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Long non-coding RNA AC245100.4 activates the PI3K/AKT pathway to promote PCa cell proliferation by elevating PAR2

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ABSTRACT

Background: Prostate cancer (PCa) is among the most generally diagnosed cancers in males. A long non-coding RNA (lncRNA) called AC245100.4 has been discovered and linked to PCa carcinogenesis. However, its specific and potential mechanism is uncertain in PCa. In this research, we investigated the role of AC245100.4 in cell proliferation and the underlying mechanism in PCa cells.

Methods: qRT-PCR assays were utilized to detect AC245100.4 expression and confirm its downstream target. The pathways related to AC245100.4 were identified by RAP-MS. PCa cell proliferation was experimented by Cell Counting Kit-8 and Colony formation assays. Western blot was performed to detect PAR2, AKT, p-AKT, Cyclin D1 and PCNA expression.

Results: AC245100.4/PAR2 overexpression promotes PCa cell proliferation and the opposite results are obtained after AC245100.4/PAR2 knockdown. Mechanistically, we found that PAR2 is confirmed as the AC245100.4 downstream target and AC245100.4 promotes PCa cell proliferation by regulating PAR2. AC245100.4 promotes PCa cell proliferation via PI3K/AKT pathway. Rescue assays validated that PAR2 knockdown reversed the impact of AC245100.4 overexpression on increasing p-AKT protein levels.

Conclusion: This research revealed that AC245100.4 enhances cell proliferation in PCa cells through modulating the PAR2/PI3K/AKT axis, which may offer novel tumor markers and potential therapeutic targets for PCa.

1. Introduction

Prostate cancer (PCa) becomes the most widespread malignant tumor and the fifth significant cause of cancer-related fatality in males overall [1,2]. Due to the lack of effective therapies, the prognosis of PCa patients tends to be poor [3]. Thus, for the sake of

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improving patient outcomes, it seems imperative to discover novel molecular biomarkers and investigate the molecular mechanism of PCa progression.

Long non-coding RNAs (lncRNAs) prove RNAs that are longer than 200 nucleotides and cannot be translated into functional proteins [4]. Previous studies have proven that lncRNAs contribute to numerous tumor regulatory processes, emphasizing their huge potentials in tumor screening, prognosis and therapies [5]. For instance, lncRNA SNHG1 and RNA binding protein hnRNPL form a complex that promotes PCa growth and metastasis by coregulating CDH1 [6]; lncRNA HOTAIR modulates PCa invasion and metastasis by targeting hepaCAM [7]; lncRNA CCAT1 regulates miR-490-3p/FRAT1 axis to promote PCa cell proliferation, migration, and invasion [8]. In our previous study, we identified lncRNA AC245100.4, which is highly expressed in PCa, promoted PCa cell proliferation by binding with HSP90. However, the novel mechanism by which AC245100.4 affects PCa cell proliferation remains unclear.

Protease-activated receptor 2 (PAR2), encoded by F2RL1, is a member of subfamily of GPCRs, which also plays vital roles on tumor progression [9]. It has been reported that PAR2 can be regulated by lncRNA. For instance, lncRNA MEG3 induces PAR2 expression, leading to the activation of NF-κB pathway [10]. Our previous study also found that AC245100.4 contributes to prostate cancer migration by regulating PAR2 and activating the p38-MAPK pathway [11]. However, the role of AC245100.4/PAR2 axis in prostate cancer cell proliferation and related mechanism remains to be determined.

In this study, based on our previous research, we intended to explore the mechanism for AC245100.4/PAR2 axis mediated PCa cell proliferation, maybe providing novel advances in the molecular mechanism of PCa treatment.

2. Methods

2.1. Cell culture

Prostate epithelial cells（RWPE-1）and prostate cancer cells（DU145，PC3 and PC3M）were obtained from the American Type Culture Collection. The cells were plated in DMEM/RPMI-1640 containing 10% fetal bovine serum and 1% penicillin-streptomycin and incubated at 37 °C with 5% CO2 in a humidified cell-culture incubator.

2.2. Cell transfection

GenePharma was the source for the si-*AC245100.4* and si-*PAR2*. The sequence of siRNAs which target AC245100.4 or PAR2 is available upon request from the authors. GenePharma produced the pcDNA3.1-*AC245100.4* (OE-*AC245100.4*) and pcDNA3.1-*PAR2* (OE-*PAR2*). Following the manufacturer's recommendations, cells were transfected using Lipofectamine 3000 (Invitrogen, Carlsbad, California, United States).

