The novel long non-coding RNA PRNCR1-2 is involved in breast cancer cell proliferation, migration, invasion and cell cycle progression

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Abstract. Long non-coding RNAs (lncRNAs) have recently been reported to act as important mediators of tumor initiation and progression. The present study aimed to investigate the expression and pathogenic roles of the lncRNA prostate cancer-associated non-coding RNA (PRNCR)1-2 in breast cancer. The expression levels of PRNCR1-2 were detected in breast cancer tissues and numerous breast cancer cell lines using reverse transcription-quantitative polymerase chain reaction. Depletion of PRNCR1-2 expression in breast cancer cells was conducted through small interfering RNA-mediated silencing. Subsequently, cell proliferation was assessed by MTS assay, cell migration and invasion capacities were evaluated using the Transwell culture system, and cell cycle progression and apoptosis were analyzed by flow cytometry. Protein expression levels of the signaling components checkpoint kinase 2 (CHK2), protein kinase B (AKT), phosphorylated (p)-CHK2 and p-AKT were measured by western blotting. The results demonstrated that PRNCR1-2 expression was significantly elevated in breast cancer tissues compared with in adjacent normal tissues. Furthermore, depletion of PRNCR1-2 in HS-578T and MDA-MB-231 breast cancer cells markedly suppressed their proliferation rates, migration and invasion capacities, and cell cycle progression; however, it had no effect on cell apoptosis. In addition, PRNCR1-2 depletion increased CHK2 phosphorylation and decreased AKT phosphorylation in HS-578T and MDA-MB-231 cells. In conclusion, the lncRNA PRNCR1-2 may promote breast cancer cell proliferation, migration, invasion and cell cycle progression.

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Introduction

Breast cancer is a major human malignancy and is the second leading cause of cancer-associated mortality in women worldwide, following lung cancer (1). As estimated by the American Cancer Society, there will be >266,000 new cases of breast cancer and >40,000 cases of breast cancer-associated mortality in the next 10 years in the United States, thus accounting for 30 and 14% of total cancer cases and cases of cancer-associated mortality, respectively (1). Survival rates of patients with breast cancer is relatively low, particularly in developing countries; however, a 5-year survival rate of >80% has been achieved in England and the United States (2). As a complex heterogeneous malignancy, breast cancer remains a severe public health concern, due to ambiguities regarding the stepwise pathological processes from normal breast tissue to metastatic cancer tissue (3,4). Previous studies have revealed that the pathogenesis of breast cancer is accompanied and driven by a series of successive mutations in genetic and epigenetic networks in breast cells, finally resulting in activation of various hallmarks, malignant transformation and metastasis (5,6). In recent years, it has been suggested that noncoding RNAs (ncRNAs) are responsible for genetic and epigenetic dysregulation, which is associated with various developmental and pathological processes, including tumorigenesis (7,8). However, the specific roles of ncRNAs in breast cancer remain to be investigated.

Long ncRNAs (lncRNAs) refer to ncRNA transcripts containing >200 nucleotides, which are involved in post-transcriptional regulation of gene expression by interfering with microRNA functioning (9). Through modulating gene expression at the transcriptional, post-transcriptional and epigenetic levels, lncRNAs have been reported to be associated with various physiological and pathological processes, including stem cell development, neurogenesis and oncogenesis (8,9). In various types of human cancer, lncRNAs act as promoters and

maintainers of cancer initiation and progression; therefore, they have been considered potential biomarkers for clinical diagnosis, and also as therapeutic targets for the development of novel treatments (10,11). Based on functional studies using cellular and animal models, it has been reported that lncRNAs are involved in various cancer-associated cellular phenotypes, including cell death inhibition, activation of invasion, proliferation maintenance, dysregulated cellular energetics, genomic instability and evasion of growth suppressors (12). LncRNAs have also been identified as key regulators in breast cancer. For example, the lncRNA HOX transcript antisense RNA, which promotes cancer metastasis by modulating chromatin states, has been identified as a potential prognostic marker of cancer metastasis for estrogen receptor (ER)-positive breast cancer (13). Furthermore, the lncRNA urothelial cancer-associated 1 has been reported to enhance breast tumor growth through suppression of p27 protein levels via competitive inhibition (14). An accumulating number of lncRNAs have been demonstrated to be associated with breast cancer initiation and progression, thus suggesting that functional elucidation of key lncRNAs in breast cancer cells may provide valuable information for clinical screening and management. Prostate cancer-associated ncRNA 1 (PRNCR1) is closely associated with resistance of prostate cancer to castration (15), colorectal cancer cell proliferation and cell cycle progression (16), and progression of other types of cancer (17). However, to the best of our knowledge, the implications of PRNCR1 in breast cancer progression have not been previously addressed.

In the present study, the expression levels of PRNCR1 were detected in clinical tissues from patients with breast cancer, as well as in numerous breast cancer cell lines. Subsequently, PRNCR1 expression was knocked down in breast cancer cells and a functional analysis was conducted. The present study identified an association between PRNCR1 and breast cancer, and therefore provided a novel candidate for ncRNA-based breast cancer diagnosis and treatment. These findings may also result in future elucidation of the pathological mechanisms underlying breast cancer initiation and progression.

