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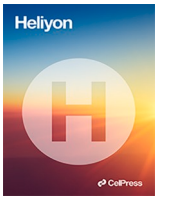

Long non-coding RNA PCAT5 regulates the progression of Esophageal Squamous Cell Carcinoma via miR-4295/PHF20

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ABSTRACT

Long non-coding RNAs (lncRNAs) have been discovered through many studies to play a crucial role in tumor progression. LncRNA PCAT5 has been identified as a human cancer-related gene in diverse cancers. However, the specific role of PCAT5 in esophageal squamous cell carcinoma (ESCC) still needs further study. The study aimed to test the PCAT5 expression and find its biological function in ESCC. Functional experiments, including EdU, transwell and TUNEL, were done in the chosen ESCC cell lines under silenced PCAT5. Luciferase reporter and Western blot experiments were implemented to ensure the possible regulatory mechanism involved in ESCC. PCAT5 presented higher expression in ESCC cells in comparison to normal cells. The silence of PCAT5 restrained ESCC cell abilities of proliferation, migration and invasion. On the contrary, it accelerated ESCC cell apoptosis. The results of rescue experiments showed that PCAT5 regulated ESCC cell proliferative, migrated, invasive and apoptotic abilities via sponging miR-4295 to up-regulate PHF20.

1. Introduction

Human esophageal cancer is one of the leading causes of cancer-related deaths worldwide, and the incidence rate varies greatly depending on geographic location [1–3]. Esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma based on the histological classification [4]. ESCC has a high incidence worldwide, especially in China [5]. In spite of significant improvements that have been achieved in ESCC treatment, the overall long-term survival rate of patients with ESCC is still poor [6]. This may be attributed to the late diagnosis and lack of individualized treatment [7]. Hence, it is urgent to find more valid biomarkers and novel treatment targets for ESCC.

Non-coding RNAs (ncRNAs) are becoming the new regulators of the cancer paradigm. According to their length, ncRNAs consist of long non-coding RNAs (lncRNAs) and small non-coding RNAs. Among the ncRNAs related to cancer, lncRNAs have attracted far more attention. LncRNAs are multipurpose molecules regulating gene expression at transcriptional and post-transcriptional levels [8]. Numerous evidences indicate that lncRNAs act as a tumor driver or tumor suppressor of various cancers [9–11]. When lncRNA HOTAIR was proven to participate in the progress of breast cancer [12], studies in this field aroused great interest. More and more literatures show that the lesion of lncRNAs is closely related to the growth of tumor cells, in addition to the formation and metastasis of

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tumors [13–16]. Therefore, the identification of cancer-linked lncRNAs and the underlying lncRNAs-interceded regulatory networks are of far-reaching significance for cancer biology. More knowledge of tumorigenesis mechanism could be provided to help develop valid cancer diagnosis and treatment methods for cancer. Additionally, analysis of abnormally expressed lncRNAs elucidates the lncRNA-regulated competitive endogenous RNA (ceRNA) network as a feasible option to comprehend pathogenesis of various tumors including ESCC [17,18]. Previous studies have proved that lncRNA could modulate ESCC through ceRNA. Specifically, Li Z et al. demonstrated that lncRNA ZFAS1/miR-124/STAT3 axis regulated the malignant behaviors of ESCC cells [19]. Li Y et al. also concluded that lncRNA NEAT1 modulated ESCC cell proliferation and invasion through targeting miR-129/CTBP2 in their study [20]. Nevertheless, the specific functions and regulatory mechanism of PCAT5 in ESCC remain to be clarified.

In our study, PCAT5 expression was tested in ESCC cell lines. Then, the influence of the silenced PCAT5 on cell proliferative, migrated, invasive and apoptotic capabilities in ESCC was specially analyzed. Meanwhile, we also established a PCAT5-mediated ceRNA modulatory mode in ESCC cell proliferation and mobility. Our work may provide new thoughts for constructing effective therapies for ESCC.

2. Materials and methods

2.1. Ethical statement and tissue samples

This study was approved by the Research Ethics Committee of Hainan Hospital of PLA General Hospital (No. 202212574). The ESCC and ANM tissue samples were obtained from patients admitted for surgery at the Hainan Hospital of PLA General Hospital. The patients were not subjected to radiotherapy or chemotherapy before surgery. All patients signed informed consent.

2.2. Cell culture

Cell lines were grown at 37 °C in an incubator with 5 % CO_2 in DMEM (Invitrogen, Carlsbad, CA, USA) harboring 10 % FBS (Invitrogen) and 1 % penicillin/streptomycin (Sigma-Aldrich, Milan, Italy).

2.3. Cell transfection

EC109 and KYSE450 cells were transfected with specific shRNAs against PCAT5 (sh-PCAT5#1 and sh-PCAT5#2) or their negative control (sh-NC), and pcDNA3.1/PHF20 or pcDNA3.1 vector, severally. The miRNA mimics, miR-4295 inhibitor, NC mimics and NC inhibitor were synthesized by GenePharma (Shanghai, China). shRNA lentiviral plasmid targeting PCAT5 (sh-PCAT5) was constructed by inserting annealed shRNA template DNA sequence into the pLKO.1 vector (Addgene, Cambridge, USA). Each plasmid was transfected into cells via Lipofectamine 2000 (Invitrogen). All the oligo sequences used in this study are listed in Table S1.

