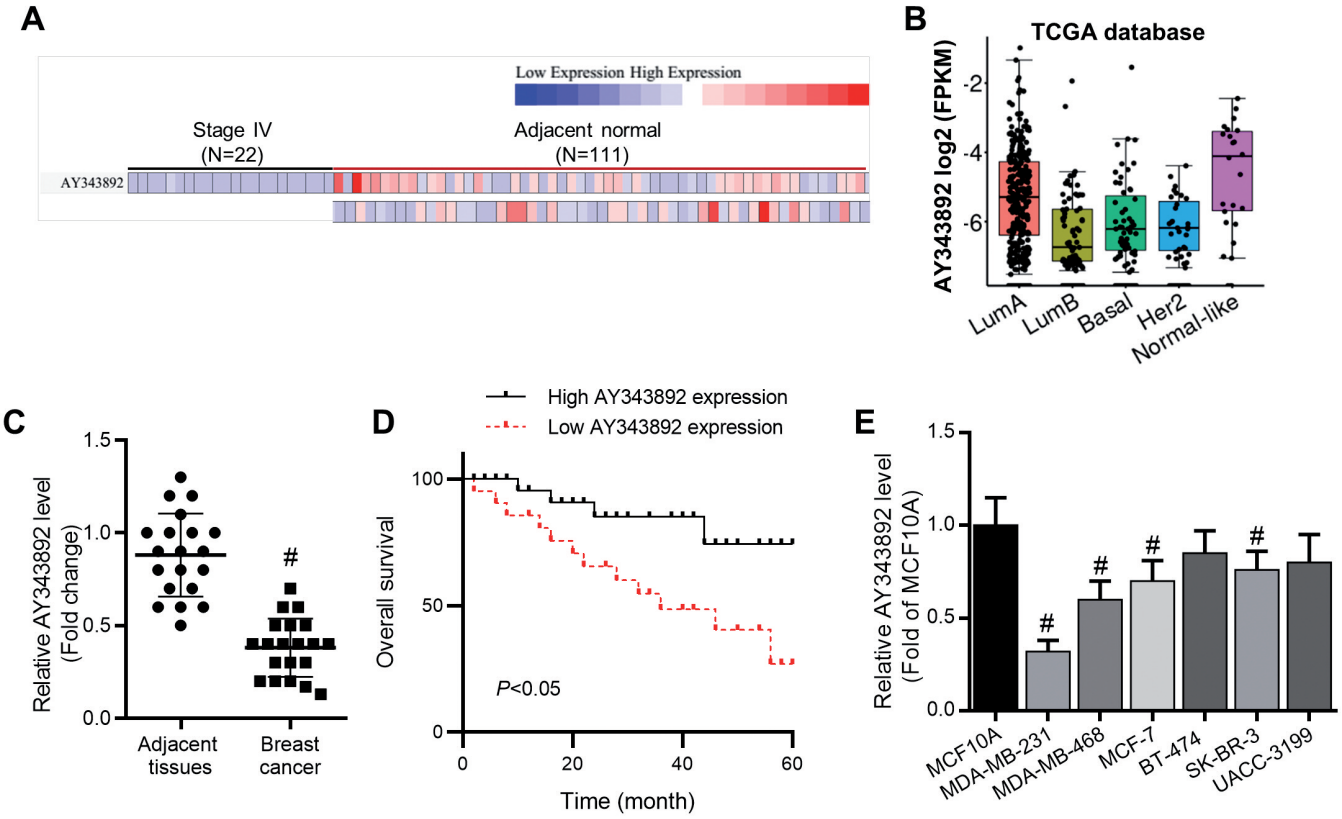


Fig. 1. Down-regulation of lncRNA AY343892 is relevant to breast cancer. **A.** Oncomine dataset analyses of AY343892 expression in breast cancer tissues and adjacent normal tissues. **B.** The expression of AY343892 in different types of breast cancer in TCGA database. **C.** RT-qPCR was conducted to evaluate the expression of AY343892 in breast cancer tissues and adjacent tissues. #p<0.05 was compared with adjacent tissue groups. **D.** The overall survival rate of breast cancer patients with high or low AY343892 expression. **E.** RT-qPCR was performed to detect the expression of AY343892 in breast cancer cells and MCF10A cell line. #p<0.05 was compared with MCF10A cell line. Data are shown as means ± SD for three independent experiments.

PTEN promoter. The impact of BRCA1 was elevated by AY343892 up-regulation and declined by AY343892 silencing (Fig. 3G).