Materials and methods

Cancer tissues and cell lines. Breast cancer tissues and paired adjacent normal tissues were collected from 20 patients at the Department of Breast Surgery, Foshan First People's Hospital (Foshan, China) between June 2015 and July 2017. Breast cancer was confirmed by pathological diagnosis post-operation, and the clinicopathological data of patients are displayed in Table I. The experimental procedures were approved by the Ethics Committee of Foshan First People's Hospital, and written informed consent was obtained from each participant prior to surgery. The breast cancer cell lines HS-578T (cat. no. HTB-126), MCF-7 (cat. no. HTB-22), MDA-MB-468 (cat. no. HTB-132), MDA-MB-231 (cat. no. HTB-26) and BT-549 (cat. no. HTB-122), and the mammary epithelial cell line MCF10A (cat. no. CRL-10317) were obtained from the American Type Culture Collection (Manassas, VA, USA). Breast cancer cells and the mammary epithelial cell line were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 10% fetal bovine serum (Biowest, Nuaillé, France) in a humidified atmosphere supplied with 5% CO_2 at 37˚C.

Table I. Clinicopathological data of patients with breast cancer.

Characteristic	Number
Age (years)	
<55	12
>55	8
Sex	
Male	0
Female	20
Distant metastasis	
Absent	10
Present	10
Histological grade	
1	3
2	11
3	6
Tumor size (cm)	
≤2	6
2-5	13
>5	1

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Relative lncRNA expression levels were measured in total RNA samples extracted from breast cancer tissues and cell lines by RT-qPCR. Prior to RNA extraction, breast tissues were homogenized in liquid nitrogen and breast cell lines were collected by centrifugation at 800 x g for 10 min at room temperature, followed by three washes with PBS. Total RNA samples were extracted using TRIzol® solution (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. RNA concentrations were determined by spectrophotometer (NanoDrop™ 1000; NanoDrop; Thermo Fisher Scientific, Inc., Wilmington, DE, USA). Subsequently, 2.0 μg RNA was used for cDNA synthesis by Moloney Murine Leukemia Virus reverse transcriptase (Promega Corporation, Madison, WI, USA) according to the manufacturer's protocol, and PCR analysis was performed using the 2X EasyTaq PCR SuperMix kit (cat. no. AS111-03; Beijing Transgen Biotech Co., Ltd., Beijing, China) according to the manufacturer's protocol. The thermocycling conditions for qPCR were: An initial denaturation at 95˚C for 120 sec, followed by 40 cycles of 95˚C for 15 sec and 60˚C for 30 sec for primer annealing and elongation, ending with a melting curve step of 65˚C to 95˚C with an increment of 0.5˚C/5 sec. β-actin was used as an internal standard for quantification. The relative expression levels of genes were calculated using the $2^{-\Delta\Delta Cq}$ method (18). For statistical analysis, at least three biological and technical replicates were performed. The following primers were used for detection of PRNCR1-2 expression: Forward, 5-CCTTTC CCTCATGACCCAGT-3 and reverse, ATTGGTGTGAGG GGAGTCTG. β-Actin: Forward, CATGTACGTTGCTAT CCAGGC and reverse, CTCCTTAATGTCACGCACGAT.

Cell transfection. For knockdown of PRNCR1-2 expression, breast cancer cells were digested with trypsin solution, seeded into 6-well plates and cultured overnight at 37˚C in an atmosphere containing 5% CO_2. Once cells reached 80% confluence, they were washed twice with PBS and added to 1.5 ml fresh basal DMEM. Subsequently, the cells were mixed with 10 μl 20 μM small interfering RNA (siRNA; sequence: 5'-CCATTA AGCTTGAGGCAAT-3'; 5'-ATTGCCTCAAGCTTAATG G-3'), or negative control siRNA (sequence: 5'-UUCUCCGAA CGUGUCACGUTT-3'; 5'-ACGUGACACGUUCGGAGA ATT-3'), synthesized by Guangzhou Forevergen Biosciences Co., Ltd., Guangzhou, China and dissolved in 250 μl Gibco™ Opti-MEM (Gibco; Thermo Fisher Scientific, Inc.). The cells were then incubated with 5 μl Lipofectamine® 2000 transfection reagent (Invitrogen; Thermo Fisher Scientific, Inc.), which was pre-mixed with 250 μl Opti-MEM, at 37˚C for 4 h in an atmosphere containing 5% CO_2. Finally, cells were cultured in fresh DMEM 48 h prior to the subsequent assays.

Cell proliferation assay. Proliferation rates of breast cancer cells were determined using the MTS method. A single-cell suspension was prepared by digesting breast cancer cells with trypsin solution, followed by cell counting. Cell density was adjusted to $3x10^4$ cells/ml, and cells were seeded into 96-well plates (100 μl/well). MTS solution (10 μl; cat. no. ab197010; Abcam, Cambridge, UK) was added to the cultured breast cancer cells at a 1:10 ratio, and cells were incubated at 37˚C for 4 h in an atmosphere containing 5% CO_2. Finally, cell proliferation rates were measured by detecting the absorbance at 490 nm using a microplate reader. At least three biological repeats were performed for statistical analysis.