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

Utilizing Trizol reagents, total RNAs were extracted from cells (TaKaRa, Japan). And to use a Reverse Transcription Kit, RNA was converted into cDNA (TaKaRa, Japan). The SYBR Green PCR Master Mix (Applied Biosystems, USA) was used for qRT-PCR detection. All steps were performed strictly according to manufacturer instructions. The level of mRNAs or lncRNAs was evaluated with the $2^{-\Delta\Delta CT}$ method after normalizing to GAPDH.

2.4. Cell Counting Kit-8 (CCK8) assay

In 96-well culture plates, transfected PCa cells were incubated. Then, 10 μL of CCK8 solution were added to each well, cultivated at 37 °C without light for an additional 120 min. Finally, the optical densities of the wells were read on a microplate reader at 450 nm.

2.5. Colony formation assay

Adding transfected PCa cells in wells and changing the media every four days. Two weeks later, the colonies were counted (>50 cells was considered a clone) after being immobilized by methanol for 15 min and stained by crystal violet at 0.1% for another half an hour.

2.6. Western blot

After washing cells with PBS solution, protein was extracted using RIPA lysis buffer. Protein content was measured utilizing BCA protein assay (Takara). Each lane was loaded with 50 μg of protein. 10% SDS-PAGE gel were used to separate equal amounts of proteins, and then the proteins were transferred to nitrocellulose membranes (Merck Millipore, USA). Membranes were blocked with 5% non-fat milk before being treated with primary and secondary antibodies. PAR2 (Proteintech, USA), p-AKT (Cell Signaling, USA), AKT (Cell Signaling, USA), Cyclin D1 (Biyuntian, China), and PCNA were the primary antibodies used for Western blot (Biyuntian, China). The molecular weight (MW) of each protein shown on immunoblots was estimated based on the pre-stained Protein Standard. The proteins in the membrane were detected with ECL enhanced chemiluminescence and the protein bands were taken on film by scanning in accordance with the manufacturer's instructions. For p-AKT and AKT, two separate gels were used for phosphorylated and unphosphorylated protein instead of stripping the membrane to detect the other to avoid the inefficiency of stripping. GAPDH or

Tubulin were used as the sample-loading control.

2.7. Bioinformatics analysis

The STARBASE database (https://starbase.sysu.edu.cn/panGeneDiffExp.php) was utilized to compare the expression of AC245100.4 in 499 PCa and 52 adjacent normal samples. The UALCAN database (http://ualcan.path.uab.edu/) was utilized to analyze the differential expression of PAR2 in 24 tumoral and their adjacent tissues. GEPIA2 database (http://gepia2.cancer-pku.cn/) and GEO Date Analysis Module within the ACBI Bioinformation tool (https://www.aclbi.com/static/index.html#/geo), the expression of PAR2 was analyzed in PCa and adjacent normal samples. Drug Susceptibility Assessment Module within the ACBI Bioinformation tool (https://www.aclbi.com/static/index.html#/drug_allergy) was used to predict the distribution of ZSTK474 (a PI3K inhibitor) IC_{50} score of each group of samples. Gene-Pathway Analysis Module within the ACBI Bioinformation tool (https://www.aclbi.com/static/index.html#/functional_analysis) was used to analyze the relationship between PAR2 expression and the PI3K/AKT pathway in PCa.

2.8. Statistical analysis

Each experiment was repeated with three times. Graph Pad Prism software (Graph Pad 8.0) was used for experimental statistical analysis. Differences between two groups was analyzed using Student's *t*-test, and the differences between three or more groups were using one-way ANOVA. Bioinformatics analysis was performed using the analysis methods recommended by the database website. For example, the bioinformatics differences between the two groups were analyzed using the Wilcox test. The correlation between genes and pathway scores was analyzed by Spearman correlation. P values of 0.05 or less were considered to denote significance.