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

TRIzol reagents (Invitrogen) were applied to obtain total RNAs from cultured cells. The manufacturer instructions were strictly followed. Later, RNAs were reversely transcribed into cDNAs with usage of Reverse Transcription kits (Promega, Madison, WI, USA). QRT-PCR was subsequently carried out with SYBR Green PCR Kit (Invitrogen) on a Bio-Rad CFX96 System (Applied Biosystems, Foster City, CA, USA). GAPDH or U6 was regarded as internal reference, and fold expression changes were calculated via $2^{-\Delta\Delta Ct}$ method. Primer sequences are shown in Table S1.

2.5. Colony formation assay

1×10^3 transfected EC109 and KYSE450 cells were cultured in fresh 6-well plates. After being incubated for 2 weeks, colonies were fixed using paraformaldehyde (Sigma-Aldrich) and dyed in crystal violet (Sigma-Aldrich). Visible colonies were then counted manually.

2.6. 5-Ethynyl-2′-deoxyuridine (EdU) incorporation assay

The Click-iT EdU Imaging Kit (Invitrogen) was employed for assaying cell proliferation based on the manufacturer guidelines. In short, transfected EC109 and KYSE450 cells were cultured in 96-well plates. Then 50 μM EdU was added prior to fixation, permeabilization for cell staining. Nuclei were subsequently stained by DAPI (Invitrogen). Finally, EdU positive cells were visualized under a fluorescence microscope (Olympus, Tokyo, Japan).

2.7. Transwell assay

The invasive or migratory ability of cells was determined utilizing transwell chambers (Corning, MA, USA). As for migration assay, transfected cells were suspended in serum-free medium and placed in the top compartment. Meanwhile, medium with 10 % FBS was added to the bottom chamber. Methanol or crystal violet (both from Sigma-Aldrich) was applied for fixation or coloration after 48 h incubation. The experimental steps of invasion assay were similar to migratory assay except that we pre-coated Matrigel onto the plate