It was well known that PTEN can negatively regulate PI3K/AKT/NF-κB signaling pathway, and thereby inhibit cancer progression (Zhang et al., 2015). To further verify whether AY343892 regulated PTEN-mediated signaling in breast cancer, we detected levels of PTEN protein, NF-κB singling-relevant proteins (NF-κB, p-NF-κB) and PTEN-mediated proteins (PI3K, p-PI3K, AKT, p-AKT) in MDA-MB-231 cells transfected with pcDNA-AY343892. The results illustrated that AY343892 overexpression increased PTEN protein level, decreased p-PI3K, p-AKT and p-NF-κB protein levels, while protein levels of PI3K, AKT and NF-κB were unchanged (Fig. 3H). Taken together, these findings demonstrated that AY343892 increased PTEN transcription by regulating BRCA1.

AY343892 inhibited breast cancer cell proliferation and induced apoptosis by up-regulating PTEN expression

To further confirm the role of AY343892/BRCA1/PTEN axis in breast cancer, we performed rescue assays. The results of the colony formation assay indicated that knockdown of AY343892

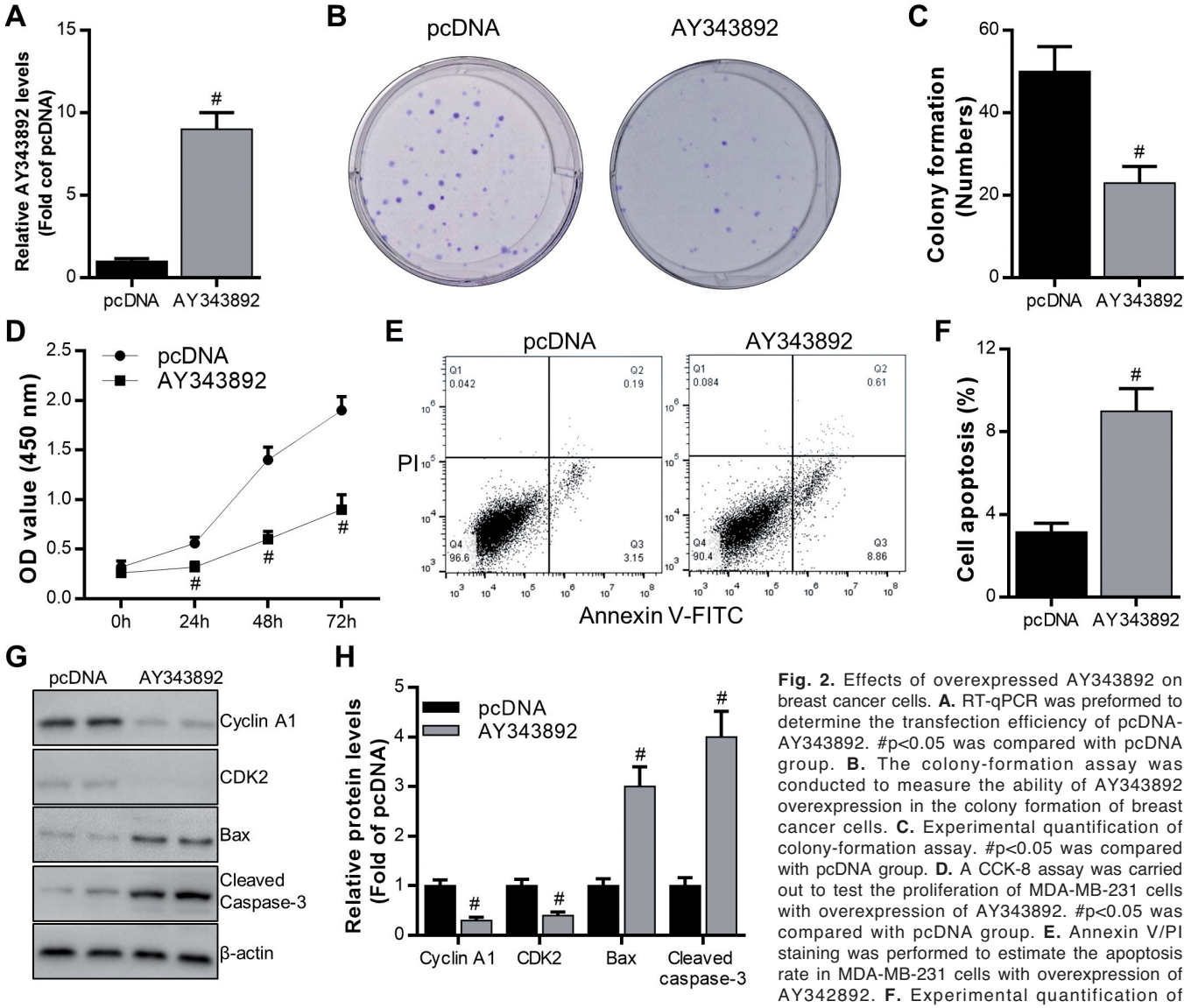
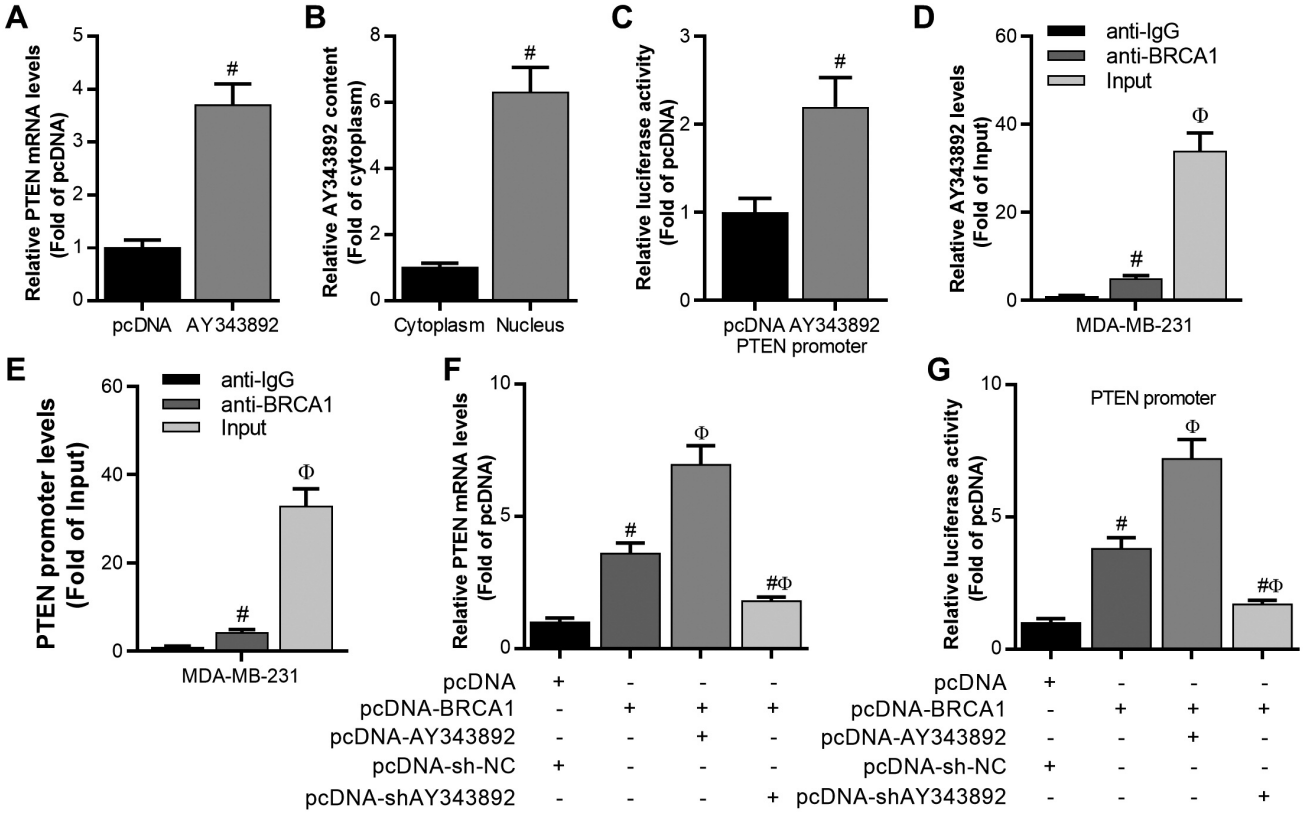


