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PHACTR1 promotes the mobility of papillary thyroid carcinoma cells by inducing F-actin formation

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ABSTRACT

Papillary thyroid carcinoma (PTC) limits effective biomarkers for predicting prognosis and targeted therapy. Phosphatase and actin regulator 1 (PHACTR1) is a mobility-promoting molecule due to its regulation on F-actin formation, which is valuable for the investigation of PTC. Our study aimed to investigate the relationship between PHACTR1 and PTC carcinogenesis, especially mobility. Our results displayed that PHACTR1 expression was elevated in metastatic or larger PTC tissues. In addition, PTC cells K1 with more obvious mobility had higher PHACTR1 expression whereas weakly mobile cells TPC-1 was contrary. Moreover, PHACTR1 silencing inhibited the invasion, migration and tumorigenicity of K1 cells, while PHACTR1 overexpression promoted the invasion, migration and tumorigenicity in TPC-1 cells. Furthermore, PHACTR1 overexpression increased the fluorescent intensity of F-actin in TPC-1 cells. Importantly, the enhanced invasion and migration in TPC-1 cells caused by PHACTR1 overexpression were significantly reversed by the disruption of F-actin assembly with swinholide A. In conclusion, PHACTR1 can promote the mobility of PTC cells, which results in the carcinogenesis of PTC. PHACTR1-regulated F-actin formation determines the mobility of PTC cells. Therefore, PHACTR1 can function as a potential biomarker for predicting prognosis and targeting therapy in PTC.

1. Introduction

The morbidity of papillary thyroid carcinoma (PTC) was the most marked in thyroid cancer patients, attaining 70–80 % [1]. Although the overall prognosis of PTC is satisfactory, some PTCs still have strong invasiveness or tend to dedifferentiate [2]. Therefore, exploring differentially expressed biomarkers with progressive prediction in PTC is of important significance for studying the onset and progression of PTC, and is helpful to screen effective therapeutic targets. The above investigation is of great significance in further improving the cure rate and viability of PTC patients.

Actin is the main component responsible for cell mobility in the cytoskeleton. Actin includes dual forms: filamentary F-actin and spherical G-actin. F-actin serves as a functional structure for cell mobility. Actin-binding proteins are capable of regulating the dynamics of actin and play a central role in the assembly of F-actin infrastructure [3,4]. PHACTR1 is a typical actin-binding protein,

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which has RPEL repeats for actin-binding and has strong actin-binding ability [5,6]. PHACTR1 overexpression can induce actin cytoskeleton reorganization of fibroblasts and make the cells show strong F-actin fluorescence [7]. PHACTR1 knockout can lead to the instability of actin cytoskeleton reorganization in breast cancer cells, thereby weakening TGF-β-induced tumor cell migration [8]. PHACTR1 knockout can also lead to defective neuronal migration during corticogenesis [9]. *PHACTR1* gene knockdown also inhibits cell migration in mouse brain capillary endothelial cells [10]. In conclusion, PHACTR1 is a key actin-binding protein, which plays a key role in regulating F-actin as well as the reorganization and migration of cytoskeleton. However, so far, there is no report on the relationship between PHACTR1 and PTC carcinogenesis. The GEPIA of TCGA database, a network server for cancer and normal gene expression profiles [11], showed that the overall viability in PTC patients with lower *PHACTR1* expression is significantly better than that of PTC patients with higher *PHACTR1* expression, suggesting that PHACTR1 is linked to the poor prognosis in PTC (Fig. 1A and B). Therefore, we speculate that as an actin-binding protein related to cell mobility, PHACTR1 may participate in PTC cell aggression and migration, thereby promoting the progression of PTC.

Our study first investigated the expression pattern of PHACTR1 in PTC samples. Then, the relationship between the mobility of PTC cells and PHACTR1 expression was analyzed by using different PTC cell lines. Next, the effects of PHACTR1 on the function and tumorigenicity of PTC were confirmed by gene-silencing and gene-overexpression techniques. Furthermore, the relationship between F-actin formation regulated by PHACTR1 and PTC cell mobility was also clarified.

2. Materials and methods

2.1. Clinical tissue samples

60 pairs of PTC and adjacent noncancerous tissue samples were obtained by surgical excision at second hospital of Hebei Medical University (Shijiazhuang, Hebei, China) from January 2015 to October 2020. The clinicopathological characteristics of patients with PTC were presented in Table 1. Obtained samples were histopathologically tested by three pathologists without interest connections. Fresh samples were freezing in liquid nitrogen and stored at −80 °C until application. The acquisition of samples was based on the

