LncRNA PRNCR1 promotes breast cancer proliferation and inhibits apoptosis by modulating microRNA-377/CCND2/MEK/MAPK axis

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Abstract

Background: Long non-coding RNAs (lncRNAs) have recently become the vital gene regulators in diverse cancers. In our study, we purposed to inquiry into the mechanisms of lncRNA PRNCR1 in breast cancer via microRNA-377 (miR-377)/CCND2/MEK/MAPK axis.

Methods: PRNCR1 expression in breast cancer tissues was detected, and the correlation between PRNCR1 expression and prognostic survival was analyzed. The expressions of PRNCR1 and miR-377 in breast cancer cell lines were detected. Relationships among PRNCR1, miR-377 and CCND2 were confirmed by luciferase activity, RNA pull-down or RIP assays. Breast cancer cells were introduced with silenced PRNCR1 or restored miR-377 to explore their functions in breast cancer’s malignant phenotype. Related proteins expression in MEK/MAPK pathway was determined by western blot analysis.

Results: PRNCR1 was abnormally highly expressed and miR-377 was poorly expressed in breast cancer, and patients with high expression of PRNCR1 had a poor prognosis. PRNCR1 silencing or miR-377 overexpression resulted in suppressed breast cancer cell proliferation ability, blocked cell cycle process and induced apoptosis. PRNCR1 may participate in the modulation of CCND2 by competitively binding with miR-377. CCND2 activated the MEK/MAPK pathway, and after treatment with Mirdametinib, the MEK/MAPK pathway was inhibited, which was found to retard breast cancer growth.

Conclusion: Our study highlights that lncRNA PRNCR1 may competitively bind to miR-377, leading to upregulated CCND2, which in turn leads to MEK/MAPK pathway activation, thereby promoting breast cancer growth.

Introduction

Breast cancer is a heterogeneous malignancy that contributes to uncontrolled propagation and diffusion of breast tissue cells [1, 2]. In recent years, the mortality of breast cancer has been decreased in most high-income countries thanks to the developed therapy and earlier diagnosis., while an enhanced mortality still exists in certain countries [3]. Breast cancer is mainly caused by gender, age, alcohol consumption, oral contraceptive, hereditary tendency and family history [4]. Great achievements have been made in the diagnostic and therapeutic methods for breast cancer,
resulting in the decrease of mortality rate. Nevertheless, over 500,000 deaths have been found each year around the world [5]. Surgical resection, chemotherapy and radiotherapy are vital treatment regimens for breast cancer, but these methods are not suitable for advanced metastatic breast cancer [6]. Therefore, more efficient therapeutic strategies of breast cancer are needed to further investigate the functions associated with tumor growth and development of breast cancer.

Long non-coding RNAs (lncRNAs) have been affirmed in diverse cancers and exert critical functions through interacting with DNA, RNA, or protein molecules [7]. LncRNA prostate cancer non-coding RNA 1 (PRNCR1) is a ~ 13 kb intron-less lncRNA, and it is transcribed from the 8q24 ‘gene-desert’ region [8]. LncRNA PRNCR1 is a newly gene to be an oncogene in prostate cancer [9], colorectal cancer [10], as well as gastric cancer [11]. It is reported that lncRNA PRNCR1-2 takes part in breast cancer cells [12]. MicroRNAs (miRNAs) post-transcriptionally modulate gene expression via targeting the 3’untranslated region (3’UTR) of mRNAs [13]. MiRNAs are being underlined for their roles in pathological processes, including tumor formation and development in breast cancer [14, 15]. The microRNA miR-377 is located in the 14q32.31 locus, and this locus harbors genes that coding for a large number of miRNAs involved in controlling biological functions in metastatic cancer cells [16]. Evidence has shown that miR-377-3p plays a role in triple-negative breast cancer cell lines [17].

CyclinD2 (CCND2) is a unique gene among the three D-type cyclins, which is up-regulated in the growth arrest in normal fibroblasts [18]. An article has demonstrated that upregulated CCND2 increases proliferation in breast cancer cells [19]. Based on aforementioned evidence, we could conjecture that lncRNA PRNCR1/miR-377/CCND2 axis participates in breast cancer growth.