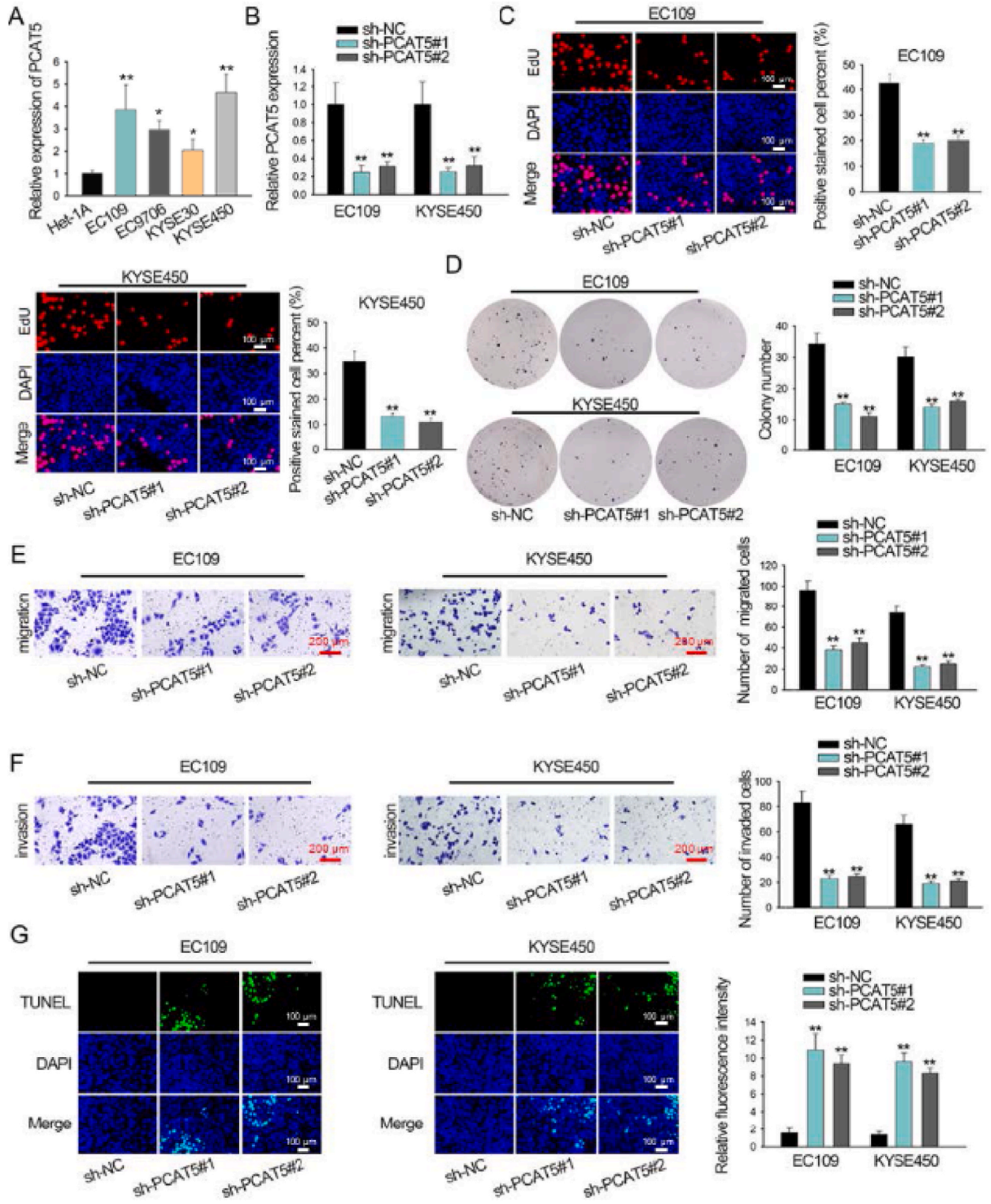

Fig. 1. The knockdown of PCAT5 inhibited cell proliferation, migration, invasion and promoted cell apoptosis in ESCC
A. The expression of PCAT5 was examined in one noncancerous cell line and four ESCC cell lines by qRT-PCR. B. The knockdown efficiency of sh-PCAT5#1 and sh-PCAT5#2 in EC109 and KYSE450 was examined. Sh-NC was a control. C-D. EdU and colony formation assay were used to test the effect of PCAT5 on cell proliferation. Scale bar, 100 μm. E-F. Transwell assays were done to test the effect of PCAT5 on cell migration and invasion. Scale bar, 200 μm. G. TUNEL assay was implemented to test the influence of PCAT5 on cell apoptosis. Scale bar, 100 μm. One-way ANOVA was applied for analysis. *P < 0.05, **P < 0.01.

surface. Migratory or invasive cells were captured under a microscope (Olympus).

2.8. Terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling (TUNEL) assay

Via In Situ Cell Death Detection Kit (Roche, Mannheim, Germany), cell apoptosis was assessed according to the manufacturer instructions. DAPI (Sigma-Aldrich) or TUNEL (Gene-denovo, Guangdong, China) was used for dyeing EC109 and KYSE450 cells. Relative fluorescence intensity was detected via an EVOS FL microscope (Thermo Fisher Scientific, Waltham, MA, USA).

2.9. Subcellular fractionation

The fractions of cytoplasm and nucleus were separated using NE-PER™ Nuclear and Cytoplasmic Extraction Reagents (Invitrogen) and then gathered by an RNeasy Midi Kit (Qiagen, Hilden, Germany). Afterward, relative expression levels of PCAT5, U6 (nuclear control) and GAPDH (cytoplasmic control) were separately explored by qRT-PCR.

2.10. Western blot

Via RIPA lysis buffer (Invitrogen), cells were lysed and then proteins were obtained. Protein concentrations were examined through BCA Kit. SDS-PAGE was adopted for accomplishing the isolation of proteins. Then, proteins were shifted onto PVDF membranes (Millipore, Billerica, MA, USA). Following that, membranes were blocked in 5 % fat-free milk and severally incubated with primary antibodies against PHF20 (ab67796, Abcam, Cambridge, USA) and GAPDH (ab8245, Abcam). Secondary antibodies were added to culture for 1 h. Amount of protein was detected via chemiluminescence detection system.

2.11. Luciferase reporter assay

The wild-type (WT) or mutant (Mut) binding sites of miR-4295 in PCAT5 sequence or PHF20 3′UTR were sub-cloned into pmirGLO dual-luciferase vector to produce PCAT5-WT/Mut or PHF20 3′UTR-WT/Mut, and then co-transfected with miR-4295 mimics or NC mimics into EC109 and KYSE450 cells. Dual-Luciferase Reporter Assay System (Promega) was employed to examine the luciferase activity.

2.12. RNA binding protein immunoprecipitation (RIP) assay

Based on the experimental instructions, Thermo Fisher RIP Kit (Thermo Fisher Scientific) was utilized to conduct RIP assay. Ago 2 antibodies (Millipore) coated with magnetic beads in RIP buffer were added to cell lysis buffer. IgG (Millipore) was involved as negative control (NC). The PHF20 and miR-4295 level was assayed using qRT-PCR.

2.13. RNA pull-down assay

In a nutshell, cell lysates were incubated with biotinylated RNAs including Bio-miR-4295-WT, Bio-miR-4295-Mut, Bio-PHF20-WT, Bio-PHF20-Mut and Bio-NC. Afterward, cells were cultivated with streptavidin-coupled agarose beads (Sigma-Aldrich) to pull down the complex. Relative expression level of RNAs in KYSE450 and EC109 was evaluated, respectively.

2.14. Statistical analysis

Data of experiment was manifested as mean ± SD and analyzed by GraphPad Prism 7.0 software (Graph Pad, La Jolla, CA, USA). All experiments were required to do in triplicate at least. Student's t-test, one-way ANOVA (Analysis of Variance) and two-way ANOVA were used for 2 or more groups. $P < 0.05$ was deemed as statistically significant.