3. Results

1. AC245100.4 facilitates the PCa cell proliferation.

Our previous study demonstrated that AC245100.4 affects PCa proliferation by binding with HSP90 [12], and AC245100.4 knockdown inhibits the tumorigenesis of PCa cells [13]. However, whether AC245100.4 affects PCa cell proliferation by new mechanisms is unknown. To further investigate this issue, we re-verified the impact of AC245100.4 on PCa cell proliferation. For validating the association of AC245100.4 with PCa, we firstly assessed the AC245100.4 expression in 499 prostate cancer tissue samples and 52 normal prostate tissue samples utilizing STARBASE database (http://starbase.sysu.edu.cn/) , and we discovered that the levels of AC245100.4 in prostate cancer tissue samples were highly expressed in comparison to those in prostate tissue samples

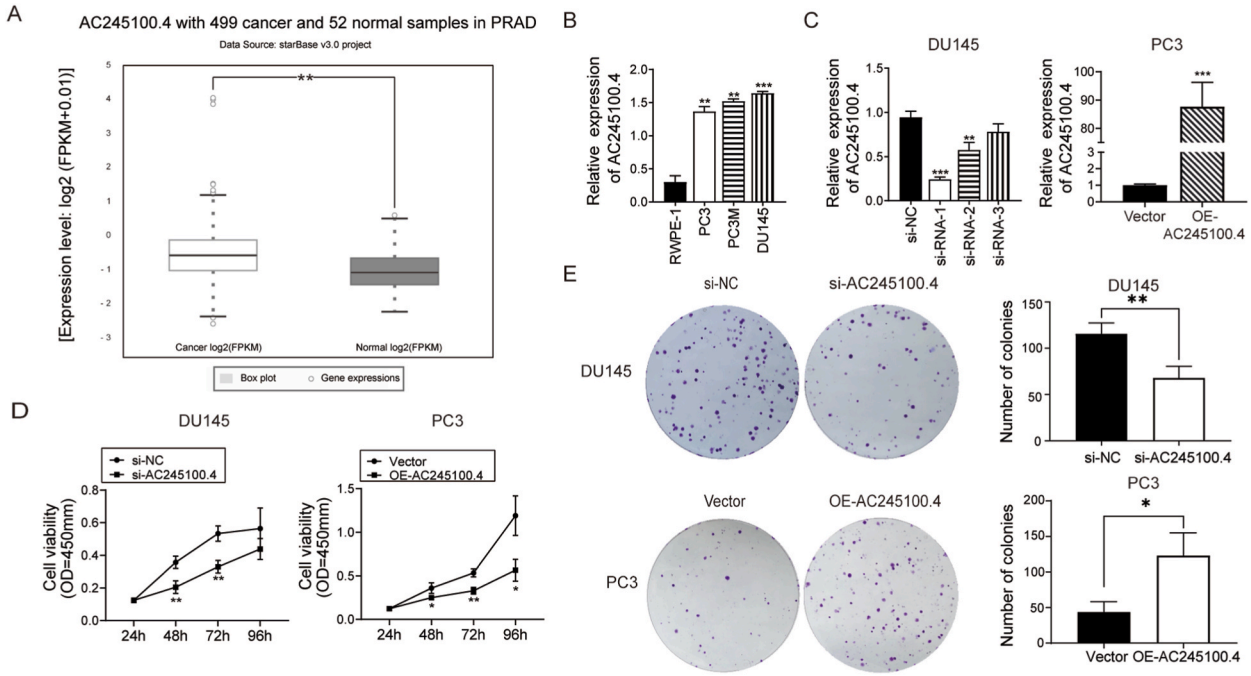

Fig. 1. AC245100.4 facilitates the cell proliferation in prostate cancer. (A) The levels of AC245100.4 in prostate cancer tissues (n = 499) and normal tissues (n = 52) was from STARBASE. (B) AC245100.4 expression in PC3, PC3M, DU145 and RWPE-1 cell lines was measured using qRT-PCR. (C) The levels of AC245100.4 were tested via qRT-PCR in PCa cells with si-*AC245100.4* or OE-*AC245100.4*. (D) and (E) Cell proliferation was assayed by CCK8 and clone formation assay. *$P<0.05$, **$P<0.01$, ***$P<0.001$, n = 3 for each group.

(Fig. 1A). After that, qRT-PCR was implemented to explore AC245100.4 expression in various PCa cell lines and prostate epithelial cell lines. We found that AC245100.4 expression in PC3, PC3M and DU145 cell lines was also higher in comparison to prostate RWPE-1 cell lines ((Fig. 1B). Our findings suggested that AC245100.4 is highly expressed in prostate cancer tissues and cells, which is in agreement with prior findings.