Fig. 2. Effects of overexpressed AY343892 on breast cancer cells. **A.** RT-qPCR was preformed to determine the transfection efficiency of pcDNA-AY343892. #p<0.05 was compared with pcDNA group. **B.** The colony-formation assay was conducted to measure the ability of AY343892 overexpression in the colony formation of breast cancer cells. **C.** Experimental quantification of colony-formation assay. #p<0.05 was compared with pcDNA group. **D.** A CCK-8 assay was carried out to test the proliferation of MDA-MB-231 cells with overexpression of AY343892. #p<0.05 was compared with pcDNA group. **E.** Annexin V/PI staining was performed to estimate the apoptosis rate in MDA-MB-231 cells with overexpression of AY342892. **F.** Experimental quantification of Annexin V/PI staining. #p<0.05 was compared with pcDNA group. **G.** Western blot detected protein expressions of Cyclin A1, CDK2, Bax and cleaved-caspase-3. **H.** Optical density analysis of western blot. #p<0.05 was compared with pcDNA group. Data are shown as means ± SD for three independent experiments.

increased cell colony formation, while this effect was rescued by PTEN overexpression (Fig. 4A,B). The results of the CCK-8 assay manifested that cell proliferation enhanced by depletion of AY343892 was counteracted by up-regulation of PTEN (Fig. 4C). In addition, results of flow cytometry indicated that



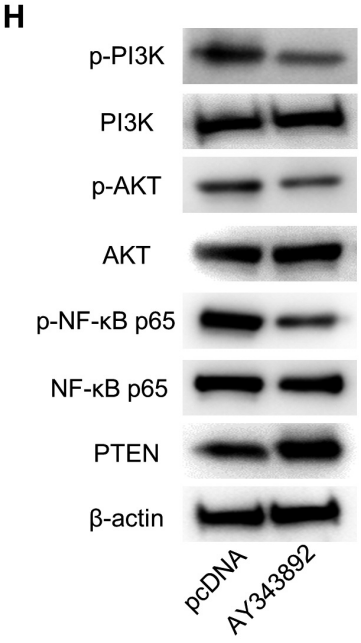


Fig. 3. The mechanism of AY343892 regulates the progress of breast cancer. **A.** RT-qPCR was implemented to evaluate the mRNA level of PTEN upon AY343892 overexpression. #p<0.05 was compared with pcDNA group. **B.** RT-qPCR was preformed to quantify the expression of AY343892 in cytoplasm and nucleus. #p<0.05 was compared with cytoplasm group. **C.** Luciferase reporter assay was performed to evaluate the activity of PTEN promoter in pcDNA group and pcDNA-AY343892 group. #p<0.05 was compared with pcDNA group. **D.** RIP assay was conducted to evaluate the combination of AY343892 with BRCA1. #p<0.05 was compared with anti-IgG group; Φp<0.05 was compared with anti-BRCA1 group. **E.** RT-qPCR was preformed to assess the binding of PTEN promoter to BRCA1. #p<0.05 was compared with anti-IgG group; Φp<0.05 was compared with anti-BRCA1 group. **F.** The PTEN mRNA level in MDA-MB-231 cells transfected with indicated plasmids was tested. #p<0.05 was compared with pcDNA group; Φp<0.05 or #Φp<0.05 was compared with pcDNA-BRCA1 group. **G.** Luciferase reporter assay was conducted to evaluate the activity of PTEN promoter in each group. #p<0.05 was compared with pcDNA group; Φp<0.05 or #Φp<0.05 was compared with pcDNA-BRCA1 group. **H.** Levels of PTEN protein, NF-κB singling-relevant proteins and PTEN-mediated proteins in pcDNA or pcDNA-AY343892 group were tested. Data are shown as means ± SD for three independent experiments.