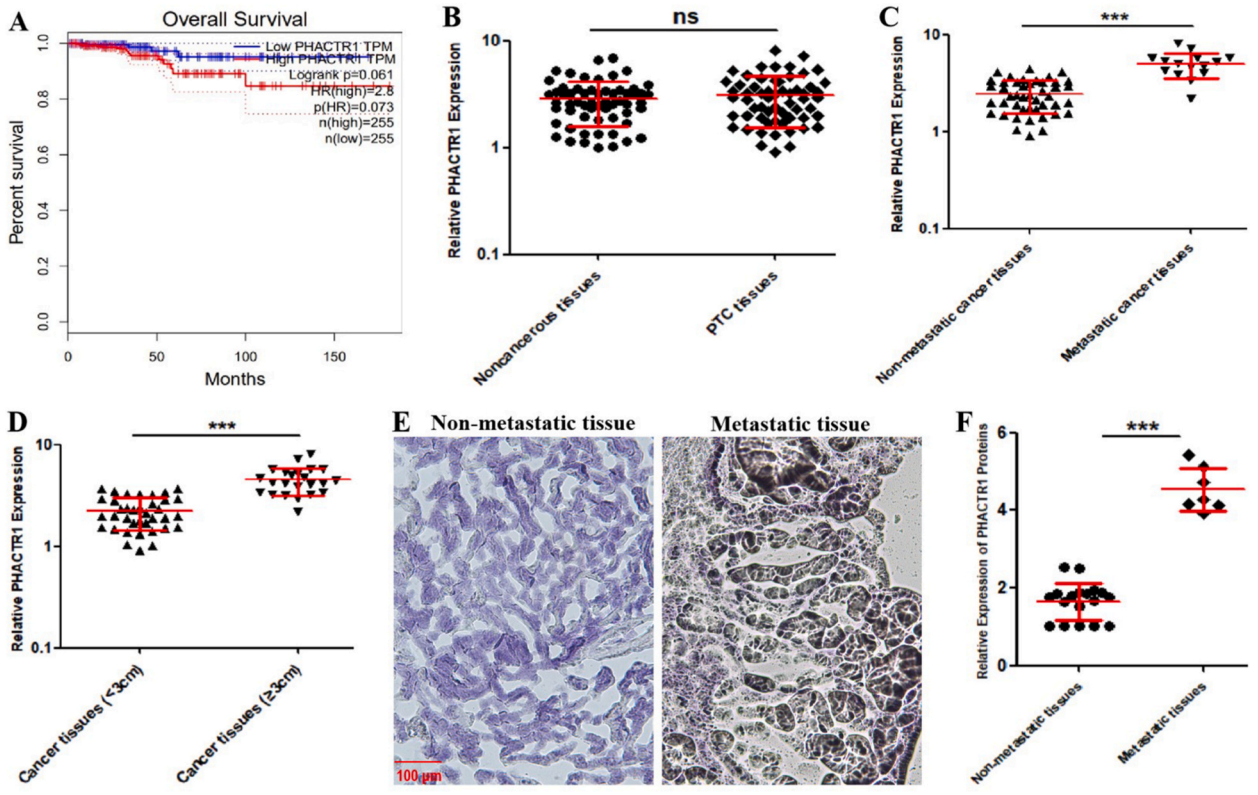

Fig. 1. PHACTR1 was overexpressed in larger and metastasis PTC tissues. (A) The comparison of overall survival between PTC patients with low *PHACTR1* mRNA expression and high *PHACTR1* mRNA expression in GEPIA database (http://gepia.cancer-pku.cn/index.html). (B) *PHACTR1* levels in 60 pairs of PTC tissues and paracancerous tissue. Results are expressed as median with interquartile range. ns (not significant) by Wilcoxon signed-rank test. (C) *PHACTR1* expression in 15 PTC samples with metastasis and 45 non-metastatic PTC samples. (D) *PHACTR1* expression in 23 PTC samples with a diameter greater than 3 cm and 37 PTC samples with a diameter less than 3 cm. (E–F) Protein expression of PHACTR1 in 7 metastatic PTC samples and 18 non-metastatic PTC samples was evaluated by immunohistochemical assays. Scale bar: 50 μm. (B–F) Results are presented as median with interquartile range. ***$P < 0.001$ by Wilcoxon rank sum test.

informed consent of all participants. All procedures related to human body research complied with the ethical standards of second hospital of Hebei Medical University (2016-R269) and with Helsinki declaration in 1964 and its subsequent amendments or similar ethical standards.

2.2. Immunohistochemistry (IHC) assays

The sections from various tissues were prepared, incubated with primary antibody overnight at 4 °C against human PHACTR1 (GTX122251, 1:200; GeneTex, CA, USA), and then visualized by PV-9000 DAB detection kit according to manufacturer's instructions. All sections were observed by using IX81 microscopy. DUSP4 expression levels were semi-quantitatively graded through observing staining intensity as following: graded 1 (no stain), graded 2 (weak stain), graded 3 (clear stain), or graded 4 (strong stain). The total immunoreactivity scorings were gained by multiplying the abundance (expressed as a fraction) and staining intensity.

2.3. Cell lines and their culture

Various PTC cell lines (K1, TPC-1, BCPAP and IHH-4) were obtained from Cell Bank of Chinese Academy of Sciences. The cells were incubated in RPMI-1640 medium (Invitrogen, CA, USA) supplemented with 10 % fetal bovine serum (FBS, Invitrogen). These cells were all grown in a humid environment with 37 °C and 5 % CO_2.

2.4. Quantitative real-time PCR (qRT-PCR) analyses

The extraction and purification of total RNA depended on the Trizol method. The cDNA syntheses and qRT-PCR detections were carried out according to manufacturer's instructions. The sequences from designed primer for qRT-PCR detections were as following:

PHACTR1, 5′-TGTGGTGGCTCATGCTTGTATTCC-3' (Forward) and 5′-GATGCGGTCTCAGTATGTTGTCCAG-3' (Reverse).

GAPDH, 5′-CCTGCCTCTACTGGCGCTGC-3' (Forward) and 5′-GCAGTGGGGACACGGAAGGC-3' (Reverse).

The $2^{-\triangle\triangle Ct}$ method was used to calculate relative expression. Relying on ABI7500 PCR system (Applied Biosystem, Thermo Fisher Scientific, MA, USA), the SYBR PreMix Ex TaqTM kit (Takara, Tokyo, Japan) was used to perform qRT-PCR detections.

Table 1
The clinicopathological characteristics of patients with papillary thyroid carcinoma. The clinicopathological parameters of PTC patients include gender, age, CDFl classification grade, degree of invasion, PTC size, pathological stage (T), pathological stage (N), and clinical outcome. The table shows the number of patients with corresponding characteristics among 60 cases.

Characteristics	Cases
Total	60
Sex	
Male	18
Female	42
Age, years	
≥65	26
<65	34
CDFl classification grade	
I	35
II-III	25
Degree of invasion	
Intrathyroid	40
Extrathyroid	20
PTC diameter, cm	
<3.0	37
≥3.0	23
Pathological stage (T)	
T2	45
T3-T4	15
Pathological stage (N)	
N0	51
N1	9
Outcome	
Persistent/recurrent	5
Death	1
Cured	54

2.5. RNA interference (RNAi) assays related to siRNA

The designed siRNA against human *PHACTR1* and matched control-siRNA were gined from Santa Cruz Biotechnology (sc-95456; Santa Cruz, CA). siRNAs (100 pmol/well) were transfected into K1 cells incubated in 6-well plates by using RNAiMAX (Invitrogen) according to manufacturer's instructions. After 48 h of incubation, the silencing efficiency was confirmed by Western-blotting assays.

2.6. Lentiviral infection

The construction of recombinant lentiviruses based on *PHACTR1*-cDNA or *PHACTR1*-shRNA relied on homogenous recombination between pEX-Puro-Lv105 vector and cDNA or shRNA from 293 cells by using relevant construction kit according to manufacturer's instructions (GeneCopoeia). The same method was used to construct and package matched control vectors. As the supernatant was harvested two days later, TPC-1 and K1 cells were treated with the medium supplemented with lentiviruses and 8 mg/L of polybrene at multiplicity of Infection of 30 (MOI 30) for two days. The selection of infected cells relied on the usage of purinomycin (7.5 μg/mL) The overexpression efficiency was confirmed by Western-blotting assays.

2.7. Analyses of cell invasiveness and migration

Cell invasion ability was estimated via Transwell assays as following: Cells suspended in serum-free DMEM supplemented with 1 mg/mL of proliferation inhibitor, mitomycin C, were seeded into the upper lumen of Transwell. Matrigel was used to coat the filter membrane for 30 min at 37 °C. DMEM supplemented with 20 % FBS was added to the lower lumen of Transwell. After incubation for 36 h, the cells that migrated to the lower surface were fixed and stained using 1 % crystal violet. Meanwhile, the corresponding images are obtained. Cell invasion ability was measured by counting the specific number of stained cells.

Cell migration ability was estimated via Scratch test as following: After 75 % confluence, a wound were generated in cells cultured in 12-well plates by manually scraping the cell monolayer with the tip of a 200 μL pipette. Floating cells are removed by washing the cultures, and the adherent cells were incubated in DMEM supplemented with 1 % FBS. The migration of cells into the wound was visualized at 0 and 24 h in each group. ImageJ software was used to calculate the scratch area.