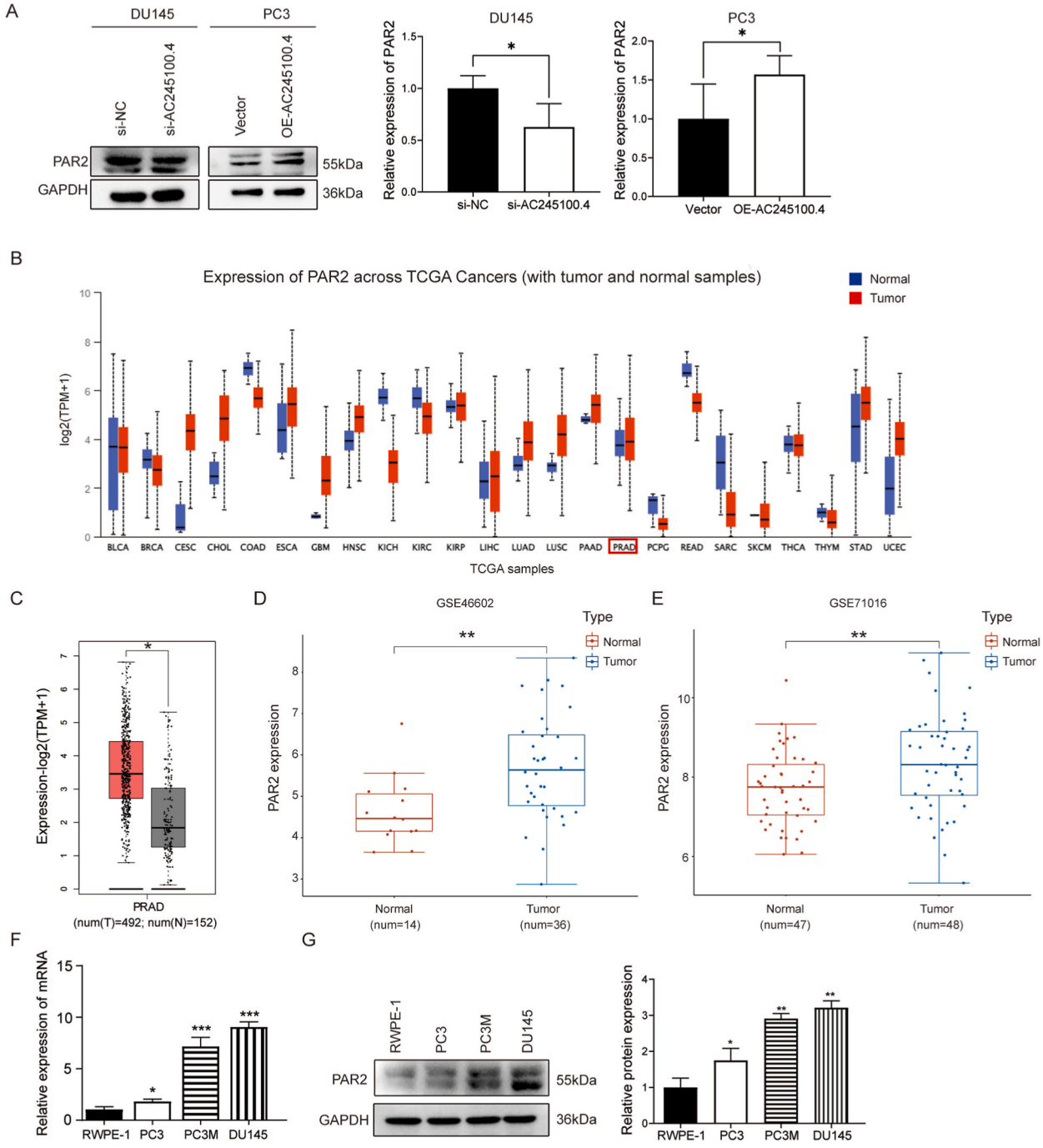

Fig. 2. PAR2 is positively regulated by AC245100.4 and highly expressed in prostate cancer tissues and cells. (A) PAR2 protein expression in PCa cells with si-*AC245100.4* or OE-*AC245100.4* was tested via Western blot. (B)The expression of PAR2 in 24 tumor and 24 tumor and their adjacent tissues was from UALCN. (C) The expression of PAR2 in prostate cancer tissue samples (492) and prostate tissue samples (152) was from GEPIA2. (D) and (E) The expression of PAR2 in prostate cancer tissue samples and prostate tissue samples was from GSE46602 or GSE71016. (F) and (G) qRT-PCR and Western blot were used to detect PAR2 mRNA and protein expression in PC3, PC3M, DU145 and RWPE-1 cell lines. $*P<0.05$, $**P<0.01$, $***P<0.001$, n = 3 for each group.