3. Results

3.1. The knockdown of PCAT5 inhibited ESCC cell proliferation, migration, invasion and promoted cell apoptosis in ESCC

Firstly, qRT-PCR was performed to determine the levels of PCAT5 in 20 ESCC and matched ANM tissues. The findings confirmed that PCAT5 was significantly and frequently under up-expressed in ESCC tissues compared to paired ANM tissues (Fig. S1A). Then, PCAT5 expression was examined in ESCC cell lines (EC109, EC9706, KYSE30 and KYSE450) and paired normal cell lines (Het-1A), and the results unveiled that in comparison with normal noncancerous cells Het-1A, the expression of PCAT5 was greatly higher in ESCC cells, especially in EC109 and KYSE450 cell lines. Thus, the two cell lines were chosen for subsequent assays (Fig. 1A). Then we examined the knockdown efficiency of PCAT5 transfected with sh-PCAT5#1 and sh-PCAT5#2 in EC109 and KYSE450 cells (Fig. 1B). According to the results, the expression of PCAT5 notably decreased after PCAT5 depletion. After that, EdU experiment was utilized to test the influence of PCAT5 silencing on cell proliferation (Fig. 1C). The cell proliferation ability was highly restrained owing to the knockdown of PCAT5 in EC109 and KYSE450. This phenomenon revealed that the silence of PCAT5 had an inhibitory impact on cell proliferation. Then, the colony formation assay also presented the similar outcomes which further confirmed that the knockdown of

