Non-coding RNA Research 9 (2024) 421–428

Contents lists available at ScienceDirect

Non-coding RNA Research

journal homepage: www.keaipublishing.com/en/journals/non-coding-rna-research

Original Research Article

LncRNA PCAT6 mediates UBFD1 expression via sponging miR-545-3p in breast cancer cells

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ARTICLE INFO

Keywords:
LncRNA PCAT6
UBFD1
Post-translational regulation

ABSTRACT

Background: LncRNA PCAT6 has been shown to involve in carcinogenesis of different tumors. In this study, we investigated underline mechanism by which PCAT6 promoted breast cancer cell progression.
Methods: RIP was used to identify lncRNAs associated with IMP1. Bioinformatics assays were used to predict potential miRNAs that interact with PCAT6 and mRNAs that are targeted by miR-545-3p. RNA-seq and RT-qPCR were used to analyze differential expression of lncRNAs and miRNA-targeted genes. Luciferase reporter and RNA pull-down assays were performed to identify the molecular interactions between PCAT6 and individual miRNAs. The role of PCAT6-mediated cell proliferation and invasion were tested by CCK-8 and transwell assays following loss-of-function and gain-of-function effects.
Results: We identified that PCAT6 is one of the lncRNAs that associated with IMP1. PCAT6 not only binds to IMP1, but also acts as a ceRNA to interact with multiple miRNAs, including miR-545-3p. Binding of IMP1 destabilized PCAT6, while competitive interaction with miR-545-3p allowed PCAT6 to positively regulate UBFD1 expression. Silencing UBFD1 mRNA could effectively rescue PCAT6-induced cell proliferation and invasive abilities.
Conclusions: Our study provided evidence that PCAT6 activates UBFD1 expression via sponging miR-545-3p to increase carcinogenesis of breast cancer cells. Based on the nature of UBFD1 as a polyubiquitin binding protein, our study suggested that ubiquitin pathway might contribute to breast cancer progression.

1. Background

With numerous ncRNAs (non-coding RNA transcripts) have been identified over the past years, investigation of their biological functions becomes an attractive research field [1,2]. NcRNAs generally contain two main groups: the long non-coding RNAs (lncRNAs) and the microRNAs (miRNAs) [3]. The lncRNAs are more than 200 nucleotides long and miRNAs are usually 18 to 25 nucleotides in length, both of them perform roles in regulating mRNA expression via various mechanisms [4,5]. To data, many ncRNAs have been identified to be involved in pathological diseases, including autoimmune disease, neurological disorders and cancer [4,6,7]. Particularly, regulatory networks between lncRNA and miRNA involved in cancer progression have been received extensive consideration [8].

LncRNAs and miRNAs are important regulators to mediate gene expression through transcriptional and post-transcriptional mechanisms [5]. For examples, lncRNA HOTAIR plays a vital role in regulation of chromatin state through interacting with polycomb repressive complex 2 [9]. In breast cancer cells, elevated expression of NEAT1 (nuclear paraspeckle assembly transcript 1) increases ZEB1 expression by sponging miR-448, thereby promoting cell growth and invasion capacity [10]. Emerging evidence has shown that lncRNAs act as "miRNA-sponges" or competing endogenous RNAs (ceRNA) to decrease the miRNA-induced repression of mRNAs [4]. While miRNAs negatively regulate gene expression via either translational repression or mRNA degradation [5]. MiRNAs also bind to a large and heterogeneous class of functional lncRNAs to conduct their regulations and their interactions frequently reverse lncRNA-directed biological functions [11]. Therefore,

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https://doi.org/10.1016/j.ncrna.2024.01.019
Received 19 January 2024; Accepted 30 January 2024
Available online 8 February 2024

lncRNA-miRNA interactions are important aspects in the processes of gene expression in addition to already recognized steps of mRNA splicing, decay and translational control [12–14]. Further characterization of novel lncRNA-miRNA interactions during breast cancer progression, will reveal useful information for developing potential prognostic markers and therapeutic targets.

LncRNA PCAT6 (Prostate cancer-associated transcript 6) has been reported to involve in carcinogenetic process of colon, ovary, liver and breast tumors [15]. In breast cancer cells, PCAT6 has been shown to enhance angiogenesis by sponging miR-4723-5p to up-regulate expression of VEGFR2 [16]. While another study indicated that silence of PCAT6 suppressed cell proliferation and induced cell apoptosis [17]. Here, we show that lncRNA PCAT6 is a binding partner of IMP1. IMP1 destabilized PCAT6 and decreased ability of PCAT6 to associate with target miRNAs. We demonstrated that PCAT6 acted as a ceRNA for miR-545-3p and prevented the miRNA from association with UBFD1 mRNA. Accordingly, either silencing PCAT6 or UBFD1 reduced cell proliferation and invasive abilities. Our study provided evidence that the sponge effect of PCAT6 to miR-545-3p eventually increased UBFD1 expression and carcinogenesis of breast cancer cells.

2. Methods

2.1. Cell lines and culture conditions

Breast cancer cell lines MDA-MB-231, BT-549, T47D and human embryonic kidney 293T cell line were obtained from ATCC (American Type Culture Collection). A pair of stable cell lines MDA-MB-231/GFP-IMP1 and MDA-MB-231/GFP were established previously [18]. Stable BT-549 cell lines expressing PCAT6 or PCAT6-MS2 were established by infection of lentivirus as previously described [19]. Cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 °C in a humid chamber of 5% CO2.