To determine the influence of AC245100.4 in PCa cell proliferation, we designed and synthesized 3 interfering fragments of AC245100.4 and constructed pcDNA3.1-*AC245100.4* (OE-*AC245100.4*) and pcDNA3.1 (Vector), and then performed qRT-PCR on PCa cells to measure interference and overexpression efficiency. The results showed that si-*AC245100.4*-1, which was chosen to use in all subsequent experiments, had the highest interference efficiency, and AC245100.4 expression in PC3 cells with pcDNA3.1-*AC245100.4*

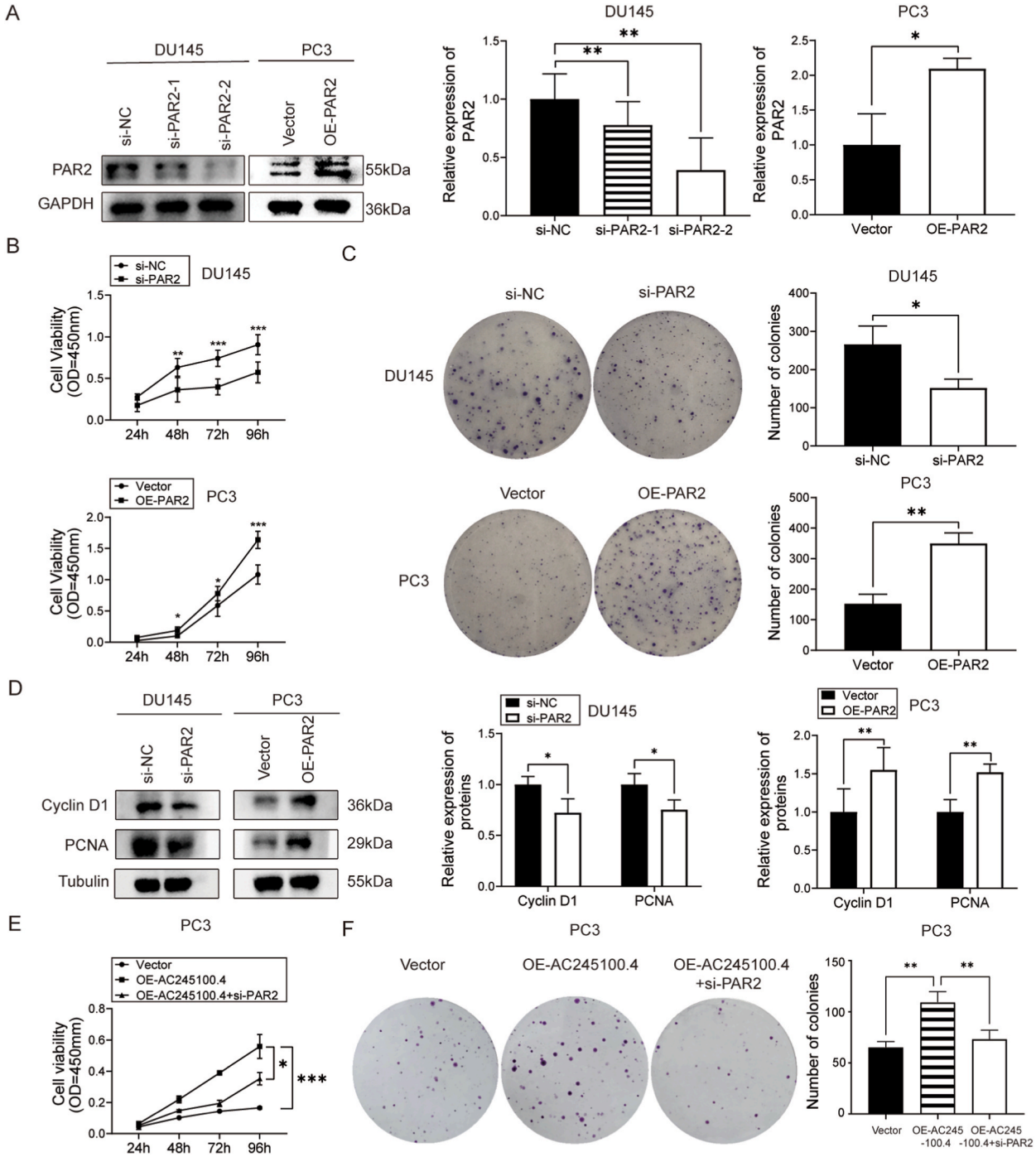

Fig. 3. AC245100.4 promotes cell proliferation via regulating PAR2 in PCa cells. (A) PAR2 protein expression in PCa cells with si-*PAR2* or OE-*PAR2* was tested via Western blot. (B) and (C) The cell proliferation was tested by CCK8 assay and clone formation assay in PCa cells with si-*PAR2* or OE-*PAR2*. (D) Western blot was used to detect Cyclin D1, PCNA protein expression in PCa cells with si-*PAR2* or OE-*PAR2*. (E) and (F) The cell proliferation was tested by CCK8 assay and clone formation assay in PCa cells with OE-*AC245100.4* and si-*PAR2*. *$P<0.05$, **$P<0.01$, ***$P<0.001$, n = 3 for each group.