Materials And Methods
Clinical sample collection
From February 2013 to June 2014, 64 patient samples from breast cancer resections along with the corresponding paracancerous tissues were collected from Pingxiang Health Vocational College. The pathological diagnosis was obtained based on histology or biopsy of the tumor specimen and examined by experienced pathologists. The breast cancer tissues and paracancerous tissues were stored in liquid nitrogen.

Cell culture and transfection
Human breast epithelial cell line (MCF-10A) and breast cancer cell lines (MDA-MB-231, MCF-7, BT-549, MDA-MB-468 and SK-BR-3) without mycoplasma and other contamination were selected. The above cells were purchased from American Type Culture Collection (Manassas, VA, USA). All cells were cultured in 90% RPMI-1640 medium, which were appended with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Waltham, MA, USA), 100 U/mL penicillin and 100 mg/mL streptomycin, and then cultured in a saturated incubator containing 5% CO₂ at 37 °C.

MCF-7 cells were divided into groups, and the vector used for transfection was purchased from Guangzhou RiboBio (Guangdong, China). siRNA was designed and synthesized by Thermo Fisher Scientific Inc. MCF-7 cells were seeded at 1 × 10⁶ cells/well in 6-well plates, and transduced with Lipofectamine 2000 transfection reagent (Invitrogen, Carlsbad, CA). According to different experimental requirements, cells were collected at different time periods after transfection for subsequent experiments.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Cells were harvested 48 h post transfection and total RNA was extracted using Trizol (Sigma-Aldrich, St Louis, MO, USA). RNA sample (5 µL) was diluted 20-fold with RNase-free ultrapure water, and the absorbance value at 260 nm and 280 nm was read by an ultraviolet spectrophotometer to determine the concentration and purity of RNA. According to the reverse transcription kit instructions (Beyotime, Shanghai, China), reverse transcription was performed on a PCR amplification instrument for the synthesis of a cDNA template. The required qPCR primers were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). The primer information is shown in Table 1. Glyceraldehyde phosphate dehydrogenase (GAPDH) was the loading control of PRNCR1 and CCND2, and U6, the loading control of miR-377. \(2^{-\Delta\Delta Ct}\) method was adopted for gene expression analysis.
Western blot assay

Cells were harvested 48 h post transfection and then washed with precooled phosphate buffered saline (PBS) and lysed with radioimmunoprecipitation assay buffer containing 10% protease inhibitor (MCE, USA). The cell sample was transferred to a 1.5 mL centrifuge tube, centrifuged for 10 min at 13,000 g for acquiring the supernatant. The measurement of protein concentration was implemented by bicinchoninic acid method and stored at -20 °C until use. The kit of sodium dodecyl sulfate polyacrylamide gel electrophoresis was utilized for preparing 10% separation gel and 4% concentrated gel. Next, the protein was separated by electrophoresis on polyacrylamide gel, and transferred to the nitrocellulose membrane by wet transfer method, and the membrane was blocked by 5% defatted milk for 1 h. After that, the membrane was probed with the primary antibodies CCND2 (ab207604), MEK1 (ab32091), phosphorylated (p)-MEK1(ab96379), p38 MAPK (ab31828), p-p38 MAPK (ab4822) (all from Abcam, Cambridge, USA), and re-probed with secondary antibody IgG (ab150077, Abcam). The image was developed by a Bio-Rad gel imaging system (MG8600, Beijing Thmorgan Biotechnology Co., Ltd., Beijing, China), and quantitative analysis was performed using IPP7.0 software (Media Cybernetics, Singapore).

Cell counting kit (CCK)-8 assay

According to the kit's operating instructions, CCK-8 (Bimake) was adopted to determine cell proliferation capacity. Twenty-four hours post cell transfection, the cells were re-seeded at 3000 cells per well onto a 96-well plate. Optical density value was detected using a microplate reader at 450 nm. Each experiment was executed in triplicate and repeated independently three times.