Cell migration and invasion assays. To evaluate breast cancer cell migration, cultured cells were collected by trypsin digestion and centrifugation at 100 x g for 1 min at room temperature, diluted in serum-free medium to obtain $1x10^5$ cells/ml, and a 100-μl cell suspension was added to the upper chambers of a Transwell system. The lower chambers of the Transwell system were filled with 600 μl fresh DMEM. After being cultured under normal conditions for 48 h, migrated cells were fixed with 4% paraformaldehyde for 15 min, stained with 1% crystal violet for 10 min both at room temperature, washed with PBS, and finally observed and counted under an inverted fluorescence microscope (Leica Microsystems GmbH, Wetzlar, Germany). To assess cell invasion, Matrigel matrix (Corning Incorporation, Corning, NY, USA) was mixed with DMEM at a ratio of 1:3, and was added to the Transwell chambers and incubated at 37˚C for 2 h. Cells were then cultured for 48 h at 37˚C, fixed with 4% paraformaldehyde for 15 min, stained with 1% crystal violet for 10 min, both at room temperature, and finally analyzed under a microscope.

Cell cycle progression analysis. Breast cancer cells (80% confluence) were washed three times with PBS, and a single-cell suspension was generated through trypsin digestion. Subsequently, ~$1x10^6$ cells were fixed with 70% ethanol at -20˚C overnight. Cells were then collected by centrifugation at 500 x g for 5 min and were washed twice with PBS. Cell precipitates were then resuspended in 500 ml Cell Cycle staining buffer (cat. no. F559763; Guangzhou Forevergen Biosciences Co., Ltd.), incubated at 37˚C for 30 min, and finally detected by flow cytometry (Sysmex Partec GmbH, Görlitz, Germany). Three biological replicates were performed for statistical analysis.

Cell apoptosis assay. Cell apoptosis was determined using the Annexin V-allophycocyanin/7-aminoactinomycin D (7-AAD) Apoptosis Detection kit (cat. no. 40309ES20; Yeasen, Shanghai, China), according to the manufacturer's protocol. Breast cancer cells were collected by digestion with EDTA-free trypsin solution and were centrifuged at 800 x g for 5 min. Cell precipitates were then resuspended in 1X binding buffer, and cell density was adjusted to $1x10^6$ cells/ml. Finally, 100 μl cells were incubated with 5 μl Annexin V and 5 μl 7-AAD in the dark for 15 min at room temperature, incubated with 400 μl binding buffer for 1 h, and analyzed by flow cytometry (Sysmex Partec GmbH). Data from three biological repeats were used for statistical analysis.

Western blotting. Protein was extracted using RIPA Lysis Buffer (Beyotime Institute of Biotechnology, Haimen, China). The concentration was determined using the Bicinchoninic Acid Kit for Protein Determination (Sigma-Aldrich; Merck KGaA). Total proteins were extracted from breast tissues and breast cancer cell lines for immunoblotting. Briefly, 35 μg total proteins were boiled at 100˚C for 5 min, loaded and separated by 12% SDS-PAGE, and electroblotted onto polyvinylidene fluoride (PVDF) membranes (EMD Millipore, Billerica, MA, USA). PVDF membranes were then blocked with 5% lipid-free milk solution for 1 h at room temperature, and incubated with primary antibodies against phosphorylated (p)-checkpoint kinase 2 p-CHK2 (cat. no. ab59408; 1:1,000), CHK2 (cat. no. ab47433; 1:1,000), p-protein kinase B p-AKT (cat. no. ab38449; 1:500) and AKT (cat. no. ab179463; 1:500; all Abcam) at room temperature for 1 h. The membranes were then washed three times with PBS, incubated with horseradish peroxidase-conjugated secondary antibodies (cat. no. ab6940; 1:10,000; Abcam) at room temperature for 1 h, and finally detected with enhanced chemiluminescence solutions (Thermo Fisher Scientific, Inc., Waltham, MA, USA). GAPDH (cat. no. ab181602; 1:10,000; Abcam) was used as an internal standard, and three biological repeats were performed. Signals were densitometrically assessed using Quantity One® software version 4.5 (Bio Rad Laboratories, Inc., Hercules, CA, USA).

Statistical analysis. Data were presented as mean ± standard error of the mean. The present findings were statistically analyzed using SPSS 18.0 software package (SPSS, Inc., Chicago, IL, USA) via Student's t-test or one way analysis of variance, least significant difference test was used to analyze the differences among more than two groups. $P<0.05$ was considered to indicate a statistically significant difference.

Results

PRNCR1-2 expression is elevated in breast cancer. To identify a novel lncRNA associated with breast cancer pathogenesis, the expression levels of PRNCR1-2 were analyzed