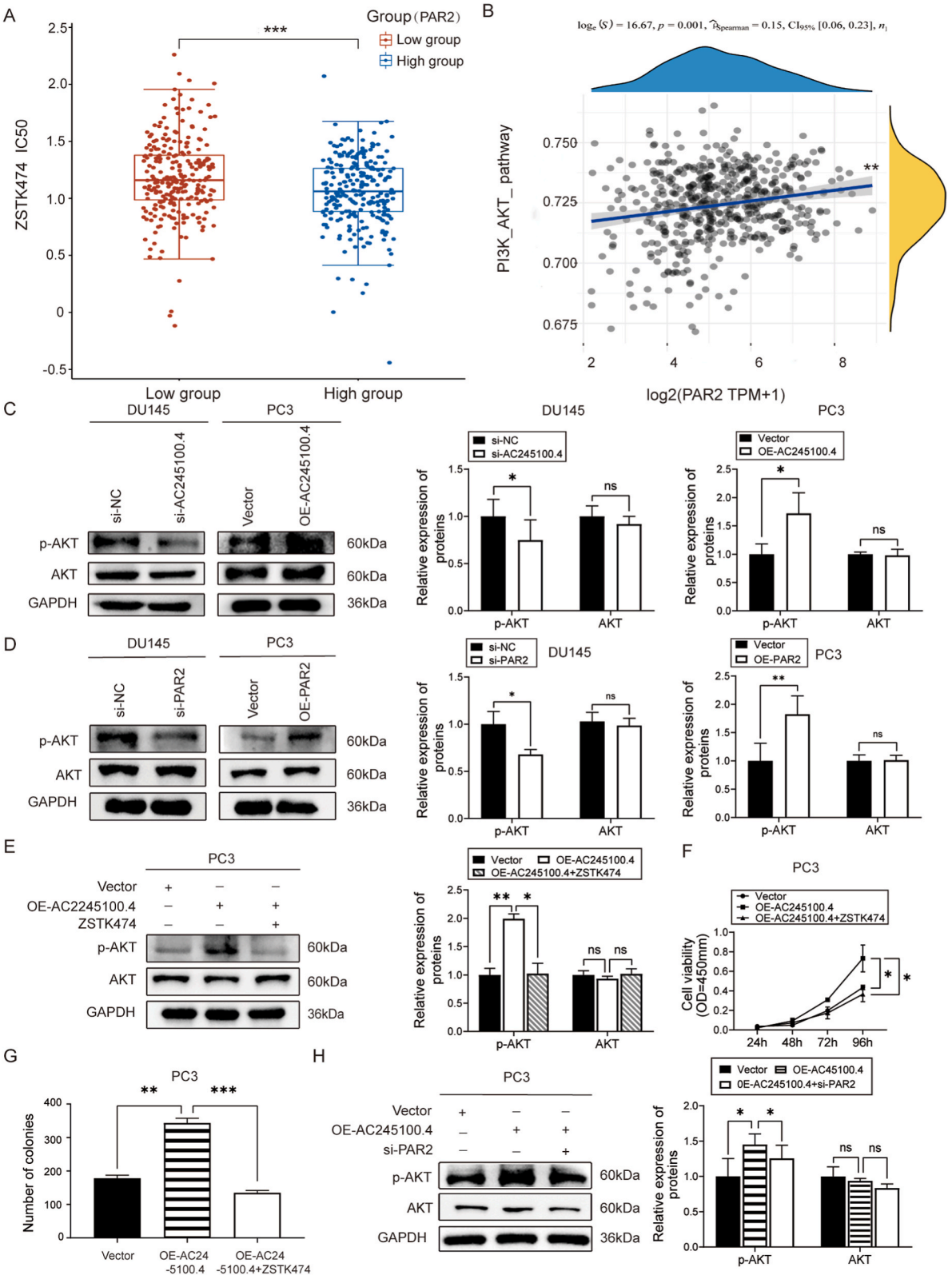

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Fig. 4. AC245100.4 promotes cell proliferation via PAR2/PI3K/AKT axis in prostate cancer cells. (A) The distribution of ZSTK474 IC_{50} score of each group of samples was predicated. (B)The correlation between PAR2 expression and PI3K/AKT/mTOR pathway scores was calculated. (C) AKT, p-AKT protein expression in PCa cells with si-*AC245100.4* or OE-*AC245100.4* were tested via Western blot. (D) AKT, p-AKT protein expression in PCa cells with si-*PAR2* or OE-*PAR2* was tested via Western blot. (E) ZSTK474 was added in PC3 cells with AC245100.4 overexpression , and the AKT, p-AKT protein expression were detected by Western Blot. (F) and (G) ZSTK474 was added in PC3 cells with AC245100.4 overexpression , and the proliferation levels in prostate cancer cells were measured using the CCK8 assay and clone formation assay. (H) AKT, p-AKT protein expression were tested in PCa cells with OE-*AC245100.4* and si-*PAR2* via Western blot. $*P<0.05$, $**P<0.01$, $***P<0.001$, n = 3 for each group.

was elevated markedly in comparison to the Vector group. (Fig. 1C). To evaluate the influence of AC245100.4 on PCa cell proliferation, CCK8 and clonal formation assays were conducted. The Data demonstrated that AC245100.4 knockdown significantly decreased the cell viability and the numbers of colony in PCa cells. Conversely, we obtained opposite results after AC245100.4 overexpression (Fig. 1D and E). The results suggested that AC245100.4 has the intimate connection to PCa and affects PCa cell proliferation, which are in concordance with the previous study.

2. PAR2 is positively regulated by AC245100.4 and highly expressed in prostate cancer tissues and cells