Flow cytometry
The cells at 48 h post transfection were rinsed with PBS-balanced salt solution, and detached with 0.25% trypsin. The trypsin was removed when the cells were observed to be shrinkage round under the microscope, and the detachment was terminated by adding serum-containing medium. Afterwards, the cells were triturated for cell suspension, which was centrifuged to remove the supernatant. Afterwards, the cells were fixed with 70% ice-cold ethanol for 30 min, and dyed with 1% propidium iodide (PI) solution containing RNA enzyme for 30 min. With PI removal, the cells were adjusted to the concentration of 1 mL. The samples were positioned in a BD-Aria FACS Calibur flow cytometer (FACSCalibur, Beckman Coulter, USA) for detecting the cell cycle.

The cells at 48 h post transfection were altered to the concentration of $1 \times 10^6$ cells/mL. Next, the cells were appended with 70% precooled ethanol solution and rinsed with PBS two times. Cell suspension (100 µL, no less than $10^6$ cells/mL) was suspended in 100 µL Binding Buffer, supplemented with added with 10 µL Annexin V-fluorescein isothiocyanate (FITC), 5 µL PI as well as 300 µL Binding Buffer. A flow cytometer (Attune NxT, Thermo Fisher, USA) was implemented to detect apoptosis at 488 nm.

**Fluorescent in Situ Hybridization (FISH)**

PRNCR1 expression was detected in situ in MCF-7 cells using a FISH Kit (C10910, RiboBio). The cell slide was placed on the 24-well plate’s bottom, and MCF-7 cells were detached onto the slide (approximately $6 \times 10^4$/well). When reaching 60–70% confluence, cells were fastened in 4% paraformaldehyde, appended with 1 mL pre-cooled permeable fluid. After discarding the permeable fluid, cells were supplemented with 200 µL prehybridization solution. Meanwhile, the hybridization solution was added with 2.5 µL 20 µM FISH Probe Mix storage fluid. Afterwards, the pre-hybridization solution was removed and hybridization solution containing the PRNCR1 probe was appended. Next, the cells were washed with different kinds of wash solution and incubated devoid of light. The nucleus was stained with 1',6-diamidino-2-phenylindole 2hci solution and washed with PBS three times. Under dark conditions, the cell slide was carefully removed from the well, which was fixed on a glass slide with a mounting plate and perform fluorescence detection. PRNCR1 specific probe was synthesized by RiboBio.
Dual luciferase reporter gene assay
The biological prediction website was utilized to analyze the binding site of PRNCR1 and miR-377, and the binding site of miR-377 and CCND2, thereby obtaining the fragment sequence containing the action site. Next, the full length PRNCR1 and 3’UTR of CCND2 were cloned and amplified into pmiR-GLO luciferase vectors (E1330, Promega, Madison, WI, USA) and named as PRNCR1-wild type (Wt) and CCND2-Wt. Bioinformatics software was adopted for forecasting the binding sites of miR-377 and PRNCR1, along with miR-377 and CCND2 by site-directed mutations. PRNCR1-Mut and CCND2-mutant type (Mut) vectors were constructed, and the internal reference was pRL-TK vector (E2241, Promega) expressing renilla luciferase. The 293T cells were introduced with miR-377 mimic or mimic negative control (NC) with the luciferase reporter vector respectively, and the fluorescence intensity was tested using a fluorescence detector (Glomax 20/20, Promega).

RNA pull-down assay
Cells were transduced with 50 nM biotin-labeled Wt-bio-miR-377 and Mut-bio-miR-377. Forty-eight hours later, cells were incubated for 10 min with specific lysis buffer (Ambion, Austin, Texas, USA). After that, the lysate was cultured with M-280 streptavidin magnetic beads (S3762, Sigma) pre-coated with RNase-free bovine serum albumin together with yeast tRNA (TRNABAK-RO, Sigma). Next, the beads were cultured for 3 h at 4 °C, washed with pre-chilled lysis buffer, low salt buffer, and high salt buffer. The bound RNA was purified by Trizol and then detected.