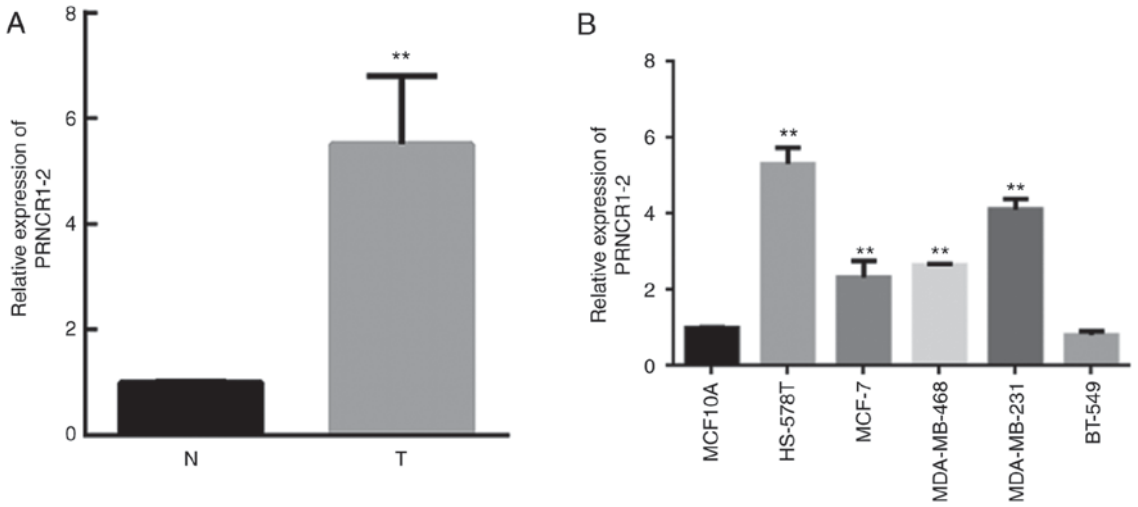

Figure 1. PRNCR1-2 expression in breast cancer. (A) PRNCR1-2 expression in breast cancer tissues and paired adjacent normal tissues. (B) PRNCR1-2 expression in MCF10A and five breast cancer cell lines. The relative expression levels of PRNCR1-2 were determined by reverse transcription-quantitative polymerase chain reaction. **P<0.01 vs. N or MCF10A. N, normal adjacent tissues; PRNCR1-2, prostate cancer-associated non-coding RNA 1-2; T, tumor tissues.

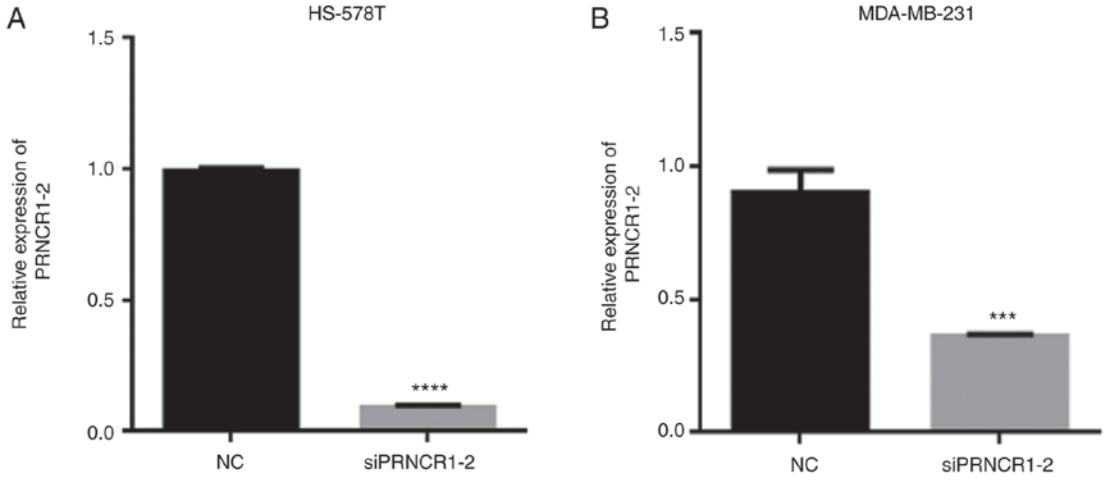

Figure 2. Depletion of PRNR1-2 expression in breast cancer cells. (A) Expression of PRNCR1-2 in HS-587T breast cancer cells transfected with siPRNCR1-2. (B) Expression of PRNCR1-2 in MDA-MB-231 breast cancer cells transfected with siPRNCR1-2. Reverse transcription-quantitative polymerase chain reaction was conducted for PRNCR1-2 expression in HS-587T and MDA-MB-231 cells. ***P<0.001; ****P<0.0001. NC, negative control; PRNCR1-2, prostate cancer-associated non-coding RNA 1; si, small interfering RNA.

in 20 pairs of breast cancer and adjacent normal tissues (Fig. 1A). Compared with the normal tissues, the expression levels of PRNCR1-2 were significantly elevated in breast cancer tissues (P<0.05; Fig. 1A). For further validation, PRNCR1-2 expression was confirmed in five breast cancer cell lines and a mammary epithelial cell line; PRNCR1-2 were successfully overexpressed in the cancer cell lines HS-587T, MCF-7, MDA-MB-468 and MDA-MB-231 compared with the mammary epithelial cell line MCF10A, but failed to overexpress in the cancer cell line BT-549 (Fig. 1B). The upregulation of PRNCR1-2 expression in breast cancer tissues suggested that PRNCR1-2 may have pathogenic roles in breast cancer. Due to the high expression level of PRNCR1-2 in HS-587T and MDA-MB-231 cells, these two cell lines were chosen for the following studies.

PRNCR1-2 depletion in HS-587T and MDA-MB-231 cells. To investigate the potential roles of the lncRNA PRNCR1-2 in breast cancer pathogenesis, HS-587T and MDA-MB-231 breast cancer cells were transfected with a specific siRNA against PRNCR1-2. It was revealed that transfection with siRNA-PRNCR1-2 markedly reduced PRNCR1-2 expression in HS-587T and MDA-MB-231 cells, compared with in the negative control group (Fig. 2A and B). siRNA-PRNCR1-2-transfected HS-587T and MDA-MB-231 cells were used for subsequent functional assays.