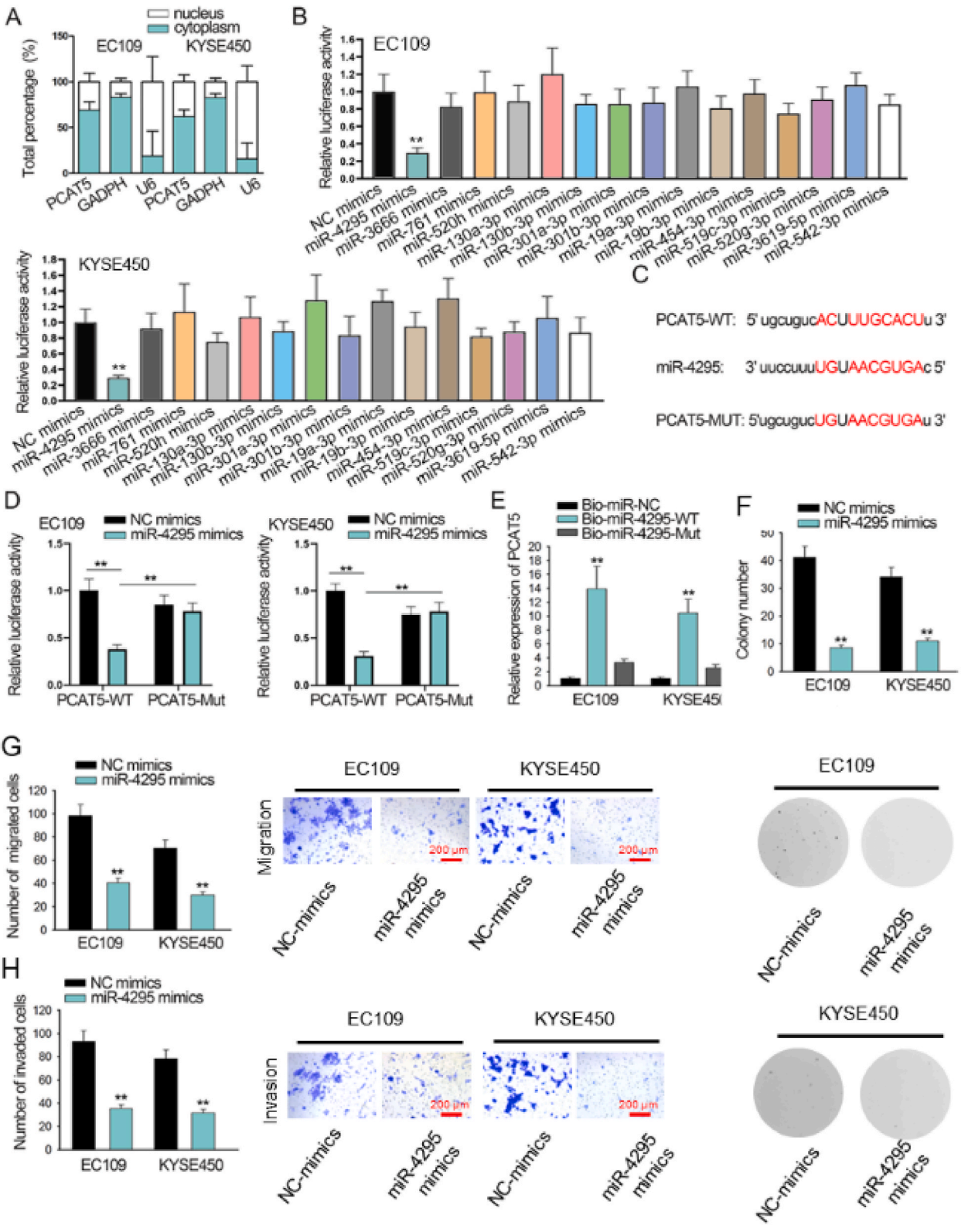

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Fig. 2. MiR-4295 could combine with PCAT5
A. The subcellular fractionation assay was used to find the location of PCAT5. T-test was applied for analysis. B. Luciferase reporter assay was implemented to test the effects of 15 candidate miRNAs on the luciferase activity of PCAT5 reporter plasmids. One-way ANOVA was applied for analysis. C. The binding sites of PCAT5 and miR-4295 were predicted by starBase database. D-E. Luciferase reporter assay and RNA pull-down assay were applied to ensure the combination and binding sites of PCAT5 and miR-4295. Two-way ANOVA was applied in Fig. D for analysis. One-way ANOVA was applied in Fig. E for analysis. F. Colony formation assay were used to test the effect of miR-4295 on cell proliferation. G-H. Transwell assays were done to test the effect of miR-4295 on cell migration and invasion. Scale bar, 200 μm. T-test was applied in Fig. F–I for analysis. **P < 0.01.

PCAT5 inhibited proliferation of EC109 and KYSE450 (Fig. 1D). Additionally, transwell migration experiment was implemented to determine PCAT5 influence on ESCC cell migration (Fig. 1E). Transwell migration result showed that EC109 and KYSE450 cell migration ability was inhibited by the knockdown of PCAT5. Hence, the knockdown of PCAT5 could restrain the cell migration in ESCC. Afterward, the cell invasion was detected by transwell invasion experiment (Fig. 1F). Based on the data collected, the number of invaded cells greatly decreased under the condition of the down-regulation of PCAT5 in cell EC109 and KYSE450. Moreover, the cell apoptosis ability was tested via TUNEL assay, and the results indicated that when PCAT5 was silenced, the apoptosis capability of EC109 and KYSE450 cells improved remarkably (Fig. 1G). Taken together, the knockdown of PCAT5 exerted an inhibitory impact on cell proliferative, migrated and invasive abilities but a promoting effect on apoptotic ability of cell EC109 and KYSE450 in ESCC.

3.2. MiR-4295 could combine with PCAT5

To support the idea of ceRNA, the miRNA that could combine with PCAT5 should be found. The subcellular fractionation assay was implemented to ensure the localization of PCAT5 and it found that PCAT5 mainly amassed in the cytosol of cells (Fig. 2A). Fifteen miRNA candidates which could possibly combine with PCAT5 were selected by StarBase (http://starbase.sysu.edu.cn/) database. To choose the most appropriate one, overexpression efficiency of these miRNAs in EC109 and KYSE450 cells was first detected (Fig. S2A-O). Furthermore, in accordance with results of the luciferase reporter assay, the relative luciferase activity of PCAT5 was decreased notably only by overexpression of miR-4295 (Fig. 2B). Subsequently, the binding sites of PCAT5 and miR-4295 were predicted on StarBase and we mutated the binding sequences of PCAT5 (Fig. 2C). Results from luciferase reporter assay revealed that luciferase activity of wild type PCAT5 was remarkably decreased by miR-4295 mimics but that of mutated PCAT5 almost had no change (Fig. 2D). Aside from that, RNA pull-down experiment further verified that PCAT5 combined with miR-4295 at predicted sites (Fig. 2E). To detect the effect of miR-4295, colony formation assay was conducted and it was illustrated that the overexpression of miR-4295 restrained the cell proliferation in ESCC (Fig. 2F). Moreover, transwell experiments were implemented to detect the impact of miR-4295 on cell migration and invasion (Fig. 2G–H). After miR-4295 overexpression, the number of migrated and invaded cells dropped and these outcomes showed the inhibitory function of miR-4295 on cell migration and invasion in ESCC. To sum up, miR-4295 could combine with PCAT5 and it was a tumor suppressor in ESCC cells.

3.3. PHF20 was a target of miR-4295

To find a full axis, the target mRNA of miR-4295 was selected by starBase. Based on four bioinformatics tools (miRmap, microT, PicTar and RNA22), two mRNAs which were possible to combine with miR-4295 were screened out (Fig. 3A). Then we examined the expression levels of mRNA PHF20 and GADD45A in ESCC cell lines. The results revealed that GADD45A was not abnormally expressed in ESCC cells and normal cells. By contrast, mRNA level and protein level of PHF20 were both higher in ESCC cell lines than in normal cell line (Fig. 3B). Afterward, binding sites of PHF20 and miR-4295 were predicted (Fig. 3C). Then, luciferase reporter assay, RIP and RNA pull-down assays were applied for exploration of the connection between PHF20 and miR-4295 and the results all proved the combination of PHF20 and miR-4295 (Fig. 3D–F). Additionally, to determine whether the expression of PCAT5 and miR-4295 in ESCC cells was sufficient enough to affect PHF20, we measured the endogenous level of PCAT5, miR-4295 and PHF20 respectively. MiR-4295 could bind to PHF20 through five possible binding sites. Based on the previous study [21], when the proportion of miR-4295 and targets was 5:1, it met the threshold for miR-4295 to effectively influence its target. The results of qRT-PCR indicated that copy number of miR-4295 was 25 times more than that of PHF20 (Fig. S3A), indicating that the endogenous level of miR-4295 was competent enough to affect PHF20 in ESCC cells. Moreover, binding site of miR-4295 on PCAT5 was simply one. And we found the endogenous level of PCAT5 in ESCC cells was 5 times more than that of PHF20 (Fig. S3B). Referring to the previous literature [22], a competitive relationship may exist between PCAT5 and PHF20. After testing the knockdown efficiency of miR-4295, the relationship of PCAT5, PHF20 and miR-4295 was examined via qRT-PCR and Western blot (Fig. 3G–H). The results showed that PCAT5 inhibition would decrease the expression of PHF20 and miR-4295 inhibitor could reverse the inhibited effect of sh-PCAT5 on PHF20. To conclude, PCAT5 could regulate the expression of PHF20 by sponging miR-4295.