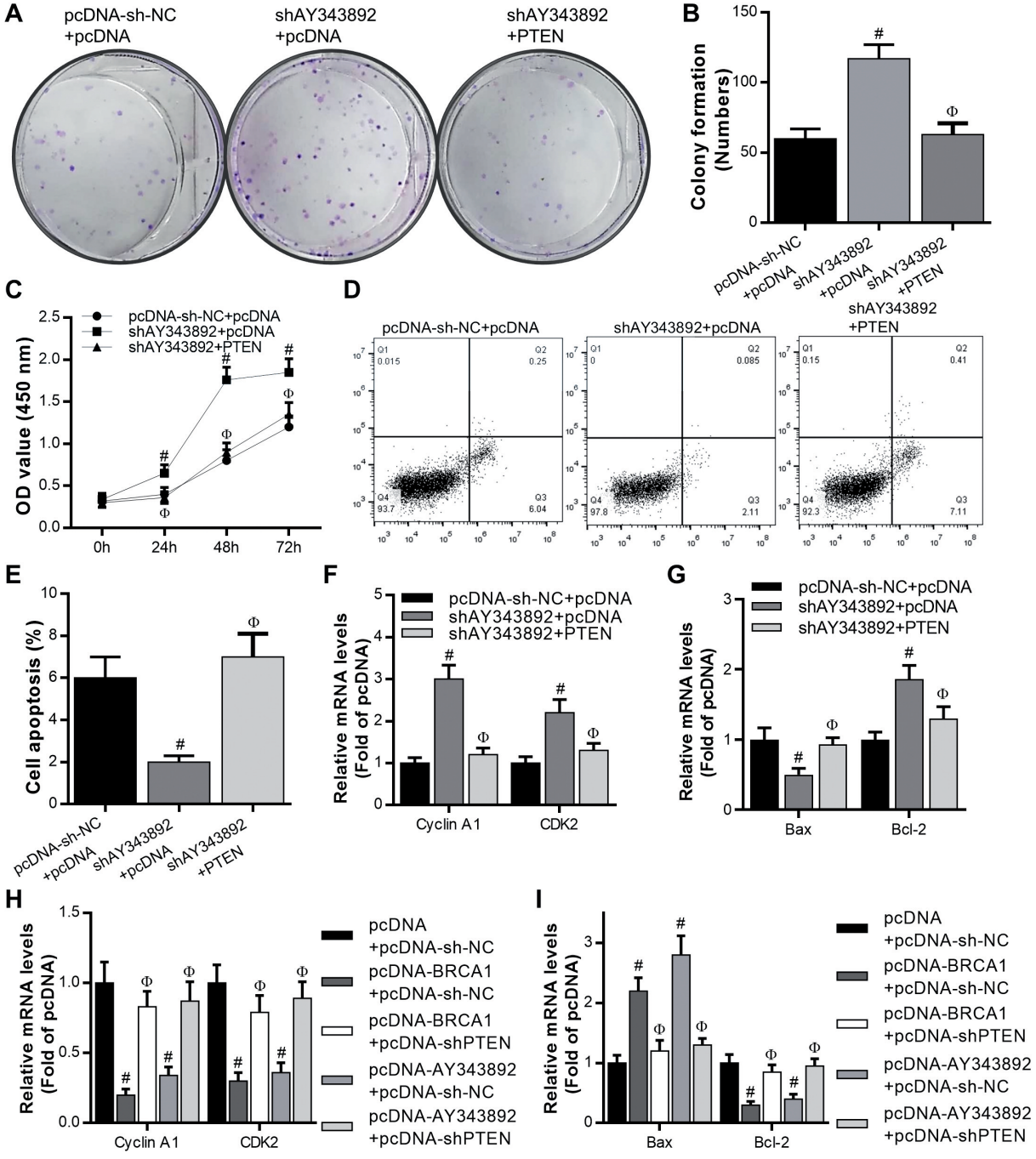


Fig. 4. PTEN is an important molecule to regulate the progress of breast cancer by AY343892 and BRCA1. **A.** The colony-formation assay was conducted to evaluate the proliferation ability of MDA-MB-231 cells in each group. **B.** Experimental quantification of colony-formation assay. #p<0.05 was compared with sh-NC group; Φp <0.05 was compared with shAY343892 group. **C.** CCK-8 assay was conducted to evaluate the proliferation of MDA-MB-231 cells in each group. #p<0.05 was compared with sh-NC group; Φp<0.05 was compared with shAY343892 group. **D.** Annexin V/PI staining was operated to estimate the apoptosis rate of MDA-MB-231 cells in each group. **E.** Experimental quantification of Annexin V/PI staining assay. #p<0.05 was compared with sh-NC group; Φp<0.05 was compared with shAY343892 group. **F.** RT-qPCR was preformed to detect mRNA levels of Cyclin A1 and CDK2 in MDA-MB-231 cells transfected with indicated plasmids. #p<0.05 was compared with sh-NC group; Φp<0.05 was compared with shAY343892 group. **G.** The mRNA levels of Bax and Bcl-2 in MDA-MB-231 cells with indicated transfection were determined by RT-qPCR. #p<0.05 was compared with sh-NC group; Φp<0.05 was compared with shAY343892 group. **H.** The mRNA levels of Cyclin A1 and CDK2 in MDA-MB-231 cells with appointed transfection were assessed with application of RT-qPCR. #p<0.05 was compared with pcDNA group; Φp<0.05 was compared with pcDNA-BRCA1 or pcDNA-AY343892 group. **I.** The mRNA levels of Bax and Bcl-2 in MDA-MB-231 cells transfected with appointed plasmids were detected by RT-qPCR. #p<0.05 was compared with pcDNA group; Φp<0.05 was compared with pcDNA-BRCA1 or pcDNA-AY343892 group. Data are shown as means ± SD for three independent experiments.