Radioimmunoprecipitation (RIP) assay
Cell were lysed with lysis buffer (25 mM Tris-HCl pH 7.4, 150 mM NaCl, 0.5% NP-40, 2 mM EDTA, 1 mM NaF and 0.5 mM dithiothreitol) containing RNasin (Takara, Dalian, China) and a protease inhibitor mixture (B14001a, Roche, USA). The lysate was centrifuged to collect the supernatant. Then, anti-Ago-2 magnetic beads (BMFA-1, Biomarker, Beijing, China) were added, and the control group was added with anti-IgG magnetic beads. Then the beads were rinsed three times with wash buffer (50 mM Tris-HCl, 300 mM NaCl pH 7.4, 1 mM MgCl2, 0.1% NP-40). RNA was extracted from magnetic beads by Trizol method.

Statistical analysis
All data were processed by SPSS 21.0 statistical software (IBM Corp. Armonk, NY, USA). All data were
reported as mean ± standard deviation for no less than three independent experiments. The statistical significance of the average was determined by the student t test. For skewed distribution data, non-parametric tests were used to determine statistical significance. P < 0.05 was indicative of statistical significance.

Results
LncRNA PRNCR1 is enhanced in breast cancer, and downregulated PRNCR retards breast cancer growth
First, we collected 64 samples of breast cancer and paracancerous tissues for detecting PRNCR1 expression by RT-qPCR. As indicated in Fig. 1A, we discovered that PRNCR was elevated in breast cancer tissues. Patients were followed up after surgery to collect the prognostic survival time. At the same time, the breast cancer tissues were divided into PRNCR1 high expression group (n = 32) and PRNCR1 low expression group (n = 32) based upon the median PRNCR1 expression, and the result of which indicated that high expression of PRNCR1 had poor prognostic survival (Fig. 1B). Human breast epithelial cell line MCF-10A and breast cancer cell lines MDA-MB-231, MCF-7, BT-549, MDA-MB-468 and SK-BR-3 were obtained for detecting PRNCR1 expression in cells (Fig. 1C). The findings indicated that PRNCR1 expression was increased to varying degrees in breast cancer cell lines in comparison to MCF-10A cell line. Therefore, MCF-7 cells with the highest PRNCR1 expression were selected for further assays.
Additionally, MCF-7 cells were transfected with si-NC and si-PRNCR1 to verify the transfection efficiency by RT-qPCR (Fig. 1D). The outcomes suggested that MCF-7 cells upon si-PRNCR1 treatment exhibited reduced viability, retarded cell cycle progression and induced proportion of apoptosis (Fig. 1E-G). The above results show that PRNCR1 can promote breast cancer cell growth.

miR-377 competitively binds with LncRNA PRNCR1
Subsequently, we detected the localization of LncRNA PRNCR1 in MCF-7 cells by RNA-FISH, and the results showed that it was mainly localized in the cytoplasm (Fig. 2A). The online website predicted that a binding site existed between PRNCR1 and miR-377 (Fig. 2B). The dual luciferase reporter gene assay verified that miR-377 overexpression inhibited the activity of the PRNCR1 fluorescent reporter vector (Fig. 2C). RNA pull-down assay indicated that PRNCR1 bound by Wt-miR-377 was elevated
versus Mut-miR-377, indicating that miR-377 could directly bind with PRNCR1 (Fig. 2D). The result of RIP assay implied that compared with IgG, AGO2-bound PRNCR1 was increased (Fig. 2E), indicating that miR-377 could bind with PRNCR1.