3.4. PCAT5 regulated ESCC cell proliferation, migration, invasion and apoptosis via miR-4295/PHF20

To prove the PCAT5/miR-4295/PHF20 axis, rescue assays were implemented. In the beginning, the efficiency of pcDNA3.1/PHF20 was tested (Fig. 4A). Subsequently, EdU assay was performed and the reduced cell proliferation caused by the knockdown of PCAT5 was rescued by overexpressed PHF20 (Fig. 4B). Similar to EdU experiment, the colony formation assay also represented that cell proliferation ability weakened by silence of PCAT5 was recovered by overexpression of PHF20 (Fig. 4C). In addition, the transwell

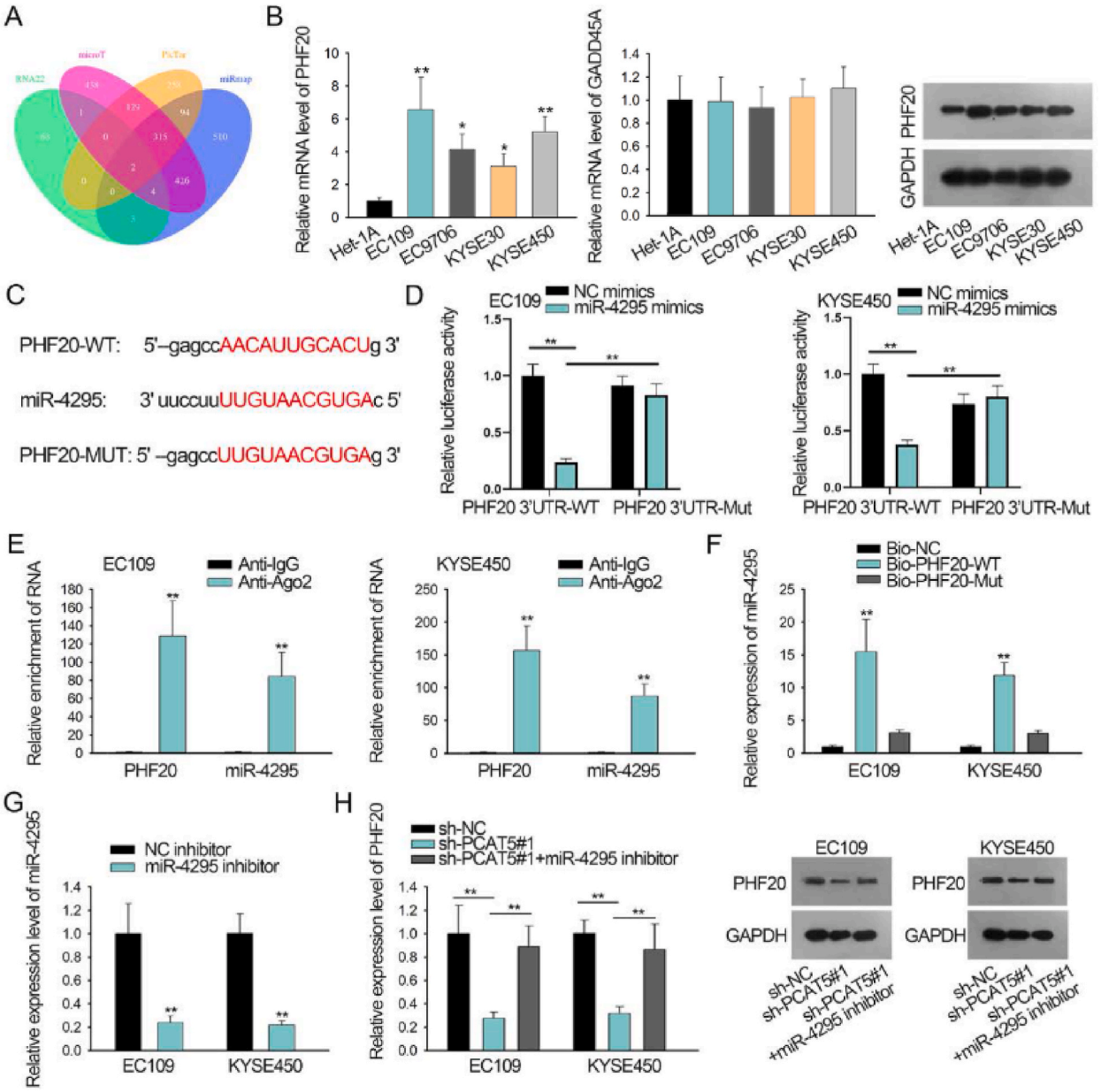

Fig. 3. PHF20 was a target of miR-4295

A. Target genes binding with miR-4295 were selected by starBase database. B. The RNA expression of PHF20 and GADD45A was test via qRT-PCR. The protein level of PHF20 was detected via Western blot. One-way ANOVA was applied for statistics analysis. C. The binding sites between PHF20 and miR-4295 were predicted by bioinformatics analysis. D-F. Luciferase reporter assay, RIP assay and RNA pull-down assay were applied to identify the interaction of PHF20 