2.8. Western-blotting assays

Total lysates were extracted from cells or tissues using cold RIPA buffer. BCA method was used to measure protein content. After blockage with 5 % non-fat milk at room temperature for 1 h, polyvinylidene difluoride membranes (PVDFs) were incubated with human anti-PHACTR1 (GTX122251, 1:2000) and anti-GAPDH at 4 °C overnight. Then, secondary antibody conjugating HRP (Proteintech, Wuhan, China) was applied at room temperature for 1 h. Relying on chemiluminescence system (Amersham Image 600; General Electric; Cytiva), enhanced chemiluminescent-substrate reagent kit (Amersham; Cytiva) was used to visualize the signals. The ImageJ software (v1.8.0) was used to semi-quantify the signal densitometry.

2.9. In vivo tumorigenesis assays

4∼6-weeks-old male athymic BALB/c nude mice were purchased from Gem Pharmatech Co., Ltd (Nanjing, China). The mice were placed in a specific pathogen-free (SPF) facility, with a room temperature of 20–30 °C and a humidity of 60–80 %, and fed with SPF mouse feed and sterile water. Indicated cells were constructed into stable overexpressed or silenced cells through the transduction of lentiviruses encoding *PHACTR1*-cDNA or *PHACTR1*-shRNA. The stable cells were injected subcutaneously on ventral side of right rib at the density of 2×10^6 cells/mouse (10 mice per group). 30 days later, these tumor-bearing mice were sacrificed upon anesthesia using thiopental (15 mg/kg), and the tumors were resected and weighted. All animal protocols were authorized by the Institutional Animal Care and Use Committee in second hospital of Hebei Medical University (2016-R269).

2.10. Immunofluorescence staining

For F-actin staining, cells were permeated, and stained with Phalloidin conjugating FITC (1:40; Sigma-Aldrich) at 4 °C overnight according to manufacturer's instructions to show actin. After permeabilization, cells were exposed to the indicated primary antibodies (FITC-conjugated Phalloidin, 1:40; human PHACTR1, GTX122251, 1:500) overnight at 4 °C to perform fluorescent double-staining of PHACTR1 and F-actin according to the manufacturer's protocols. Ultimately, DAPI was used to counterstain cell nuclei for 15 min. Images were obtained by using Olympus IX81 fluorescent microscope (Tokyo, Japan).

2.11. Coimmunoprecipitation (Co-IP) analyses

RIPA buffer was used to extract total protein. Then, we used 100 μL ice buffer to clean the beads, added 100 μL of antibody-combining buffer, rotated indicated antibody and magnetic beads for 30 min, and used 200 μL of buffer to wash the beads. The lysates and magnetic beads binding antibody were incubated at room temperature for 1 h and rinsed using 200 μL of buffer. The

magnetic beads were rinsed using 20 μL of elution buffer to remove the supernatant. The extracted lysates were used for Co-IP using anti-human PHACTR1 antibody (GTX122251, 1:500), and subsequent Western-blotting assays using anti-human F-actin antibody (ab205, 1:500; Abcam, Cambridge, UK) was applied to observe the precipitates.

2.12. Analyses of cell proliferation

Cell counting Kit-8 (CCK-8) tests were performed to estimate cell proliferation by using the related kit (Dojindo, Kumamoto, Japan). For CCK-8 tests, indicated cells plated into 96-well plates (2000 cells/well) were treated with 10 μL of CCK-8 reagent after different time. After 2 h, Varioskan Flash reader (Thermo Fisher Scientific) was used to measure the optical density at 450 nm (OD450) to assess cell proliferation level.

2.13. Statistical analysis

Statistical analysis relied on the usage of GraphPad Prism software 7. One-way ANOVA, Student's t, Wilcoxon signed rank sum and Wilcoxon rank sum tests were employed for comparison. Post-Hoc pairwise comparisons of one-way ANOVA test relied on the usage of Bonferroni test. P value < 0.05 was regarded as statistically significant.

3. Results

3.1. PHACTR1 has strong expression in larger and metastasis PTC tissues

The GEPIA in TCGA database exhibited that compared with PTC patients with higher *PHACTR1* level, PTC patients with lower *PHACTR1* level have higher overall survival (Fig. 1A). Our data also showed that although the comparison of *PHACTR1* gene expression between cancer tissues and adjacent tissues was not statistically significant, PTC samples with diameter greater than 3 cm or