2.2. Reagents

Primary antibodies against IMP1 and GAPDH were purchased from Cell Signaling Co. (USA). Rabbit polyclonal antibodies to human UBFD1 and β-actin were purchased form abcam Inc (USA). siRNAs against PCAT6 or UBFD1 mRNA, miRNA mimics and PCR primers were purchased from Tiangen Biotech Co. (Beijing, China). The sequences of all primers are listed in Suppl Table S1. The siRNA and miRNA mimics are listed in Suppl Table S2.

2.3. Cell proliferation assays

Cell viability was analyzed by CCK-8 Kit (Beyotime, Shanghai, China) based on the manufacturer's instructions. Briefly, cells were seeded at a density of 5×10^3/well (100 μl) in a 96-well microplate (Corning, USA). After cultured for 24 h, CCK-8 reagent (10 μl) was added to each well and cultured for additional 2 h. All experiments were performed in triplicate. Cell viability was indicated by the absorbance at 450 nm using a microplate reader.

2.4. Cell invasion assays

The procedures of cell invasion assays (transwell) were performed as previously described [20].

2.5. Bioinformatic studies

Potential miRNA target sites on the PCAT6 lncRNA were predicted using online public databases TargetScan 7.2 (https://www.targetscan.org) and micro-RNA.org-target program. We have also selected three miRNAs known for PCAT6 binding from published literatures [21]. Using these approaches, total six miRNAs were used for assays.

2.6. Identification of IMP1-associated lncRNAs

The procedure for identifying lncRNAs that associate with IMP1 was performed as previously mentioned [20]. Briefly, T47D expressing ectopic IMP1 and T47D control cells were lysed in an ice-cold lysis buffer. After high-speed centrifugation for 60 min at 4 °C, supernatants were incubated with IMP1 antibody and Protein A agarose beads (Sigma) at 4 °C overnight. The supernatant was removed by a short centrifugation and the agarose resin was extensively washed. RNAs were extracted using TRIzol and sent for RNA-seq assays in Shanghai Biotechnology Corporation (Shanghai, China) to identify lncRNAs complexed with IMP1. The RNA-seq data has been deposited as a submission number of GSE220087 in Gene Expression Omnibus (GEO).

2.7. Fluorescence in situ hybridization (FISH)

FISH experiments were performed as previously described [22]. MDA-MB-231 cells were grown on coverslips and fixed with 4% Paraformaldehyde (PFA). All reagents used in the experiments were prepared in diethylpyrocarbonate (DEPC)-treated water. Nucleotide sequences of FISH probes for detecting PACT6 were list in Table S2.

2.8. RNA-pulldown assays

Procedure for RNA-pulldown assays to detect the interaction between PCAT6 and its associated miRNAs was as previously described [23]. Briefly, cells expressing MS2-tagged PCAT6 were cultured and lysed. Lysates were incubated with a recombinant protein MBP-MCP at 4 °C overnight and then incubated with maltose beads (NEB, USA) for 4 h at room temperature. miRNAs coprecipitated with PCAT6 were isolated.

2.9. RNA isolation and RT-qPCR

RNA extraction and RT-qPCR were performed as described previously [20]. Briefly, total RNA was extracted using TRIzol Reagent (Takara, Japan). After quantitation, 1 μg RNA was reverse transcribed to cDNA using a PrimerScript kit (Takara, Japan). The qPCR was performed using a SYBR Green reaction mix (Vazyme, China). Relative expression of interested genes was measured using the $2^{-\Delta\Delta Ct}$ method.

2.10. Statistical analysis

All assays were at least bio-repeated in triplicate, and the results were indicated as means ± S.D. with *P* value below 0.05 as significant. Student's *t*-test or one-way ANOVA was applied to determine statistical probabilities.

3. Results

3.1. PCAT6 is one of the lncRNAs that associate with IMP1 in breast cancer cells

We have previously shown that ectopic expression of IMP1 (insulin-like growth factor 2 messenger RNA binding protein) repressed breast cancer growth and metastasis by mediating local translation of cell adhesion and motility-related mRNAs [22]. We assumed that IMP1 could also play role in regulation of lncRNAs. To address this, we used IMP1-RIP followed by RNA-seq analysis to identify lncRNAs that associate with IMP1 in T47D cells (The raw RNA-seq data have been deposited as submission number 220087 in Gene Expression Omnibus (GEO) database). Compared to the control (IgG-precipitates), a group of lncRNA were highly enriched in the IMP1-precipiates (Fig. 1A). To ensure whether binding of IMP1 to these identified lncRNA occurred in