Protease-activated receptor 2 (PAR2), encoded by F2RL1, was considered as the downstream target of AC245100.4 in a prior investigation [11]. For deeper validating the relationship between PAR2 and AC245100.4, we used DU145 cells with AC245100.4 knockdown and PC3 cells overexpressed AC25100.4 to detect PAR2 expression via Western blot. Obtained data indicated that the PAR2 expression was markedly decreased after knockdown of AC245100.4, while the opposite results were obtained after overexpressing AC245100.4 (Fig. 2A). Given that data demonstrated that PAR2 is the downstream of AC245100.4 , which corroborated the previous conjecture. Previous reports have demonstrated that PAR2 was related to carcinogenesis and has been promoted as a potential therapeutic target [14]. We analyzed the differential expression of PAR2 in 24 tumoral and their adjacent tissues via UALCAN database (http://ualcan.path.uab.edu/), and found that PAR2 was highly expressed in 13 cancer tissues, including prostate cancer (Fig. 2B). Subsequently, in order to investigate the correlations between PAR2 and prostate cancer further, the PAR2 level in 492 prostate cancer tissue samples and 152 normal prostate tissue samples were assessed using the GEPIA2 database (http://gepia2.cancer-pku.cn/). Additionally, we searched the Gene Expression Omnibus web portal (https://www.ncbi.nlm.nih.gov/geo/) using the following key word: "Prostate cancer", and the microarray datasets both containing PCa patients and healthy controls were primarily considered. Then, datasets containing samples with other treatments such as drugs or have a sample size of less than 10 pairs were excluded. Finally, we chose two datasets, GSE46602 and GSE71016, and compared PAR2 expression differences between prostate cancer and normal prostate tissue samples in each dataset using the ACBI Bioinformation tool (https://www.aclbi.com/). These findings all demonstrated that PAR2 is highly expressed in PCa tissues (Fig. 2C, D and E). Likewise, we also found that PAR2 is highly expressed in PCa cells in comparison to prostate epithelial cells (Fig. 2F and G). In brief, the remarkable higher expression of PAR2 is observed in prostate cancer tissues and cells, which suggests that PAR2 is strongly associated with PCa.