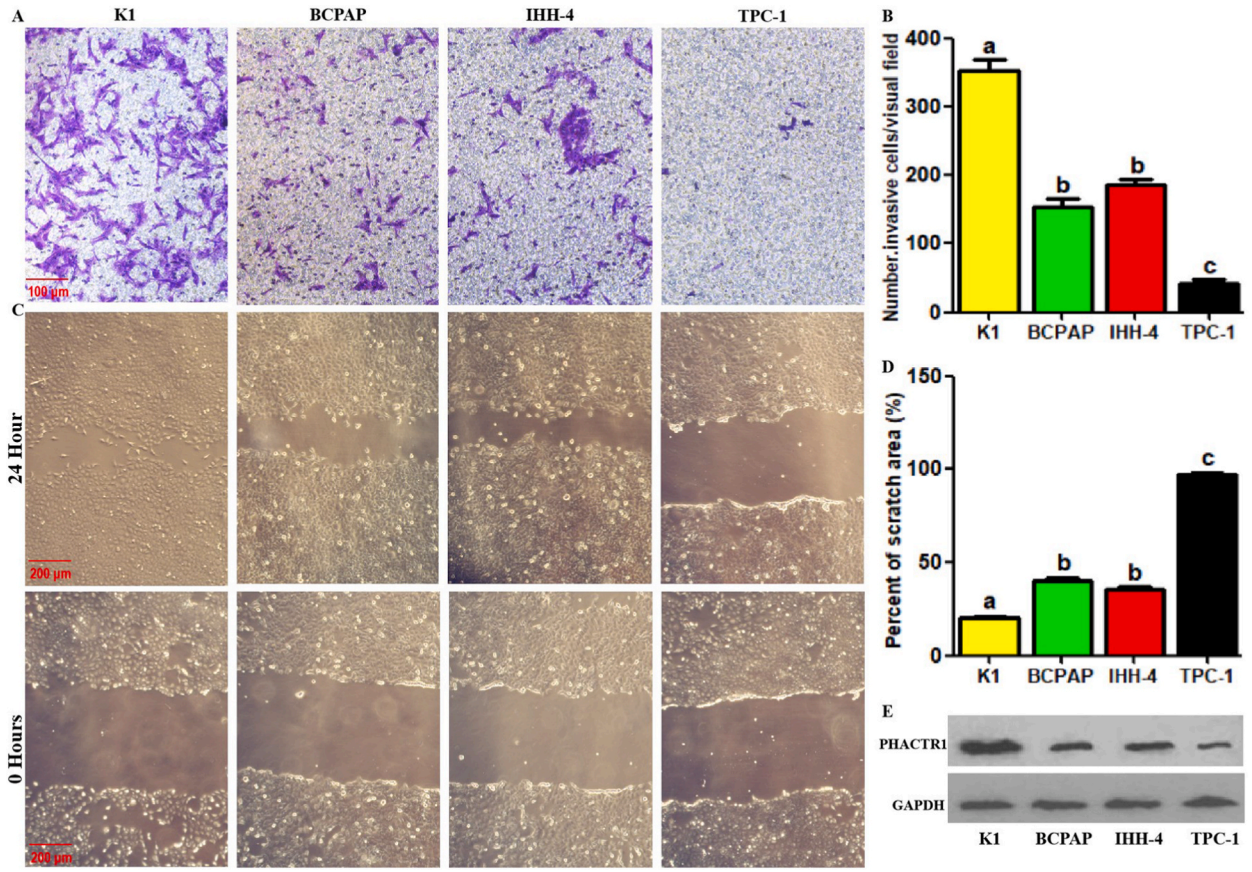

Fig. 2. The mobility and PHACTR1 protein levels of different PTC cell lines. (A–B) The invasion of K1, BCPAP, IHH-4 and TPC-1 cells was evaluated using Transwell assays. Scale bar: 100 μm. (C–D) The migration of K1, BCPAP, IHH-4 and TPC-1 cells was evaluated using scratch assays. Scale bar: 200 μm. (E) PHACTR1 protein expression in K1, BCPAP, IHH-4 and TPC-1 cells. $^{letters}P<0.05$ by one-way ANOVA with Bonferroni multiple comparison test.

metastasis had more obvious *PHACTR1* expression (Fig. 1B-D). Furthermore, Immunohistochemical (IHC) staining also revealed that metastatic PTC samples displayed significantly stronger PHACTR1 protein expression than non-metastatic PTC samples (Fig. 1E-F).

3.2. The mobility and PHACTR1 expression were discrepant in different PTC cell lines

Next, we investigated several PTC cells. As exhibited in Fig. 2A-D, K1 cells had the highest level in invasion and migration abilities, while TPC-1 cells had the lowest level in invasion and migration abilities. Moreover, the mRNA as well as protein expression levels of PHACTR1 in K1 cells were the highest, and those of TPC-1 cells were the lowest (Fig. 2E).

3.3. The alteration of PHACTR1 expression regulated the mobility of PTC cells

Then, we knocked down *PHACTR1* gene through siRNA transfection in K1 cells. The knockdown efficiency of PHACTR1 was verified by western-blotting (Fig. 3C). It could be seen that PHACTR1 knockdown reduced the invasion and migration level of K1 cells (Fig. 3A-B, D-E). TPC-1 cells were given overexpression of *PHACTR1* gene using lentiviral transduction. PHACTR1 overexpression efficiency was verified by western-blotting (Fig. 3C). PHACTR1 overexpression strengthened the invasion and migration level in TPC-1 cells (Fig. 3A-B, D-E). However, PHACTR1 knockdown slightly reduced the proliferative ability in K1 cells without statistical significance, and PHACTR1 overexpression was also ineffective in the proliferative ability in TPC-1 cells (FigS.**A**).

3.4. The alteration of PHACTR1 expression regulated the tumorigenicity in vivo of PTC cells

Additionally, *in vivo* xenograft assays also demonstrated that PHACTR1 silencing decreased the sizes and weights of xenografts with K1 cells, while PHACTR1 overexpression increased the sizes and weights of xenografts with TPC-1 cells (Fig. 4A-B). The *in vivo*

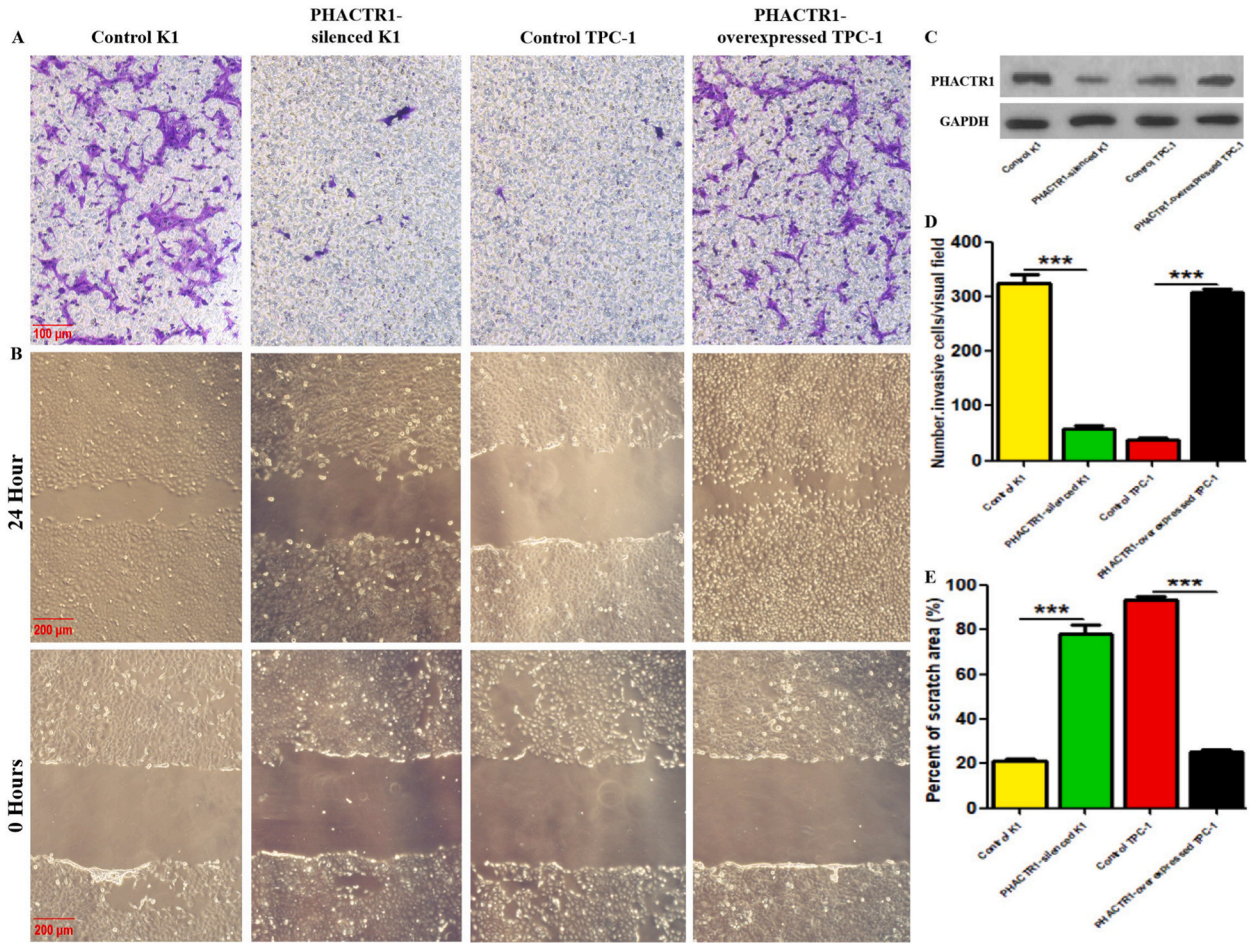

Fig. 3. The significance of PHACTR1 in the mobility of PTC cells. (A, D) The invasion of the indicated K1 or TPC-1 cells was evaluated using Transwell assays. Scale bar: 100 μm. (B, E) The migration of the indicated K1 or TPC-1 cells was evaluated using scratch assays. Scale bar: 200 μm. (C) PHACTR1 protein expression in the indicated K1 or TPC-1 cells. ***P $<$ 0.001 by one-way ANOVA test.