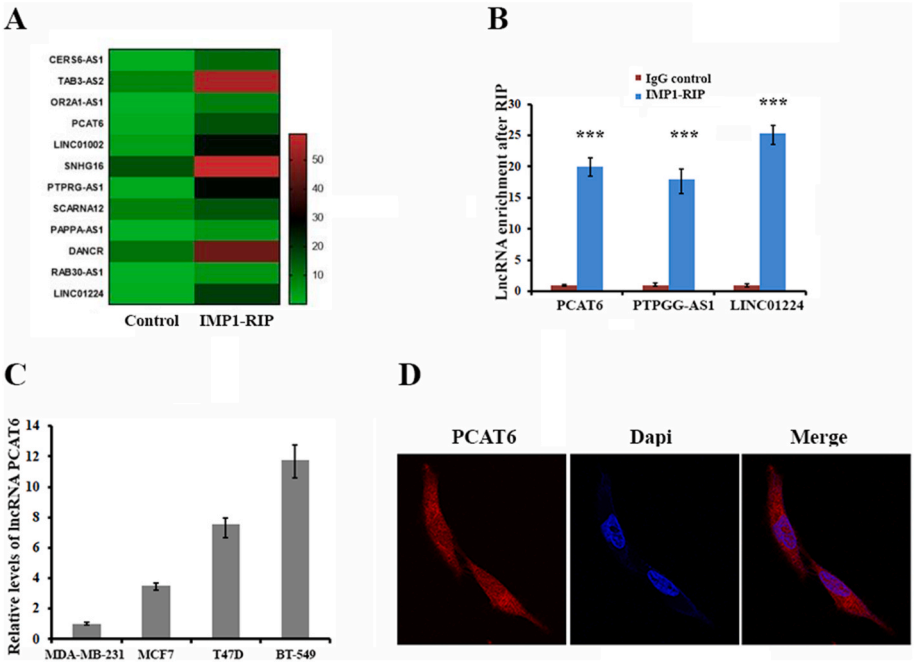

Fig. 1. pCAT6 is a lncRNA associated with IMP1 in breast cancer cells
(A) RIP (RNA immunoprecipitation) using IMP1 antibody was performed to identified IMP1-associated lncRNAs in T47D cells. The heatmap shows some lncRNAs that were highly enriched in the IMP1-precipiates. (B) IMP1-RIP followed by RT-qPCR were performed to confirm IMP1-bound lncRNAs in BT-549 cells. Three lncRNAs selected from (A) were truly bound to IMP1. $***P < 0.001$. (C) PT-qPCR indicates the relative expression of PCAT6 in four breast cancer cell lines. (D) FISH shows the cellular distribution of PCAT6 in breast cancer cells.

other breast cancer cells, we performed IMP1-RIP assays in BT-549 cell line and selected three lncRNAs, PCAT6, PTPGG-AS1 and LINC01224, to test their association with IMP1. RT-qPCR showed that all three lncRNAs, including PCAT6, were truly associated with IMP1 (Fig. 1B). Of particular interest in the lncRNAs that were involved in breast cancer progression and were not well studied, we used PCAT6 as a target to investigate its oncogenic role and the potential impact of IMP1 on PCAT6 expression. PCAT6 gene encodes a non-coding transcript with the sizes of 764 bps (NR_046325.1). Examination of the endogenous PCAT6 levels in four breast cancer cell lines showed that MDA-MB-231 cells expressed relatively lower levels of PCAT6, while BT-549 cells express highest levels of the lncRNA (Fig. 1C). FISH showed that PCAT6 predominantly localizes in the cytoplasm of breast cancer cells (Fig. 1D), indicating that PCAT6 could mainly perform cytoplasmic functions.

3.2. PCAT6 facilitates the proliferation and invasion of breast cancer cells

To investigate the biological function of PCAT6, we established stable MDA-MB-231 cell lines overexpressing PCAT6 or PCAT6-tagged MS2 chimeric RNA (PCAT6-MS2), which would allow us to perform pulldown assays using a MBP-MCP fusion protein that recognizes the MS2 hairpins structure [19]. Cell proliferation (CCK-8) and transwell assays demonstrated that expressing ectopic PCAT6 significantly increased cell proliferation and invasive abilities (Fig. 2A, B and 2C). While, silencing PCAT6 expression by siRNA treatment in BT-549 cells notably decreased the cell proliferation and invasion (Fig. 2D, E and 2F). These results indicate the oncogenic role of PCAT6 in breast cancer cells.

3.3. Identification of PCAT6-associated miRNAs in breast cancer cells

Given that PCAT6 is widely distributed in the cytoplasm, we hypothesized that PCAT6 would act as a sponge to associate with miRNAs and mediate their functions. It has previously been reported that PCAT6 could affect tumor cell proliferation, invasion and apoptosis through regulating various miRNAs, which include miR-185-5p, miR-143-3p and miR-326 [24]. By using an online RNAhybrid prediction program, we predicted additional three miRNAs, miR-545-3p, miR1306-5p and miR-543, which could potentially interact with PCAT6. To verify the in vivo binding ability of these six miRNAs to PCAT6, we performed RNA pulldown assays using amylose resin-conjugated with recombinant protein MBP-MCP in lysates prepared from MDA-MB-231 cells, which expressed ectopic PCAT6-MS2 (Fig. 3A, upper). After precipitation, miRNAs that complexed with PCAT6 were analyzed by RT-qPCR (Fig. 3A, lower). Results showed that miR-545-3p and miR-326, were highly enriched in the precipitates, while miR-185-5p was also co-precipitated with PCAT6 at a relatively lower level. No enrichment for miR-143-3p, miR-1306-5p and miR-543 was observed in the precipitates (Fig. 3B). To confirm the in vivo binding of PCAT6 to miR-545-3p and miR-185-5p, we subcloned PCAT6 into the 3′UTR of the luciferase reporter (Fig. 3B, upper). After co-transfection of the reporter with the individual miRNA mimic into HEK-293T cells, we detected that miR-545-3p mimic could significantly reduce the luciferase activity (Fig. 3C). By analyzing the nucleotide sequence, we identified a region within PCAT6 (Supp Fig. S1; 632 bp to 643 bp), which is highly matched with the nucleotide sequence of mir-545-3p. We then mutated the miR-545-3p binding site and subcloned mutant PCAT6 into the 3′UTR of the luciferase gene (Fig. 3D, upper panel). In comparison to wild-type PCAT6, co-transfection of mutant PCAT6 reporter with the miR-545-3p mimic only slightly changed luciferase activity (Fig. 3D, lower panel). Finally, we performed transwell assays in MDA-MB-231 cells expressing wild-type PCAT6 or mutant PCAT6. Results showed that compared to the PACT6-expressing cells, the invading ability of cells expressing mutant PCAT6 was largely decreased. These results indicated that PCAT6 promoted cell invasion through sequestering miR-545-3p.