PRNCR1-2 regulates breast cancer cell proliferation, migration and invasion. To explore the possible roles of PRNCR1-2 in breast cancer initiation and progression, the proliferation, migration, invasion, cell cycle progression and apoptosis of siRNA-PRNCR1-2-transfected HS-587T and MDA-MB-231 cells were analyzed. The results of the MTS assay revealed that the proliferation rates of siRNA-PRNCR1-2-transfected HS-587T cells were markedly downregulated compared with in the negative control

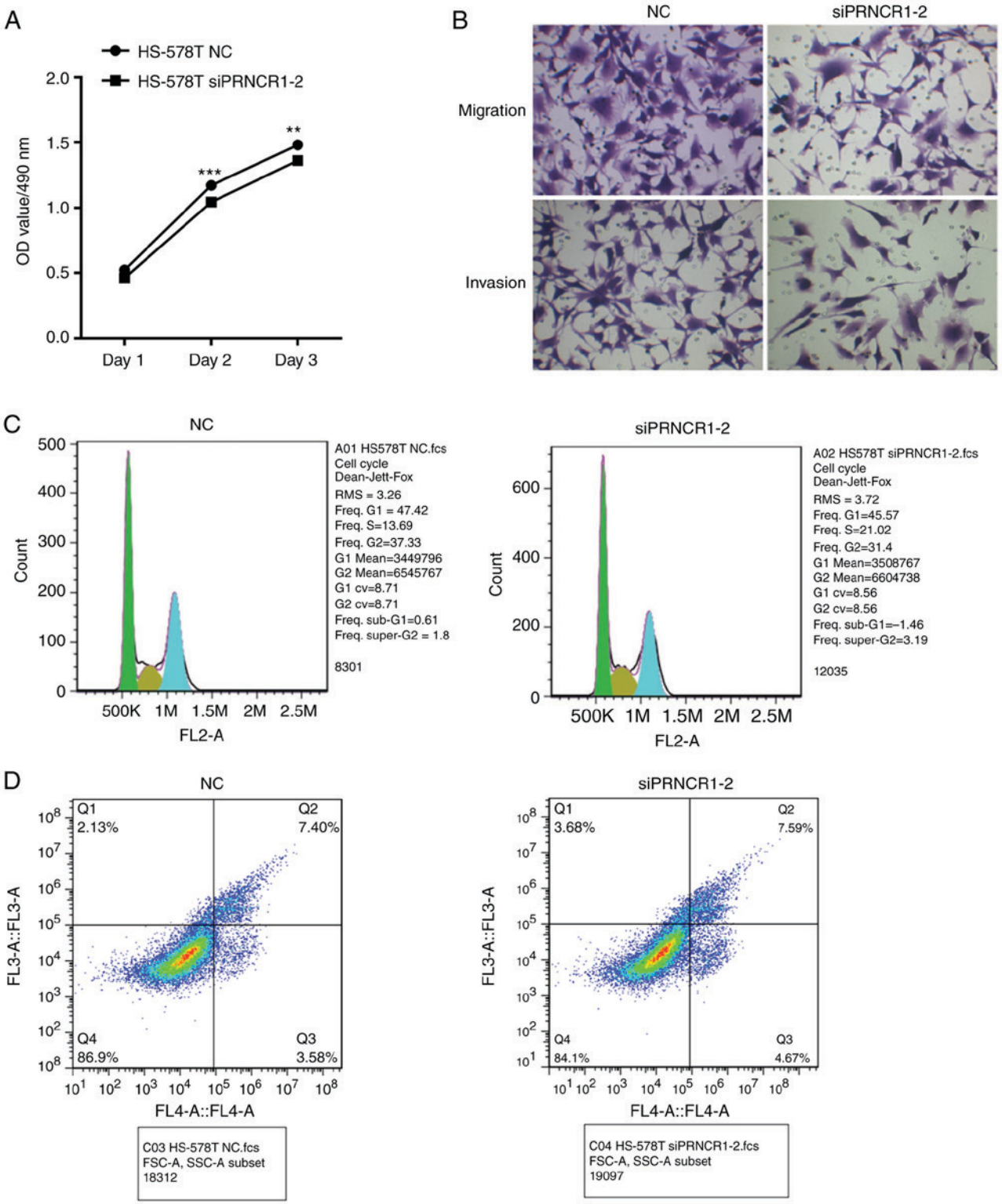

Figure 3. Cellular properties of HS-587T cells with PRNCR1-2 knockdown. (A) Proliferation of HS-587T cells with PRNCR1-2 knockdown. Cell proliferation was assessed by MTS assay. (B) Migration and invasion of HS-587T cells transfected with siPRNCR1-2. Transwell assays were used for analysis of cell migration and invasion. (C) Cell cycle progression of PRNCR1-2-depleted HS-587T cells. (D) Apoptotic cell ratios of PRNCR1-2-depleted HS-587T cells. Cell cycle progression and apoptosis were analyzed by flow cytometry. **P<0.01; ***P<0.001. NC, negative control; PRNCR1-2, prostate cancer-associated non-coding RNA 1-2; si, small interfering RNA.