**Overexpressed miR-377 inhibits breast cancer development**

Further investigation was focused on miR-377 expression in cells, and the obtained findings suggested that relative to MCF-10A, miR-377 expression in other breast cancer cells was declined in varying degrees, and MCF-7 cells had the lowest expression (Fig. 3A). Next, we introduced MCF-7 cells with mimic NC, miR-377 mimic, inhibitor NC, and miR-377 inhibitor, respectively, so as to verify the transfection efficiency by RT-qPCR (Fig. 3B). The outcomes suggested that MCF-7 cells upon miR-377 mimic treatment exhibited reduced viability, retarded cell cycle progression and induced proportion of apoptosis. On the contrary, MCF-7 cells introduced with miR-377 inhibitor presented an inverse trend (Fig. 3C-E). It is suggested that miR-377 results in suppressed growth of breast cancer cells.

**miR-377 targets CCND2 in breast cancer**

With the aim to explore the downstream regulatory mechanism of miR-377, we screened the CCND2 gene through the online website (Fig. 4A). CCND2 is one of the cell cycle regulators, and its expression is elevated in many cancers. The dual luciferase reporter gene assay verified the targeting relationship between miR-377 and CCND2, and overexpressed miR-377 restricted the luciferase activity of the CCND2 reporter vector (Fig. 4B). CCND2 protein expression was reduced after miR-377 overexpression, and CCND2 protein expression was increased after miR-377 silencing (Fig. 4C, D). In the rescue experiments, mimic NC + oe-NC, miR-377 mimic + oe-NC, mimic NC + oe-CCND2 and miR-377 mimic + oe-CCND2 were introduced into MCF-7 cells, and miR-377 and CCND2 in cells was tested by RT-qPCR and western blot analysis (Fig. 4E-G). The outcomes indicated that in contrast to the mimic NC + oe-NC group, the miR-377 mimic + oe-NC group exhibited increased miR-377 expression and reduced CCND2 expression, while the mimic NC + oe-CCND2 presented elevated CCND2 expression. In addition, elevated CCND2 expression was found in the miR-377 mimic + oe-CCND2 group versus the miR-377 mimic + oe-NC group. Furthermore, in contrast to the mimic NC + oe-NC group, the miR-377 mimic + oe-NC group exhibited reduced viability, retarded cell cycle progression
and induced proportion of apoptosis of MCF-7 cells. However, promoted viability, accelerated cell cycle progression and suppressed proportion of apoptosis of MCF-7 cells were found in the miR-377 mimic + oe-CCND2 group versus the miR-377 mimic + oe-NC group (Fig. 4H-J). The above results comprehensively show that miR-377 inhibits CCND2 to inhibit breast cancer cell growth.

**LncRNA PRNCR1/miR-377/CCND2/MEK/MAPK axis modulates breast cancer proliferation and apoptosis**

The expression of some members of the MEK/MAPK pathway (MEK1, p-MEK1, p38 MAPK, and p-p38 MAPK) in MCF-7 cells treated with oe-NC and oe-CCND2 were determined, and the results showed that overexpressed CCND2 elevated expression of p-MEK1 and p-p38 MAPK (Fig. 5A, B). Therefore, we speculated that CCND2 may regulate breast cancer proliferation and apoptosis through the MEK/MAPK pathway. Additionally, the MEK/MAPK pathway inhibitor Mirdametinib was supplemented into MCF-7 cells for detecting the expression of MEK1, p-MEK1, p38 MAPK, and p-p38 MAPK. The obtained findings suggested that suppression of the MEK/MAPK pathway (Mirdametinib) decreased the MEK1, p-MEK1, and p-p38 MAPK in MCF-7 cells (Fig. 5C, D). Furthermore, suppression of the MEK/MAPK pathway (Mirdametinib) was found to restrict viability and cell cycle entry as well as promoted apoptosis of MCF-7 cells (Fig. 5E-G). In summary, IncRNA PRNCR1 may absorb miR-377, resulting in elevated CCND2 and activated MEK/MAPK pathway, thereby leading to breast cancer growth.

**Discussion**

Recently, emerging evidence has supported the participation of lncRNAs in the human cancers’ pathogenesis and progression [12]. To develop new cancer treatments and therapeutics, it is essential to fully understand the lncRNAs’ functions and mechanisms. In this current study, we aimed to depict the mechanisms of PRNCR1 in breast cancer via miR-377/CCND2/MEK/MAPK axis.