3.4. Association of PCAT6 with miR-545-3p increases UBFD1 expression

Next, we used online bioinformatics tool (micro-RNA.org-target program) to search for mRNA candidates targeted by miR-545-3p. Five mRNAs, RREB1, UBFD1 (The ubiquitin family domain containing 1),

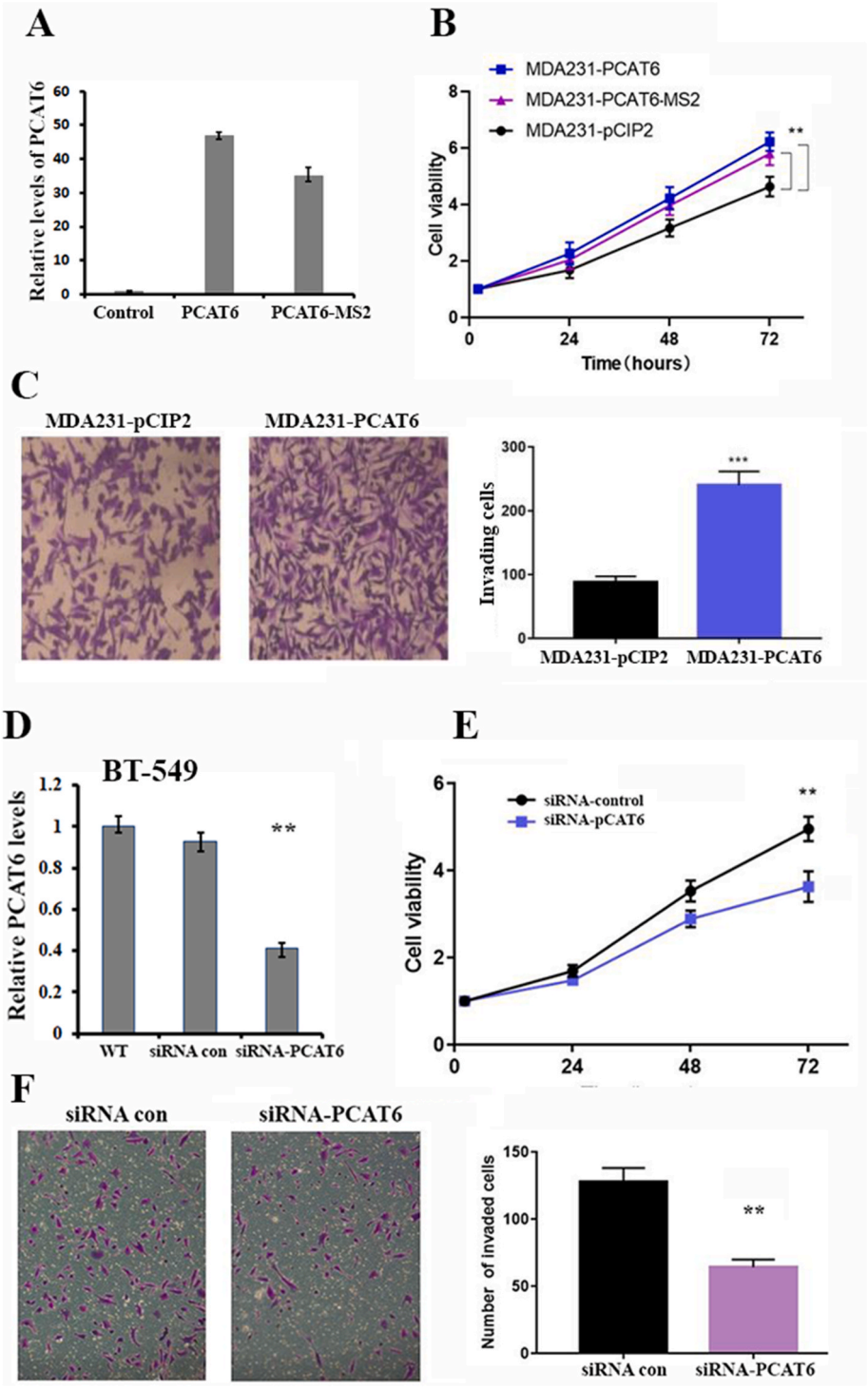

Fig. 2. PCAT6 promotes proliferation and invasion of breast cancer cells
(A) RT-PCR showing the relative levels of PCAT6 in WT and MDA-MB-231 cell lines stably expressing PCAT6 or PCAT6-MS2. (B) and (C) Cell proliferation (CCK-8) and transwell assays indicate that PCAT6 expression significantly increased cell proliferation and invasive abilities. $^{**}P < 0.01$, $^{**}P < 0.001$. (D) RT-PCR showing the relative levels of PCAT6 in BT-549 cells where PCAT6 was silenced by siRNA. (E) and (F) Knocking down PCAT6 notably reduced cell proliferation and invasion. $^{**}P < 0.01$.