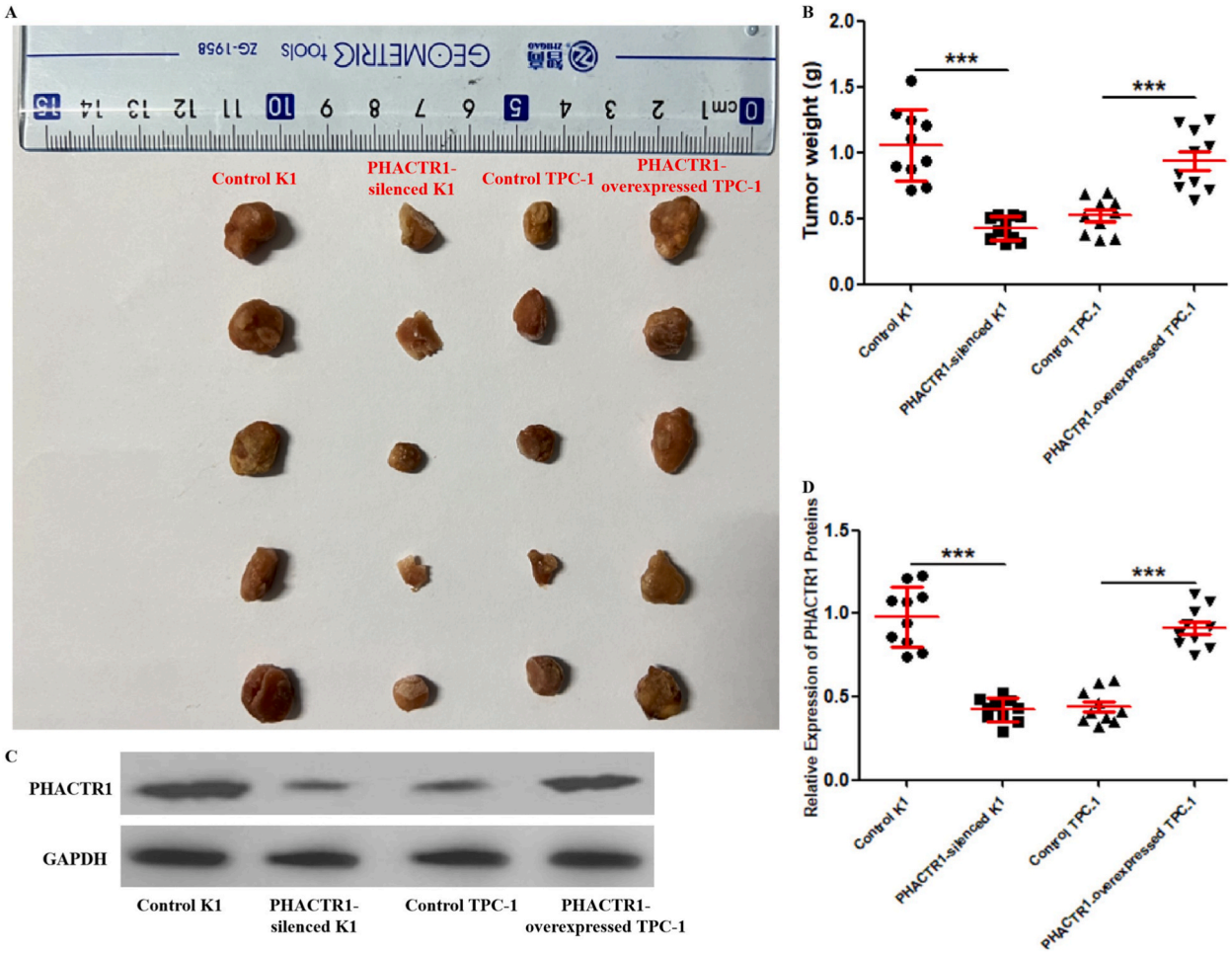

Fig. 4. The significance of PHACTR1 in the tumorigenicity *in vivo* of PTC cells. The indicated K1 or TPC-1 cells transduced by lentiviruses were inoculated into nude mice. 30 days later, all mice were killed, and the tumors were removed and weighted. (A) Representative images of the removed tumors from four groups. (B) The histogram showing the quantitative results regarding the weights of the removed tumors (n = 10). (C–D) PHACTR1 protein expression of tumor tissues in four groups (n = 10). ***P < 0.001 by one-way ANOVA test.

implantation efficiency of PHACTR1-silenced K1 cells and PHACTR1-overexpressed TPC-1 cells were also identified by western-blotting, which indicated that the *in vivo* experimental results were reliable (Fig. 4C-D).

3.5. PHACTR1 overexpression enhanced the formation of F-actin in PTC cells

PHACTR1 is known to be a typical actin-binding protein and contributes to F-actin assembly. Therefore, we need to further confirm the relationship between PHACTR1 and F-actin formation in PTC cells. As shown in Fig. 5A-B, There existed a coimmunoprecipitation and colocalization of PHACTR1 with F-actin in K1 cells, supporting the interaction between PHACTR1 and F-actin in PTC cells. Furthermore, PHACTR1 silencing sharply reduced the number of F-actins in K1 cells while PHACTR1 overexpression effectively increased the number of F-actins in TPC-1 cells (Fig. 6). It is indicated that PHACTR1 is involved in F-actin assembly in PTC cells.