From the obtained findings, we found that PRNCR1 was regulated in breast cancer. It is reported that increased IncRNA PRNCR1-2 expression is witnessed in breast cancer tissues, and depletion of PRNCR1-2 suppress their proliferation rates in breast cancer cells [12]. Another study has revealed that IncRNA PRNCR1 is enhanced in breast cancer, and knockdown of PRNCR1 restricts breast cancer cells’ malignant phenotypes from in vitro experiments [20]. Similarly, PRNCR1 is enhanced in colorectal cancer (CRC) tissues and PRNCR1 knockdown induces cell cycle arrest in the CRC cells [10].

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Nevertheless, how PRNCR1 participates in breast cancer is barely described. Competing endogenous RNAs (ceRNAs) are capable of communicating with each other through competing for shared miRNAs [21]. From our study, we demonstrated that miR-377 expression was declined in breast cancer. miR-377 was expounded to be declined in hepatocellular carcinoma (HCC), and restoration of miR-377 suppresses HCC cell growth [22]. LncRNA PRNCR1 is a sponge in non-small cell lung cancer by competing with miR-448 [23]. Also, LncRNA PRNCR1 modulates osteogenic differentiation in osteolysis through targeting miR-211-5p [24]. However, relationship between PRNCR1 and miR-377 is barely investigated.

To probe into the molecular mechanism by which miR-377 suppressed breast cancer cell growth, we identified CCND2 as a direct target of miR-377. Aberrant expression of CCND2 has been found in testicular germ cell tumor cell lines and ovarian granulosa cell tumors [25]. In addition, it is reported that activation of CCND2 is able to induce thyroid cancer growth [26]. MiR-377 was found to participate in many tumors via different genes. Dual luciferase analysis in a study has verified that miR-377-3p could binding to HOXC6 3′-UTR region, and miR-377-3p declined HOXC6 in breast cancer [17]. MiR-377 possesses the function of tumor growth suppression via decreasing Pim-3 kinase expression in order to retard pancreatic tumor growth [27]. MiR-377 has also been interpreted to directly reducing ETS1 expression, which regulates the capability of renal cell carcinoma cells to migrate and invade [16]. Similarly, overexpressed miR-377 suppresses gastric cancer progression via vascular endothelial growth factor A [28]. LncRNA and mRNA integration network is of great importance in human cancers [29]. The upregulated CCND2 was evidenced to reverse the tumor inhibitory role of miR-497, further enhancing viability in breast cancer cells [19]. Nevertheless, no article has been focused on the binding relation between miR-377 and CCND2.

Conclusion
In summary, this study reports that PRNCR1 is elevated in breast cancer, and depleted PRNCR1 results in the inhibition of breast cancer progression (Fig. 6). These findings suggest that PRNCR1 may be explored as a biomarker for breast cancer diagnostics or therapeutics. Meanwhile, our experimental data may offer a strategy for targeting miR-377/CCND2 interaction in treating breast
cancer patients.

Abbreviations
miR-103a, microRNA-103a; NSCLC, non-small cell lung cancer; OTUB1, ovarian tumor domain-containing ubiquitin aldehyde binding protein 1; CSCs, cancer stem cells; DMEM, Dulbecco’s modified Eagle’s medium; SP, side population; oe, overexpressed; NC, negative control; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetr-azolium; OD, optical density; PBS, phosphate buffered saline; RT-qPCR, real-time quantitative PCR; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; 3’UTR, 3’untranslated region; ANOVA, analysis of variation.

Declarations

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Not applicable.

Authors’ contributions
JOY conceived the study and participated in its design and coordination; ZLL, XBY and XC performed all experiments; YPW, LL, CPL and HL analyzed and interpreted the data; The draft was improved through discussion and editing by all the authors who read and approved the final manuscript.