LIMD1, GGA3 and KMT2D mRNAs were predicted to be possible targets of miR-545-3p. After examining their expression in PCAT6-expressing MDA-MB-231 cells, we observed that levels of UBFD1 and KMT2D mRNAs were notably elevated in responding to PCAT6 expression (Fig. 4A). To determine that PCAT6-promoted expression of UBFD1 or KMT2D mRNA was through the regulation of miR-545-3p, we transfected miR-545-3p mimic into either normal cells or PCAT6-expressing MDA-MB-231 cells. Results showed that levels of both UBFD1 and KMT2D mRNAs were decreased (Fig. 4B), suggesting a correlation for PCAT6/miR-545-3p/UBFD1(KMT2D) network in breast cancer cells. We characterized a potential biding site for miR-545-3p with the 3′ UTR of UBFD1 mRNA (Fig. 4C, upper panel). To ensure the importance of the binding site, we constructed luciferase reporters in which the wild-type or mutant UBFD1 3′UTR lacking the miR-545-3p binding site was

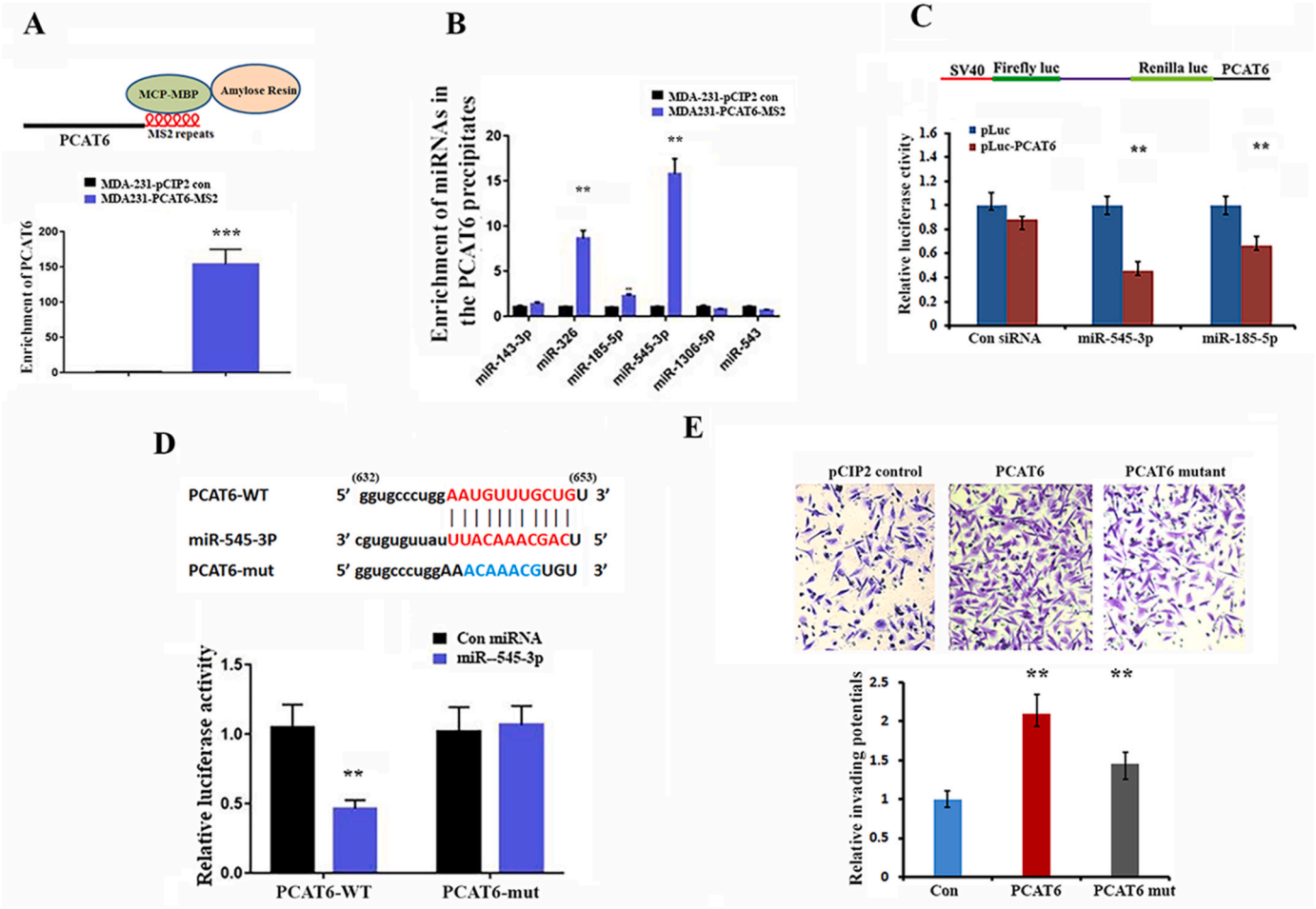

Fig. 3. miR-545-3p associates with PCAT6 with high affinity

(A) Upper panel: a representative diagram showing the PCAT6-MS2 chimeric RNA, which can bind to MBP-MCP fusion protein. Lower panel: PCAT6-MS2 was pulled down by MBP-MCP conjugated to amylose resin. ***P < 0.001. (B) RT-qPCR indicated the enrichment of miRNAs in the PCAT6 precipitates. **P < 0.01. (C) Upper: a schematic showing the luciferase reporter where the full-length of PCAT6 was subcloned into the 3′ UTR of the Renilla luciferase gene (denoted as pLuc-PCAT6). Lower panel: HEK-293T cells were transfected in combination with pLuc (control plasmid) or pLuc-PCAT6 and miR-545-3p or miR-185-5p. After 36 h transfection, luciferase activities were determined. Relative activity of renilla luciferase was normalized to firefly luciferase activity. **P < 0.01. (D) Upper panel: putative site for miR-545-3p binding within the PCAT6 sequence was shown as red-colored nucleotides. Mutated nucleotides are indicated as blue-color. Lower panel: MDA-MB-231 cells were transfected in combination with WT or mutant PCAT6 luciferase reporter and miR-545-3p mimic. Relative luciferase activities were determined after 36 h transfection. In contrast to WT PCAT6 reporter, co-transfection of mutant PCAT6 reporter with miR-545-3p mimic barely altered luciferase activity. **P < 0.01. (E) Transwell assays were performed in the cells expressing wild-type PCAT6 or mutant PCAT6, in which the biding site for miR-545-3p was mutated. Representative images for each cell group were shown. Statistical analysis indicated that mutant PCAT6 has lower invasive potential. **P < 0.01.