3.6. PTC cell mobility promoted by PHACTR1 overexpression was blocked by Swinholide A

Finally, we applied Swinholide A, a pharmacological inhibitor of F-actin cytoskeleton [12–14], to perform the rescue assays. As shown in Fig. 7A, although the addition of Swinholide A did not alter the number of F-actins in TPC-1 cells, it effectively blocked the F-actin formation in TPC-1 cells enhanced by PHACTR1 overexpression, which confirmed the efficiency of Swinholide A. Additionally, although the treatment of Swinholide A did not affect the invasive and migratory ability of TPC-1 cells, it significantly reversed the enhanced invasion and migration after PHACTR1 overexpression (Fig. 7B-E). These results suggest that the promotion of F-actin assembly is responsible for the enhanced mobility of PTC cells by PHACTR1. However, the treatment of Swinholide A had no significant

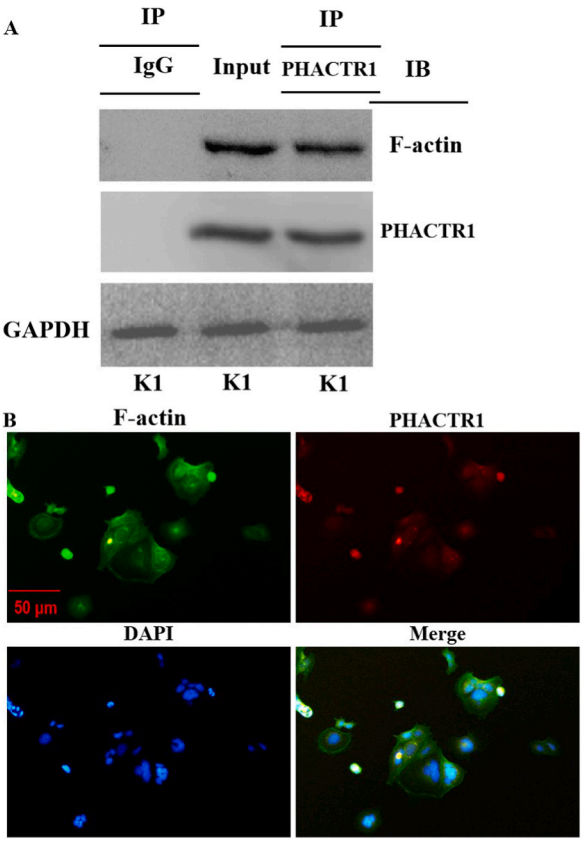

Fig. 5. Interaction between PHACTR1 and F-actin in K1 cells. (A) The lysates of K1 cells were extracted with anti-PHACTR1 antibody for coimmunoprecipitation, and then the precipitation was detected by Western-Blot analyses with anti-F-actin antibody. (B) Representative fluorescent double-staining images of PHACTR1 and F-actin in K1 cells including single and merged fluorescences. Scale bar: 20 μm. The green and red fluorescences represent F-actin or PHACTR1, respectively. The overlaps of PHACTR1 and F-actin showed yellow fluorescences. The data are representative images from three independent experiments with unanimous results. IP, the antibody for immunoprecipitation; IB, the antibody for immunoblot. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

effects on the proliferation of control and PHACTR1-overexpressed TPC-1 cells (FigS.B), which indicates that F-actin assembly is not involved in PTC cell proliferation.

4. Discussion

Through the TCGA and clinical sample data shown above, we gained knowledge that PHACTR1 is tightly related to the invasion and metastasis of PTC cancerous tissues, thus, PHACTR1 is responsible for the poor prognosis in PTC patients. It should be noted that there didnot exist significant difference in PHACTR1 expression between PTC and adjacent tissues. Therefore, PHACTR1 may promote PTC carcinogenesis by regulating cell function. As expected, due to the increased expression of PHACTR1, the invasion and migration ability in PTC cell lines are also gradually enhanced. Due to the regulatory role in F-actin assembly, PHACTR1 can lead to cell movement, which causes its contribution to the development of corresponding malignancy [7–10]. Therefore, our results suggest that PHACTR1 may function as a prognostic predictor of PTC.

Nevertheless, the strong mobility of K1 cells can be reversed by PHACTR1 knockdown, while the weak mobility of TPC-1 cells can be compensated by PHACTR1 upregulation. *In vivo* experiments also showed that the strong tumorigenicity of K1 cells can also be reversed by PHACTR1 knockdown, while the weak tumorigenicity of TPC-1 cells was recovered by PHACTR1 overexpression. PTCs with strong invasiveness or tendency towards dedifferentiation are difficult to treat [2]. A meta-analysis explored a series of prognostic biomarkers or prospective therapeutic targets for further experimental analyses and clinical tests for PTC [15]. Nevertheless, there limits effective molecular targets to repress PTC carcinogenesis in current. PHACTR1 can become a promising therapeutic target for PTC because of its contribution on PTC cell mobility. It should be noted that although PHACTR1 knockdown and PHACTR1 overexpression only slightly affected PTC cell proliferation, the *in vivo* tumorigenicity of PTC cells can also serve as a parameter for cell mobility as PTC cell invasion is also involved in the invasive growth of tumors. Accordingly, our data further confirm that PHACTR1 may exist as a molecular target in PTC therapy. Moreover, the internal mechanism underlying PHACTR1-regulated PTC cell mobility is also clarified through the elaboration on the association between PHACTR1 and F-actin. F-actin is a functional structure for cell