group (Fig. 3A), thus indicating the ability of PRNCR1-2 to modulate breast cancer cell proliferation. Furthermore, the migration and invasion of PRNCR1-2-depleted HS-587T cells were analyzed using Transwell assays; the migration and invasion of HS-587T cells were markedly suppressed by transfection with siRNAs targeting PRNCR1-2 (Fig. 3B). Consistent with PRNCR1-2 depletion-induced alterations in proliferation, the cell cycle progression of siRNA-PRNCR1-2-transfected HS-587T cells was markedly altered, with more cells arrested in S phase, thus indicating

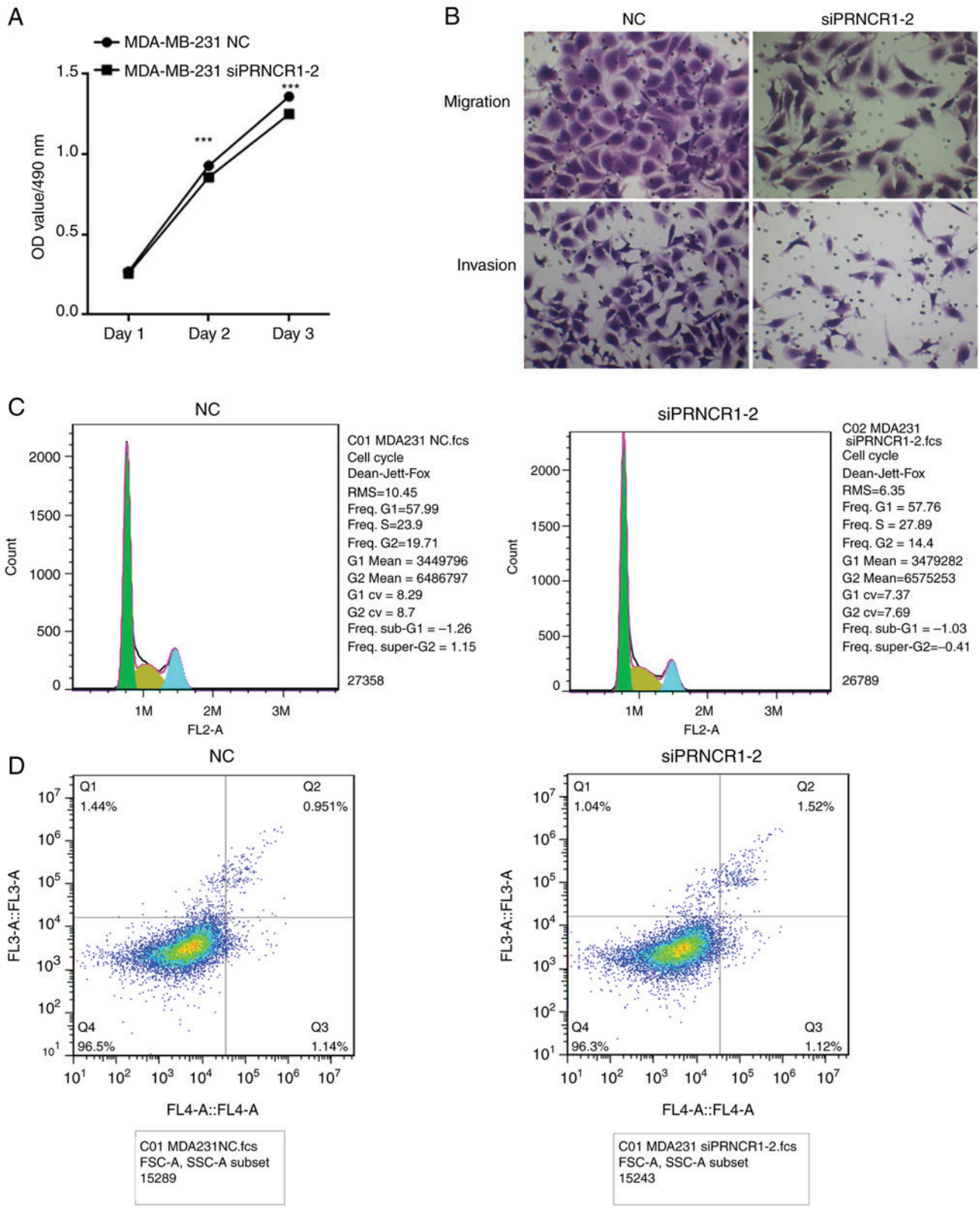

Figure 4. Cellular properties of MDA-MB-231 cells with PRNCR1-2 knockdown. (A) Proliferation of MDA-MB-231 cells with PRNCR1-2 knockdown. Cell proliferation was assessed by MTS assay. (B) Migration and invasion of MDA-MB-231 cells transfected with siPRNCR1-2. Transwell assays were used for analysis of cell migration and invasion. (C) Cell cycle progression of PRNCR1-2-depleted MDA-MB-231 cells. (D) Apoptotic cell ratios of PRNCR1-2-depleted MDA-MB-231 cells. Cell cycle progression and apoptosis were analyzed by flow cytometry. ***P<0.001. NC, negative control; PRNCR1-2, prostate cancer-associated non-coding RNA 1-2; si, small interfering RNA.

the involvement of PRNCR1-2 in promoting S/G_2 transition of breast cancer cells (Fig. 3C). The proportion of apoptotic cells in the siRNA-PRNCR1-2 group was also analyzed by flow cytometry; however, no marked alterations in cell apoptosis were observed in siRNA-PRNCR1-2-transfected HS-587T cells (Fig. 3D). The aforementioned experiments