inserted into the 3-end of luciferase gene. Co-transfection of the reporter with miR-545-3p mimic into MDA-MB-231 cells indicated that the luciferase activity of WT reporter was significantly reduced, while the mutant reporter showed very litter changes in luciferase activity (Fig. 4C, lower panel). We finally used western blots to examine the effect of PCAT6 on UBFD1 protein levels in breast cancer cells. Although PCAT6 also notably promoted UBFD1 protein levels, co-expression of PCAT6 with miR-545-3p eliminated PCAT6-induced UBFD1 upregulation (Fig. 4D). These results indicated that competitive binding of PCAT6 to miR-545-3p, which originally bound to the 3′UTR of UBFD1 mRNA, increased UBFD1 expression.

3.5. PCAT6-mediated up-regulation of UBFD1 increases the growth ability of breast cancer cells

Based on the fact that PCAT6 is one of the IMP1-associated lncRNA (Fig. 1), we hypothesized that IMP1 binding could alter the extent of the PCAT6/miR-545-3p interaction and affect UBFD1 expression. To address this, we compared cellular RNA levels of PCAT6 and UBFD1 in MDA-MB-231/GFP and MDA-MB-231/IMP1-GFP cell lines. Expression of ectopic IMP1 not only decreased levels of PCAT6, but also UBFD1 mRNA (Fig. 5A). Consistently, knocking down endogenous IMP1 in BT-549 cells increased PCAT6 and UBFD1 mRNA levels (Fig. 5B). These results indicated that binding of IMP1 destabilized PCAT6 and reduced ability of PCAT6 to sponge miR-545-3p and decrease UBFD1 expression. Thus, the role of IMP1 to repress cell proliferation could partly through down-regulation of PCAT6 and UBFD1 expression [22]. UBFD1 has been identified as a polyubiquitin binding protein [25], whose expression was abnormally high in ER-positive breast cancer [26]. To investigate the biological impact of UBFD1 upregulation in response to PCAT6 expression, we silenced UBFD1 mRNA in normal and PCAT6-expressing MDA-MB-231 cells (Fig. 5C). Surprisingly, cell proliferation was significantly reduced when UBFD1 was silenced. Moreover, the PCAT6-induced cell proliferation can also be rescued by knocking down UBFD1 mRNA (Fig. 5D). These results indicated that UBFD1 is a potential oncogenic protein and one of the roles of PCAT6 to facilitate cancer progression is via the regulation of UBFD1 mRNA.