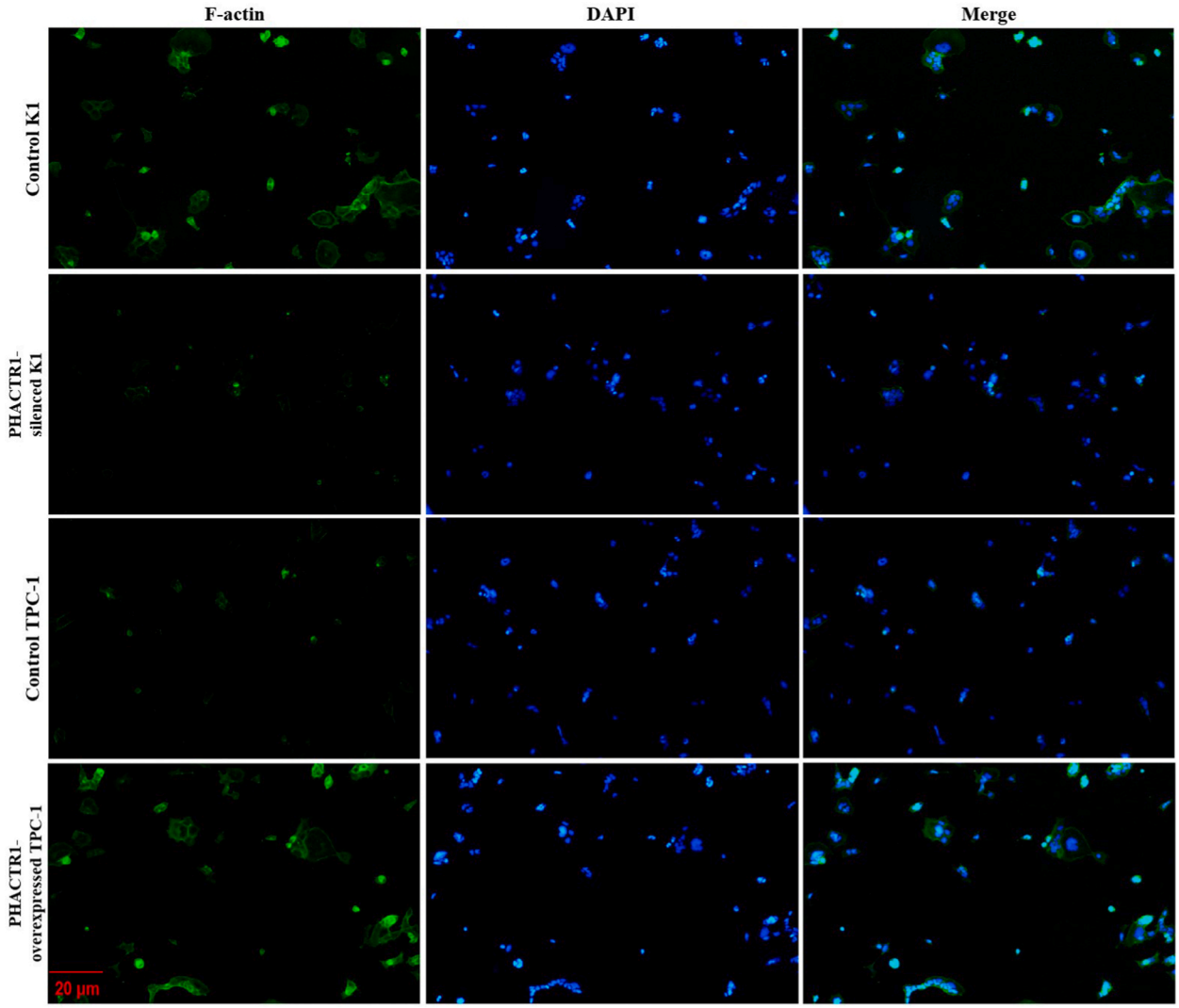

Fig. 6. The significance of PHACTR1 in the formation of F-actin in PTC cells. F-actin fluorescence of the indicated K1 or TPC-1 cells was evaluated using Immunofluorescence staining. Scale bar: 20 μm. The data are representative images from three independent experiments with unanimous results.

mobility. Myristoylated alanine-rich C kinase substrate like 1 (MARCKSL1) relies on its interaction with F-actin to strengthen the mobility of esophageal carcinoma cells [16]. Moreover, previous study demonstrated that local membrane protrusions by directed polymerization in anterior F-actin triggers cell migration [17]. The similar conclusions were also reported in other investigations [18]; Seetharaman et al., 2020). Due to the property of actin-binding protein, PHACTR1 can help the cells show strong F-actin fluorescence and induce actin cytoskeleton reorganization, which promotes the mobility of various types of cells [7–10]. The current results indicate that PHACTR1 can interact with F-actin and promote F-actin formation in PTC cells, which is involved in PHACTR1-enhanced PTC cell mobility. Therefore, the mechanism clarified in this study is consistent with previous studies [7–10]. Our working mode is described in Fig. 8. The limitation of this study is the absence of in-depth exploration regarding the underlying mechanism of PHACTR1-regulated F-actin formation in PTC cells, which requires the future research. Notably, there didnot exist marked difference in *PHACTR1* gene between cancer tissues and paracancerous tissues, which may cause PHACTR1-related targeted drugs to harm normal cells. Therefore, the relevant biological carrier materials targeting PTC cells are expected to be used in combination with PHACTR1-targeting drugs for the treatment of PTC in the future.

In conclusion, our experimental data reveal for the first time that PHACTR1, a typical actin-binding protein, can function as a mobility-promoting molecule of PTC cells. The above function not only confirms that PHACTR1 can be considered a biomarker to predict the prognosis of PTC, but also supports PHACTR1 as the therapeutic target of PTC, especially the type of high PHACTR1 expression histologically. The promotion of PHACTR1 on F-actin formation contributes to its regulation on PTC cell mobility. This study presents more potential clues for the improvement of the diagnosis and treatment of PTC patients.

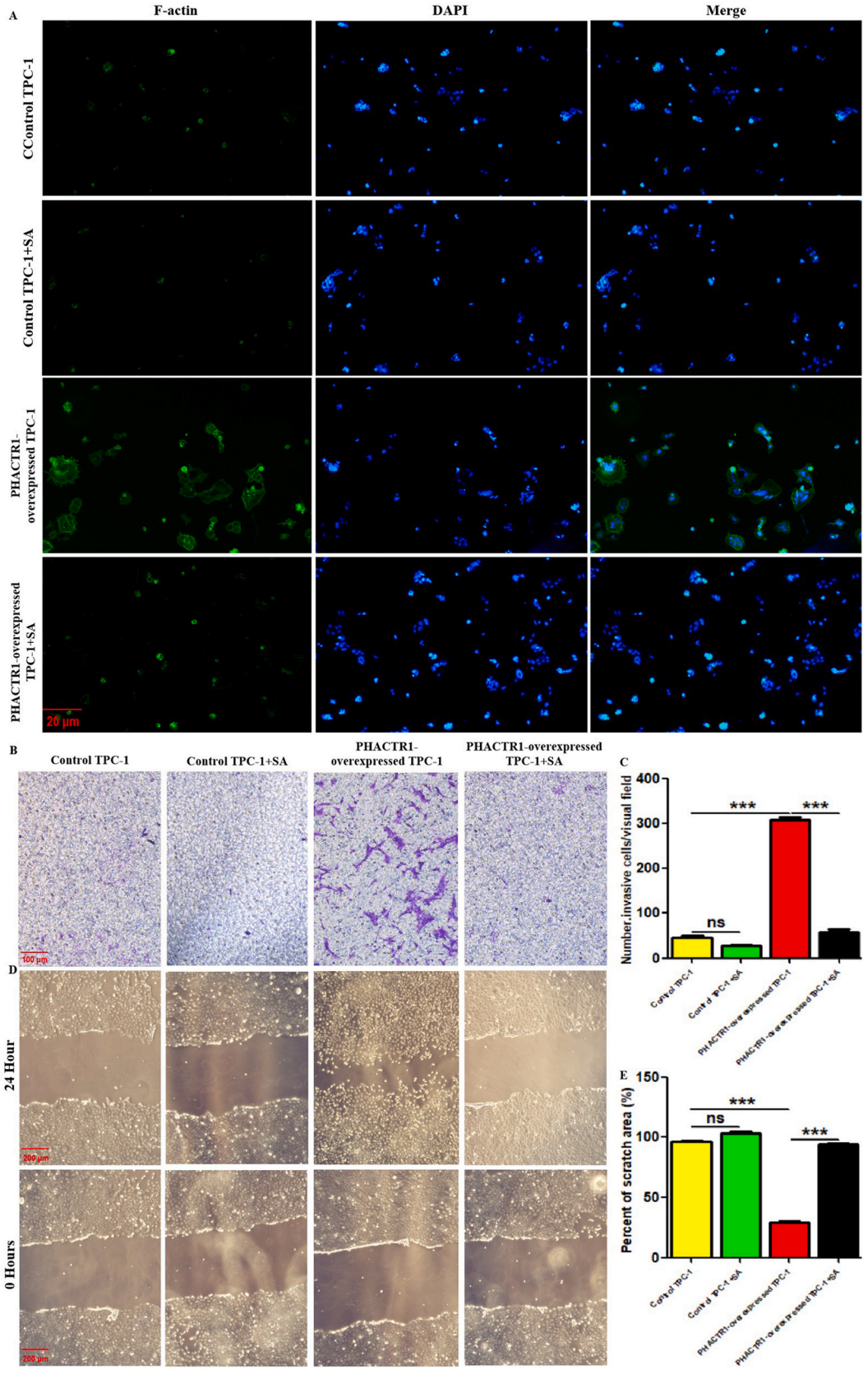

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Fig. 7. The significance of F-actin in PHACTR1-regulated PTC cell mobility. (A) F-actin fluorescence of the indicated TPC-1 cells was evaluated using Immunofluorescence staining. Scale bar: 20 μm. The data are representative images from three independent experiments with unanimous results. (B–C) The invasion of the indicated TPC-1 cells was evaluated using Transwell assays. Scale bar: 100 μm. (D–E) The migration of the indicated TPC-1 cells was evaluated using scratch assays. Scale bar: 200 μm ***P < 0.001 by one-way ANOVA test. +SA, adding the treatment of Swinholide A.