knockdown of AY343892 inhibited the apoptosis of MDA-MB-231 cells, while overexpression of PTEN reversed the inhibition of apoptosis caused by AY343892 knockdown (Fig. 4D,E). In addition, mRNA levels of Cyclin A1 and CDK2 were increased after knockdown of AY343892, while overexpression of PTEN reversed this increase (Fig. 4F). Furthermore, PTEN up-regulation also neutralized the influence of AY343892 depletion on the mRNA level of Bax and Bcl-2 (Fig. 4G). More importantly, we found that the effect of BRCA1 overexpression or AY343892 up-regulation on proliferation markers and apoptosis markers was rescued by PTEN knockdown (Fig. 4H,I). These results indicated that AY343892 inhibited cell proliferation and induced cell apoptosis in breast cancer by up-regulating PTEN expression.

Discussion

Breast cancer has been the most common cancer in the past few decades and remains the leading cause of morbidity and mortality in women. It has aroused wide public interest, and much research has been done on the pathogenesis of breast cancer to find the therapeutic targets and effective treatment methods for breast cancer patients (Torre et al., 2015; Ward et al., 2015; Siegel et al., 2018).

Long noncoding RNAs (lncRNAs) belong to non-coding RNAs, and the transcript length of lncRNAs is usually more than 200 nucleotides, without significant open reading frame (ORF). Although most lncRNAs lack an ORF, they not only directly bind and regulate their own DNA (Cecilia et al., 2018), RNA (Rodríguez-Mateo et al., 2017) and protein targets (Ding et al., 2018; Qu et al., 2018), but also regulate their targets through competing endogenous RNA (ceRNA) networks (Xian-Zi et al., 2018). Therefore, lncRNA can participate in most cellular and physiological processes by chromatin modification, transcriptional and post-transcriptional regulation. Lately, a large number of studies have shown that dysregulated lncRNAs played an important role in cancers (Fang et al., 2018), especially in breast cancers. For instance, lncRNA CASC9 enhances breast cancer progression by promoting metastasis (Zhang et al., 2019). LncRNA CTBP1-AS promotes cell proliferation, invasion and migration, and inhibit cell apoptosis in breast cancer (Cui and Geng, 2019). LncRNA SNHG7 is up-regulated in breast cancer and contributes to carcinogenesis, progression and poor prognosis of breast cancer (Sun et al., 2019). Recently, we found that lncRNA AY343892 was down-regulated in breast cancer tissues and cell lines. To further confirm the effect of AY343892 on breast cancer, we overexpressed AY343892 in MDA-MB-231 cells. It was discovered that up-regulation of AY343892 inhibited proliferation and induced apoptosis in breast cancer cells. These findings suggested that AY343892 played an inhibitory role in the progression of breast cancer.

The results have indicated that AY343892 is an essential regulator involved in the modulation of breast cancer progression. Herein, we investigated the underlying mechanism by which AY343892 regulated breast cancer. We performed a bioinformatics prediction analysis and found that PTEN, which is known as a crucial tumor suppressor, was a potent candidate. Additionally, our data showed that PTEN was upregulated by AY343892 overexpression, and overexpression of AY343892 enhances PTEN promoter activity. Furthermore, we confirmed that BRCA1, which is an anticancer gene, can bind with AY34389 and PTEN promoter. Rescue assays indicated that knockdown of AY343892 promoted proliferation and inhibited apoptosis, while overexpression of PTEN reversed promotion of cell proliferation and impairment of cell apoptosis caused by AY343892 knockdown. Moreover, down-regulation of PTEN counteracted the inhibitory effects of AY343892 overexpression on cell proliferation and its promoting effects on cell apoptosis . These findings together confirmed that AY343892 inhibited breast cancer cell proliferation and induced apoptosis by positively regulating PTEN expression.

In conclusion, our studies revealed that AY343892 served as a tumor-suppressor in breast cancer and regulated PTEN by binding to BRCA1. These findings provide a novel mechanism for the carcinogenesis and progress of breast cancer, which could be useful for subsequent research on breast cancer treatment.

Funding. This work was supported by 2019 University Outstanding Young Cadre Talents Domestic Visit and Study Program (gxgnfx2019110).

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Accepted August 5, 2020