and miR-4295. Two-way ANOVA was applied for statistics analysis of Fig. D and T-Test was used to analyze Fig. E. One-way ANOVA was applied to analyze Fig. F. G. The knockdown efficiency of miR-4295 was detected by qRT-PCR. T-Test was applied for statistics analysis. H. qRT-PCR and Western blot assay were done to examine the relation of PCAT5, PHF20 and miR-4295. One-way ANOVA was utilized for analysis. *P < 0.05, **P < 0.01.

assays were utilized to test the influence of PCAT5 on cell migration and invasion and it was found that declined cell migration and invasion due to knockdown of PCAT5 was restored by PHF20 overexpression (Fig. 4D–E). Aside from that, TUNEL experiment was conducted to discover effect of PCAT5 on cell apoptosis (Fig. 4F). The results demonstrated that the improved cell apoptosis ability caused by PCAT5 silence could be rescued by PHF20 overexpression. In a word, PCAT5 regulated ESCC cell proliferative, migrated, invasive and apoptotic abilities via miR-4295/PHF20.

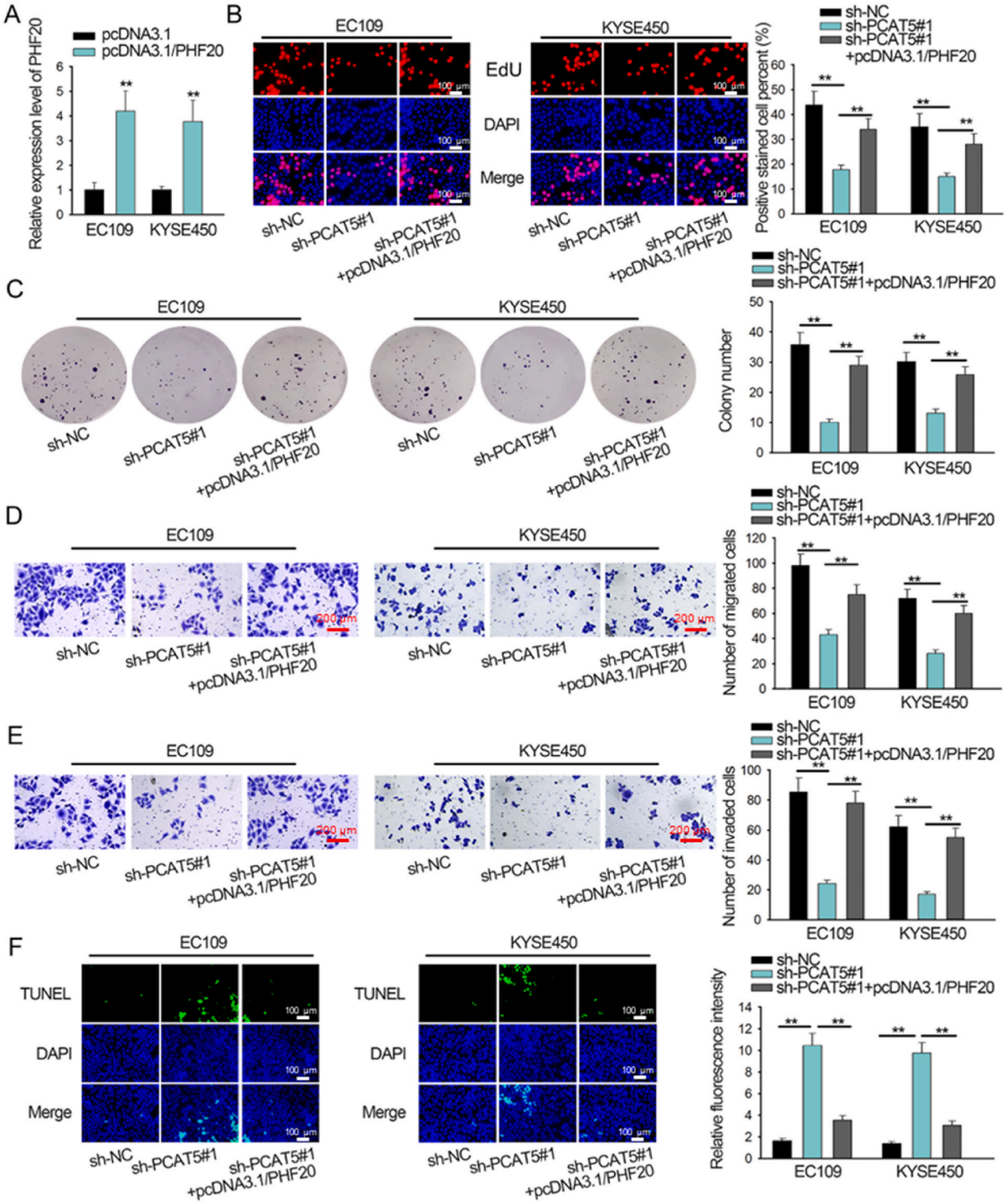

Fig. 4. PCAT5 regulated ESCC cell proliferation, migration, invasion and apoptosis via miR-4295/PHF20