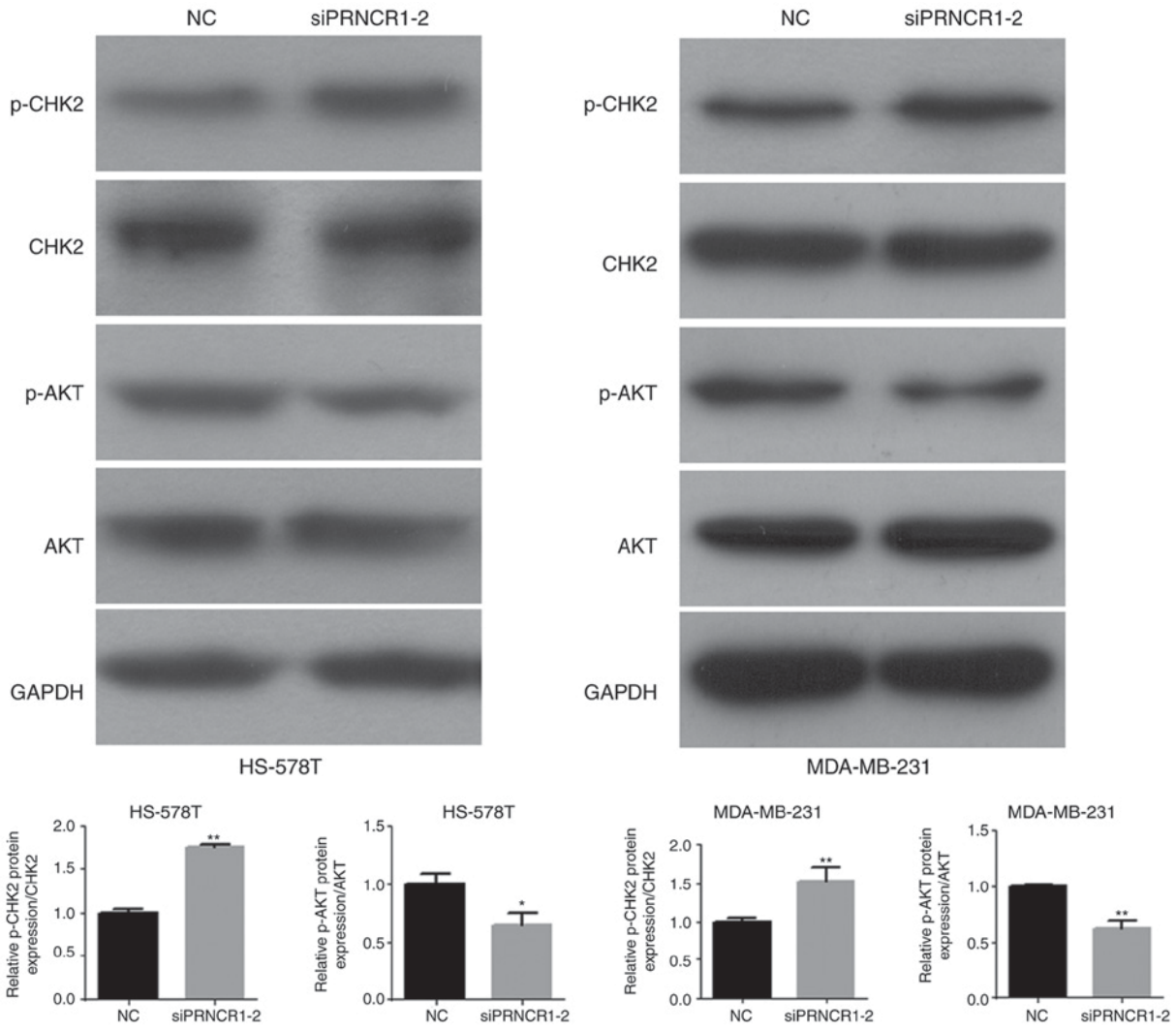

Figure 5. Signaling pathways regulated by PRNCR1-2 in breast cancer cells. The abundance and phosphorylation of key components associated with cell proliferation, migration, invasion, apoptosis and cell cycle progression in HS-587T and MDA-MB-231 cells transfected with siPRNCR1-2 were determined by western blotting. GAPDH was used as an internal standard. *P<0.05; **P<0.01. AKT, protein kinase B; CHK2, checkpoint kinase 2; NC, negative control; p, phosphorylated; PRNCR1-2, prostate cancer-associated non-coding RNA 1-2; si, small interfering RNA.

were repeated in MDA-MB-231 cells and similar results were obtained, as shown in Fig. 4. Taken together, these cellular assays suggested that PRNCR1-2 may be involved in breast cancer progression by promoting cell proliferation, migration and invasion, and by modulating cell cycle progression, but not via modulation of breast cell apoptosis.