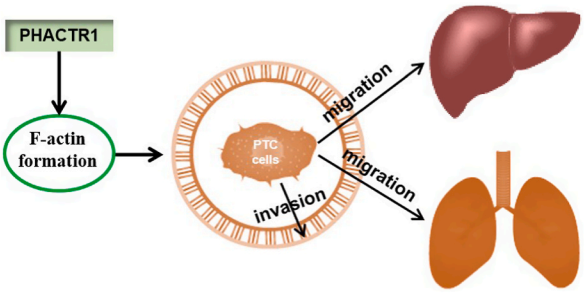

Fig. 8. The working model of the relationship between PHACTR1-regulated F-actin formation and PTC cell mobility (including invasion and migration).

Ethics statement

All procedures related to human body research complied with the ethical standards of second hospital of Hebei Medical University (2016-R269) and with Helsinki declaration in 1964 and its subsequent amendments or similar ethical standards. All animal protocols were authorized by the Institutional Animal Care and Use Committee in second hospital of Hebei Medical University (2016-R269).

Author contribution Statement

Leilei Zang: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data. Yanmei Song: Analyzed and interpreted the data. Yanhua Tian: Contributed reagents, materials, analysis tools or data. Ning Hu: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e20461.

References

[1] Z. Yang, G. Li, C. Ding, W. Sun, J. Zhang, Long non-coding RNA HULC exerts oncogenic activity on papillary thyroid cancer in vitro and in vivo, Artif. Cells, Nanomed. Biotechnol. 48 (1) (2020) 326–335.
[2] J.J. Wiltshire, T.M. Drake, L. Uttley, S.P. Balasubramanian, Systematic review of trends in the incidence rates of thyroid cancer, Thyroid 26 (11) (2016) 1541–1552.
[3] R. Dominguez, K.C. Holmes, Actin structure and function, Annu. Rev. Biophys. 40 (2011) 169–186.
[4] D. Spiering, L. Hodgson, Dynamics of the Rho-family small GTPases in actin regulation and motility, Cell Adhes. Migrat. 5 (2) (2011) 170–180.
[5] P.B. Allen, A.T. Greenfield, P. Svenningsson, D.C. Haspeslagh, P. Greengard, Phactrs 1-4: a family of protein phosphatase 1 and actin regulatory proteins, Proc Natl Acad Sci U S A 101 (18) (2004) 7187–7192.

[6] S. Mouilleron, M. Wiezlak, N. O'Reilly, R. Treisman, N.Q. McDonald, Structures of the Phactr1 RPEL domain and RPEL motif complexes with G-actin reveal the molecular basis for actin binding cooperativity, Structure 20 (11) (2012) 1960–1970.
[7] M. Wiezlak, J. Diring, J. Abella, S. Mouilleron, M. Way, N.Q. McDonald, R. Treisman, G-actin regulates the shuttling and PP1 binding of the RPEL protein Phactr1 to control actomyosin assembly, J. Cell Sci. 125 (23) (2012) 5860–5872.
[8] N. Fils-Aimé, M. Dai, J. Guo, M. El-Mousawi, B. Kahramangil, J.C. Neel, J. Lebrun, MicroRNA-584 and the protein phosphatase and actin regulator 1 (PHACTR1), a new signaling route through which transforming growth factor-β Mediates the migration and actin dynamics of breast cancer cells, J. Biol. Chem. 288 (17) (2013) 11807–11823.
[9] N. Hamada, S. Ogaya, M. Nakashima, T. Nishijo, Y. Sugawara, I. Iwamoto, et al., De novo PHACTR1 mutations in West syndrome and their pathophysiological effects, Brain 141 (11) (2018) 3098–3114.
[10] Y. Jing, L. Zhang, Z. Xu, H. Chen, S. Ju, J. Ding, Y. Guo, H. Tian, Phosphatase actin regulator-1 (PHACTR-1) knockdown suppresses cell proliferation and migration and promotes cell apoptosis in the bEnd.3 mouse brain capillary endothelial cell line, Med Sci Monit 25 (2019) 1291–1300.
[11] Z. Tang, C. Li, B. Kang, G. Gao, C. Li, Z. Zhang, GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses, Nucleic Acids Res. 45 (W1) (2017) W98–W102.
[12] V.A. Klenchin, R. King, J. Tanaka, G. Marriott, I. Rayment, Structural basis of swinholide A binding to actin, Chem Biol 12 (3) (2005) 287–291.
[13] C. Huynh, G. Ren, J. Papillon, J. Guillemette, T. Takano, A.V. Cybulsky, The cytoprotective role of Ras in complement-mediated glomerular epithelial cell injury, Clin Immunol 131 (2) (2009) 343–353.
[14] T. Ohyoshi, A. Takano, M. Namiki, T. Ogura, Y. Miyazaki, Y. Ebihara, K. Takeno, I. Hayakawa, H. Kigoshi, Development of a novel inducer of protein-protein interactions based on aplyronine A, Chem Commun (Camb). 54 (68) (2018) 9537–9540.
[15] G. Gulfidan, M. Soylu, D. Demirel, H.B.C. Erdonmez, H. Beklen, P. Ozbek Sarica, K.Y. Arga, B. Turanli, Systems biomarkers for papillary thyroid cancer prognosis and treatment through multi-omics networks, Arch. Biochem. Biophys. 715 (2022), 109085.
[16] Y. Zhao, X. Xie, L. Tian, F. Liu, Y. Sun, H. Lu, X. Zhao, Y. Mao, MARCKSL1 interacted with F-actin to promote esophageal squamous cell carcinoma mobility by modulating the formation of invadopodia, Cancer Med. 12 (3) (2023) 3299–3312.
[17] A. Bisaria, A. Hayer, D. Garbett, D. Cohen, T. Meyer, Membrane-proximal F-actin restricts local membrane protrusions and directs cell migration, Science 368 (6496) (2020) 1205–1210.
[18] M.L. Gardel, I.C. Schneider, Y. Aratyn-Schaus, C.M. Waterman, Mechanical integration of actin and adhesion dynamics in cell migration, Annu. Rev. Cell Dev. Biol. 26 (2010) 315–333.