A. The overexpression efficiency of pcDNA3.1/PHF20 was detected by qRT-PCR. T-Test was applied for analysis. B–C. EdU and colony formation experiments were used to test the effect of overexpressing PHF20 after inhibiting PCAT5 on cell proliferation. Scale bar, 100 μm. D-E. Transwell assays were done to examine the impact of overexpressing PHF20 after inhibiting PCAT5 on cell migration and invasion. Scale bar, 200 μm. F. TUNEL assay was implemented to test the influence of overexpressing PHF20 after inhibiting PCAT5 on cell apoptosis. Scale bar, 100 μm. One-way ANOVA was applied for analysis in Fig. B–F. $^{**}P < 0.01$.

4. Discussion

Based on current studies, lncRNAs are linked with regulatory networks of various cancers, functioning as oncogenes or anticancer genes. For the past few years, researches have indicated some lncRNAs are expected to serve as biomarkers in prognostic evaluation or as tumor treatment targets for human cancers [23–25]. A large number of literatures represent that the lesion of lncRNA has close connection with the metastasis and formation of tumors. For instance, lncRNA ZEB1-AS1 represses ESCC cell proliferative and invasive abilities via silencing ZEB1 expression [26]. RNA LEF1-AS1 indicates unfavorable prognosis in ESCC patients [27]. Despite increasing research revealed the pathogenesis of cancers, the regulatory role of certain lncRNA regarding the carcinogenesis of ESCC was limited. Thus, this study tried to dig into the biological functions of PCAT5 in ESCC by knockdown of PCAT5 in ESCC cell lines.

PCAT5 is proved to have a great effect on prostate cancer cells [28]. In this study, PCAT5 was abnormally overexpressed in ESCC cells compared with noncancerous cells. In addition, the silence of PCAT5 in cell EC109 and KYSE450 greatly restrained cell proliferative, invasive and migrated abilities while suppressing apoptosis ability in ESCC. A series of experiments conducted indicated that abnormal expression of PCAT5 would impact the biological behaviors of ESCC cell lines.

Since lncRNA could engage in the regulatory circuit serving as a ceRNA, we supposed that PCAT5 might also play a role of ceRNA in ESCC cells. The ceRNA axis in ESCC was widely reported. For instance, LINC00473 facilitates ESCC progression via sponging miR-497-5p and up-regulating PRKAA1 [29]. FOXD2-AS1 interacts with miR-195 by acting as a ceRNA of Akt to promote cisplatin resistance in ESCC [30].Therefore, we utilized bioinformatics analysis and a range of mechanism assays including luciferase analysis to identify the downstream miRNAs for PCAT5 and the target genes of miRNA. MiR-4295 was identified to bind to PCAT5 and to target PHF20. In existing publications, miR-4295 has been widely reported as a vital oncogenic factor in bladder cancer and gliomas [31]. MiR-4295 regulates expression of USP28 which is related to poor outcomes in patients suffering from non-small cell lung cancer [32]. PHF20 is an oncogene that influences cell proliferation, migration, invasion, and apoptosis [33,34]. For example, it may promote stemness and aggressiveness of neuroblastoma cells, whereas suppresses non-small-cell lung cancer [33]. Our study unveiled that PCAT5 functioned as a ceRNA of PHF20 via sponging miR-4295 to facilitate ESCC cell proliferation. In addition, the copy number of PCAT5, miR-4295 and PHF20 in ESCC cells met the threshold defined in previous studies, which further supported the validity of this regulatory mechanism.

In summary, our discoveries verified that lncRNA PCAT5 played an oncogenic role in ESCC. PCAT5 could promote cell proliferative, migrated and invasive capabilities while weakening cell apoptotic ability via miR-4295/PHF20 axis in ESCC. The scale and design of this study is still limited, but it might provide a new direction and idea for understanding ESCC. However, this study still has some limitations, such as the biological function of PCAT5 has not been confirmed in vivo, and the expression of PCAT5 is still lacking of clinical sample verification. These issues need further discussion in the future.

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CRediT authorship contribution statement

Hui Liu: Data curation, Methodology, Resources, Visualization, Writing – original draft, Writing – review & editing. **Hang Yin:** Formal analysis, Methodology, Supervision, Writing – original draft, Writing – review & editing. **Tao Yang:** Data curation, Writing – original draft. **Jiacai Lin:** Resources, Validation, Writing – review & editing. **Tingting Sun:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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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.e22086.

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