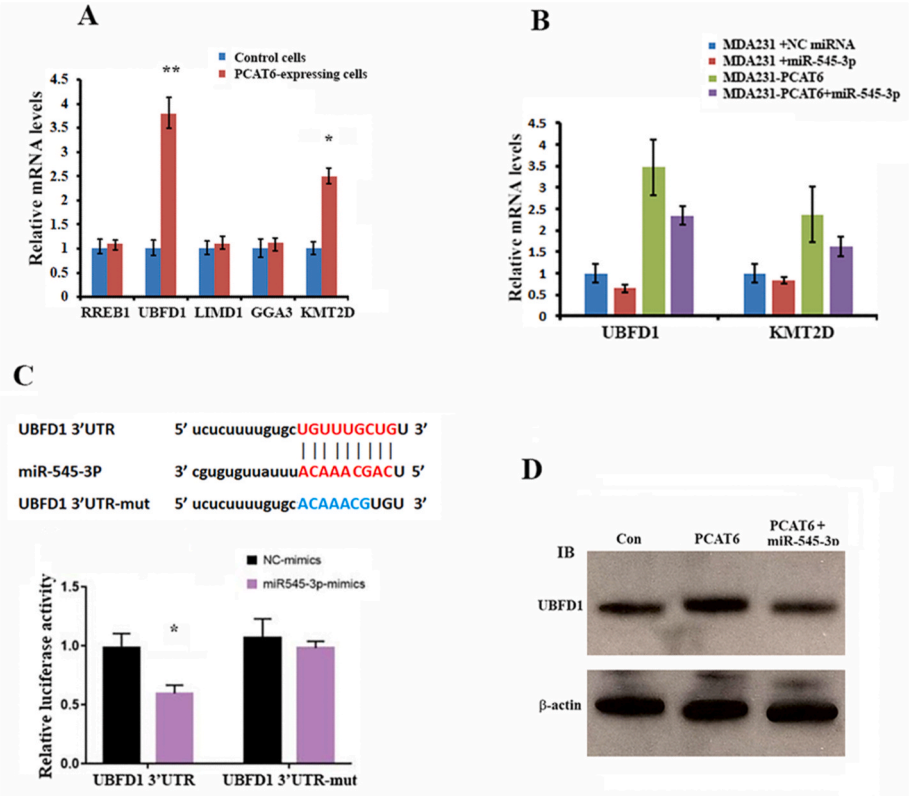

Fig. 4. Competitive binding of miR-545-3p to PCAT6 increases UBFD1 expression
(A) Five mRNAs were predicted to be the targets of miR-545-3p. Their expression was examined in WT or PCAT6-expressing breast cancer cells. Levels of UBFD1 and KMT2D mRNAs were significantly elevated when ectopic PCAT6 was expressed. **P < 0.01 and *P < 0.05. (B) Effect of PCAT6 and miR-545-3p on UBFD1 and KMT2D mRNA expression was examined in breast cancer cells. Results showed that the PCAT6-induced elevation of UBFD1 and KMT2D expression could be reduced by ectopic miR-545-3p. (C) Upper panel: putative binding site of miR-545-3p within the 3′UTR of UBFD1 mRNA was shown. Red sequences indicate the potential miRNA binding site and the blue sequences indicate mutated nucleotides on UBFD1 mRNA. Lower panel: Luciferase reporter assays indicated that in comparison to WT UBFD1 reporter, co-transfection of mutant UBFD1 reporter with miR-545-3p mimic has little effect on luciferase activity. *P < 0.05. (D) Western blots were performed in normal cells and the cells expressing ectopic PCAT6 or co-expressing PCAT6 and miR-545-3p to detect UBFD1 protein expression. β-actin was used as an internal control.

4. Discussion

Numerous studies indicated that lncRNAs play important roles in tumor biology through diverse mechanisms including post-transcriptional regulation of gene expression [1,2]. Recently, although several studies have shown that PCAT6 exerts oncogenic roles in human tumors [24], the underlying mechanisms by which PCAT6 promotes cancer progression have not been well studied. Here, we report a role of PCAT6 to upregulate UBFD1, a potent oncogene in breast cancer cells. We showed that PCAT6 functions as a ceRNA to competitively sequester miR-545-3p to enhance UBFD1 mRNA expression.

PCAT6 was reported as an intergenic lncRNA located on chromosome 1q32.1. Increasing studies showed that PCAT6 acted as a tumor promoter in various cancers. For instance, PCAT6 facilitated proliferation and invasion of cholangiocarcinoma cell via regulating miR-330-5p [27]. In osteosarcoma, PCAT6 aggravated tumorigenesis via miR-143-3p/ZEB1 axis [28]. Besides, PCAT6 abundance was elevated in colorectal tumor cells, and its knockdown eased colorectal cancer chemoresistance to 5-FU via miR-204/HMGA2/PI3K pathway [29]. In breast cancer cells, PCAT6 regulated VEGFR2 expression through sequestering miR-4723-5p and participated in the VEGFR/AKT/mTOR signaling pathway [16]. In this study, we showed that PCAT6 serves as a sponge to preferentially interact with miR-545-3p. Association of PCAT6 with miR-545-3p reduced the miRNA to target UBFD1 mRNA, thus increased UBFD1 mRNA expression and cell proliferation.

UBFD1 is one of the polyubiquitin binding proteins, which were identified in a protein array using polyubiquitin as bait [25]. Higher levels of UBFD1 protein have been found in ER-positive breast tumors and patients with higher UBFD1 expression had a poorer prognosis [26]. The role of UBFD1 in carcinogenesis has not been studied. Recently, Dong et al. has reported that in triple-negative breast tumors, PCAT6 was able to facilitated angiogenesis through binding to USP14, a deubiquitinase, to impede the ubiquitination of VEGFR2 [16]. Since the nature of UBFD1 is a ubiquitin-binding protein, and UBFD1 upregulation enhanced breast cancer cell proliferation, we hypothesize that the UBFD1-related ubiquitination pathway could be involved in breast cancer progression. Currently, how PCAT6-regulated expression of UBFD1 in participating carcinogenesis is under investigation.