Signaling in HS-587T and MDA-MB-231 cells is modulated by PRNCR1-2 depletion. For more insights into PRNCR1-2-induced regulation of cell functions during breast cancer progression, key signaling pathway components associated with cell proliferation, migration, invasion, cell cycle progression and apoptosis were detected in HS-587T and MDA-MB-231 cells transfected with siRNA-PRNCR1-2 by western blotting. The results demonstrated that the protein expression levels of p-CHK2 were significantly elevated in siRNA-PRNCR1-2-transfected HS-587T and MDA-MB-231 cells (Fig. 5). Furthermore, depletion of PRNCR1-2 expression using specific siRNA resulted into significant inhibition of p-AKT in HS-587T and MDA-MB-231 cells; however, PRNCR1-2 depletion had no effect on the abundance of total CHK2 and AKT proteins (Fig. 5). The selectively altered expression of key signaling components suggested that regulation of breast cancer cell proliferation, migration, invasion and cell cycle progression by PRNCR1-2 may be at least partially mediated by modulating phosphorylation of CHK2 and AKT.

Discussion

Non-coding components that account for large volumes of the human genome have long been predicted to have important roles in various physiological and pathological processes (19). Various types of ncRNAs, including microRNAs, siRNAs, Piwi-interacting RNAs, small nucleolar RNAs and lncRNAs critically contribute to cancer initiation and progression, much more than previously expected (8,10-12,20). Increasing evidence has suggested that lncRNAs may be involved in tumor pathogenesis, thus promoting trials of their application in breast cancer diagnosis and prognosis. The lncRNA H19 is characterized as a highly expressed biomarker in ER-positive MCF-7 breast cancer cells, which is involved in breast cell

survival and estrogen-induced cell proliferation during breast cancer development (21). In addition, its diagnostic value as a novel biomarker for breast cancer has been reported, due to its detectable expression in the urine and serum of patients with breast cancer (22,23). Accumulating numbers of such ncRNAs as candidate targets for cancer diagnosis and treatment highlight the importance of further characterization of novel ncRNAs in breast cancer pathogenesis.

Aiming to provide a novel candidate for breast cancer diagnosis and treatment, the present study analyzed the expression levels of the lncRNA PRNCR1-2 in cancer tissues collected from patients with breast cancer. PRNCR1-2 expression was significantly increased in cancer tissues, thus suggesting its potential association with breast cancer development. Subsequently, a knockdown assay was conducted to deplete PRNCR1-2 expression in breast cancer cells. The marked alterations in breast cancer cell proliferation, migration, invasion and cell cycle progression following suppression of PRNCR1-2 expression indicated that PRNCR1-2 may be considered a novel non-coding regulator in breast cancer pathogenesis. Significantly altered AKT and CHK2 phosphorylation further validated the critical roles served by PRNCR1-2 in breast cancer cells. In accordance with previous findings regarding lncRNAs in breast cancer (8), the present results revealed the cellular functions of PRNCR1-2 in breast cancer cells, which may be applied as a biomarker for breast cancer diagnosis and therapy.

The molecular mechanisms by which PRNCR1-2 promotes breast cancer cell proliferation, migration and cell cycle progression deserve further investigations. Previous studies reported that interactions of lncRNAs with proteins or RNA partners may be important for the functioning of lncRNA molecules (10,24). A recent study also demonstrated that lncRNAs can bind with Janus kinase 2 (JAK2), thus promoting JAK2 activation and signal transducer and activation of transcription 3 phosphorylation, finally mediating breast cancer brain metastases (25). Therefore, it is reasonable to speculate that PRNCR1-2 may also carry out its role in breast cancer cell proliferation and migration via its association with key protein components of these cellular processes. The large-scale identification of proteins or RNA partners interacting with PRNCR1-2 during breast cancer development may provide novel perspectives on pathogenic roles of non-coding molecules in breast cancer, as well as other types of human cancer. It is also possible that PRNCR1-2 may be directly involved in the post-translational modifications of key signaling proteins. Metastasis associated in lung adenocarcinoma transcript 1 (MALAT1) interacts with both serine and arginine-rich splicing factor 1 (SRSF1) and SRSF protein kinase 1. In colorectal cancer cells, the lncRNA MALAT1 regulates cancer cell proliferation and migration by promoting phosphorylation of SRSF1 (26). In the present study, it was revealed that depletion of PRNCR1-2 markedly altered the phosphorylation of AKT and CHK2, whereas total AKT and CHK2 protein levels were not affected. CHK2 acts as an important regulator of cell cycle progression and proliferation (27,28), and CHK2 signaling is activated by phosphorylation of itself and downstream substrates (29). AKT is another key regulator of tumor cell proliferation, cell cycle progression, migration and invasion (30-32), which is also activated by its phosphorylation (33). These observations suggested that PRNCR1-2 may modulate the phosphorylation of CHK2 and AKT by interacting with a specific kinase or phosphatase. The role of PRNCR1-2 in CHK2 and AKT phosphorylation and regulation requires further investigation.

In conclusion, this study reported that the lncRNA PRNCR1-2 is highly expressed in breast cancer tissues, and depletion of PRNCR1-2 in breast cancer cells results in the suppression of cell proliferation, migration, invasion and cell cycle progression. These findings indicated that PRNCR1-2 may be explored as a breast cancer biomarker for the development of novel diagnostic or therapeutic methods for patients with breast cancer.

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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

DP and QH conceived and designed the study, and drafted and critically revised the manuscript. XL and YXL performed the experiments and analyzed the data. HD, SC, YDL, LL, FPe and FPa participated in study design, study implementation and manuscript revision. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The experimental procedures were approved by the Ethics Committee of Foshan First People's Hospital, and written informed consent was obtained from each participant prior to surgery.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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