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Availability of data and materials
All the data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate
This study was complied with all relevant ethics of human research participants, and all participants provided written informed consent.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Figures
PRNCR1 promotes proliferation and inhibits apoptosis of breast cancer cells. A. PRNCR1 expression in breast cancer tissues and corresponding paracancerous tissues was determined by RT-qPCR. B. Relationship between PRNCR1 expression and prognosis of breast cancer patients. C. PRNCR1 expression in human breast epithelial cell line MCF-10A and breast cancer cell lines MDA-MB-231, MCF-7, BT-549, MDA-MB-468 and SK-BR-3 was determined by RT-qPCR. D. PRNCR1 expression in cells transfected with si-NC or si-PRNCR1. E. MCF-7 cell viability was determined by CCK-8 assay. F. Cell cycle distribution was detected by flow cytometry. G. Cell apoptosis was determined by flow cytometry. * P < 0.05 vs. MCF-10A cells; # P < 0.05 vs. si-NC group.

LncRNA PRNCR1 binds with miR-377. A. RNA-FISH detection of PRNCR1 localization in cells. B. Prediction of binding sites of PRNCR1 and miR-377. C. Dual luciferase reporter gene assay for validating the correlation between PRNCR1 and miR-377. * P < 0.05 vs. mimic NC group. D. RNA-pull down assay detection of PRNCR1 binding to miR-377. * P < 0.05 vs. MUT-bio-miR-377 group. E. RIP assay for validating PRNCR1 function. * P < 0.05 vs. IgG group.
Overexpression of miR-377 inhibits breast cancer cell proliferation and promotes apoptosis.

A. miR-377 expression in human breast epithelial cell line MCF-10A and breast cancer cell lines MDA-MB-231, MCF-7, BT-549, MDA-MB-468 and SK-BR-3 was determined by RT-qPCR. *P < 0.05 vs. MCF-10A cells.

B. miR-377 expression in cells of each group by RT-qPCR.

C. MCF-7 cell viability was determined by CCK-8 assay.

D. Cell cycle distribution was detected by flow cytometry.

E. Cell apoptosis was determined by flow cytometry. *P < 0.05 vs. mimic NC group; # P < 0.05 vs. inhibitor NC group.
miR-377 targets CCND2 expression in breast cancer. A. Prediction of binding sites of miR-377 and CCND2. B. Dual luciferase reporter gene assay for validating the correlation between miR-377 and CCND2. * P < 0.05 vs. mimic NC group. C. Protein band of CCND2. D. Expression of CCND2 in cells of each group determined by western blot analysis. * P < 0.05 vs. mimic NC group; # P < 0.05 vs. inhibitor NC group. E. Expression of miR-377 and CCND2 in cells of each group determined by RT-qPCR. F. Protein band of CCND2. G. Expression of
CCND2 in cells of each group determined by western blot analysis. H. MCF-7 cell viability was determined by CCK-8 assay. I. Cell cycle distribution was detected by flow cytometry. J. Cell apoptosis was determined by flow cytometry. * P < 0.05 vs. mimic NC + oe-NC group; # P < 0.05 vs. miR-377 mimic + oe-NC group.

Figure 5

CCND2 activates MEK/MAPK pathway. A. Protein bands of MEK1, p-MEK1, p38 MAPK, and p-p38 MAPK. B. MEK1, p-MEK1, p38 MAPK, and p-p38 MAPK expression levels in cells treated with oe-NC or oe-CCND2 by western blot analysis. C. Protein bands of MEK1, p-MEK1, p38 MAPK, and p-p38 MAPK. D. MEK1, p-MEK1, p38 MAPK, and p-p38 MAPK expression levels in cells by western blot analysis. E. MCF-7 cell viability was determined by CCK-8 assay. F. Cell cycle distribution was detected by flow cytometry. G. Cell apoptosis was determined by flow cytometry. * P < 0.05 vs. oe-NC group; # P < 0.05 vs. oe-CCND2 + DMSO group.
The mechanistic diagram depicts that IncRNA PRNCR1 may competitively bind to miR-377, leading to upregulation of CCND2 expression, which in turn leads to activation of the MEK/MAPK pathway, thereby promoting breast cancer cell proliferation and inhibiting apoptosis.