5. Conclusions

It was revealed in this study that PCAT6 is an IMP1-associated lncRNA. Overexpression of IMP1 destabilized PCAT6 and decreased cell proliferation ability. PCAT6 effectively sponged miR-545-3p and enhanced expression of UBFD1, a potential oncogene that is not yet characterized. Silencing UBFD1 would significantly decrease breast cancer cell proliferation, which suggest that the ubiquitin pathway might make contributions to breast cancer progression.

Ethics approval and consent to participate

Not applicable.

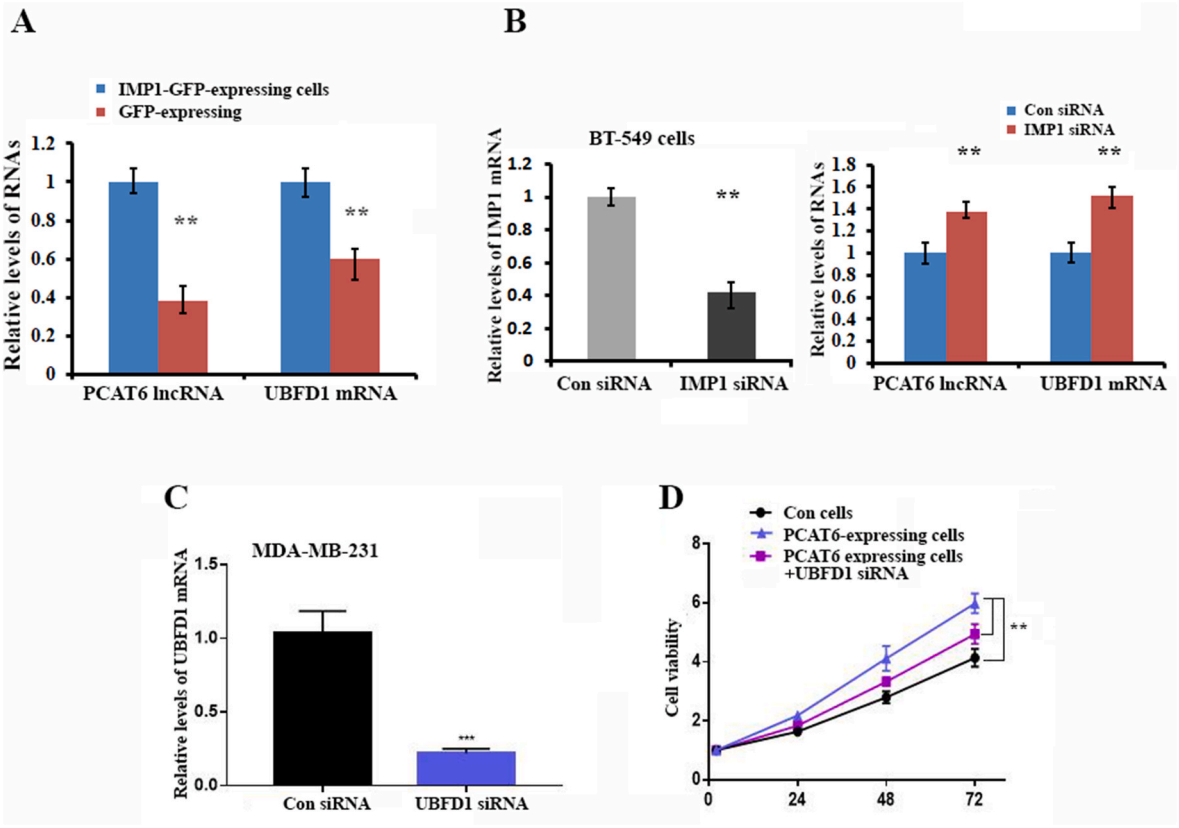

Fig. 5. PCAT6-mediated expression of UBFD1 increases the proliferation ability of breast cancer cells
(A) Expression of PCAT6 and UBFD1 mRNA were examined in stable MDA-MB-231/GFP and MDA-MB-231/IMP1-GFP cell lines by RT-qPCR. **P < 0.01. (B) Left panel, showing the relative levels of IMP1 mRNA in BT-549 cells after siRNA treatment. Right panel, IMP1 knocking down increased PCAT6, as well as UBFD1 mRNA expression. **P < 0.01. (C) RT-qPCR indicated levels of UBFD1 in PTAC6-expressing MDA-MB-231 stable cells after treatment with siRNA. (D) Cell viability assays showed that silencing of UBFD1 rescued PCAT6-induced cell proliferation ability. **P < 0.01.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

CRediT authorship contribution statement

Jun-Dong Wu: Conceptualization, Investigation, Supervision, Writing – original draft. **Liqun Xu:** Data curation, Formal analysis, Methodology, Validation. **Weibin Chen:** Conceptualization, Formal analysis, Investigation. **Yanchun Zhou:** Investigation, Supervision. **Guiyu Zheng:** Formal analysis, Investigation. **Wei Gu:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no competing interests.

Acknowledgements

We thank the members of Dr. Gu' Laboratory for their technical assistance and helpful discussion. This work was supported by a research grant from Shantou University Medical College to W.G.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ncrna.2024.01.019.

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