3. AC245100.4 promotes cell proliferation via regulating PAR2 in PCa cells

Next, to validate the influence of PAR2 on PCa cell proliferation, we firstly designed and synthesized interfering fragments of PAR2 (si-*PAR2*) and constructed pcDNA3.1-*PAR2* (OE-*PAR2*), and then we transfected them into DU145 cells or PC3 cells and detected interference and overexpression efficiency. The results showed that si-*PAR2*-2 reduced the PAR2 protein levels in PCa cells, while the results were opposite after transfecting pcDNA3.1-PAR2 (Fig. 3A). And then, the CCK8 assay and clonal formation assay were further performed to observe the influence of PAR2 on PCa cell proliferation. The results showed that the cell viability and the numbers of colony in PCa cells decreased by si-*PAR2*-2, while we got the opposite results after transfection with pcDNA3.1-*PAR2*. Additionally, Western blot was used to detect the expression of proliferation-associated genes (Cyclin D1 and PCNA) in PCa cells with PAR2 knockdown. Data from Western blot showed that PAR2 knockdown decreased Cyclin D1 and PCNA protein levels. In contrast, the opposite results were observed by overexpression of AC245100.4 (Fig. 3B, C and D). Accordingly, the study suggested that PAR2 has effects on PCa cell proliferation. We next transfected pcDNA3.1-*AC245100.4* and si-*PAR2*-2 into PCa cells and perform CCK8 assay and clonal formation assay in these cells. The results showed PAR2 knockdown reversed effects of AC245100.4 overexpression on increasing PCa cell viability and the numbers of colony (Fig. 3E and F). These findings suggested that AC245100.4 promotes prostate cancer cell proliferation by elevating PAR2 expression.

4. AC245100.4 promotes cell proliferation via PAR2/PI3K/AKT axis in PCa cells

It has been written regarding the regulation of a variety of signaling pathways by lncRNA in tumor progression [15]. Our previous RAP-MS results demonstrated that proteins interacting with AC245100.4 were enriched in 28 signaling pathways, including PI3K signaling pathway. Surprisingly, it also has been reported that PAR2 can affect PI3K/AKT signaling pathway [16]. Nevertheless, it has not been reported that PAR2 activates the PI3K/AKT pathway in prostate cancer. Therefore, we obtained the RNAseq data and corresponding clinical data of 498 prostate cancers through the TCGA dataset (https://portal.gdc.cancer.gov/), and divided them into low expression group and high expression group according to the expression of PAR2. Then we used the ACBI Bioinformation tool to predict the distribution of ZSTK474 (a PI3K inhibitor) IC_{50} score of each group of samples. The results showed that the IC_{50} score of the group with high PAR2 expression was lower than that of the group with low PAR2 expression (Fig. 4A). To investigate further the

relationship between PAR2 and PI3K/AKT in prostate cancer, we analyzed the relationship between PAR2 expression and the PI3K/AKT pathway using the ACBI Bioinformation tool and found a positive correlation in PCa (Fig. 4B). Based on the above findings, we surmise that PI3K/AKT pathway has the relationship with AC245100.4/PAR2 axis and is related to precise regulation of PCa. Here, we explored the influence of AC245100.4 and PAR2 on PI3K/AKT pathway in PCa cells. After AC245100.4 or PAR2 knockdown, p-AKT expression was significantly reduced, while the opposite was observed after AC245100.4 or PAR2 overexpression (Fig. 4C and D). These findings indicated that AC245100.4 and PAR2 both activates PI3K/AKT pathway. Furthermore, we added ZSTK474 into PC3 cells with AC245100.4 overexpression, and then detected the cell viability, clone formation ability and the expression of p-AKT in PC3 cells. As was shown in Fig. 4E, F and G, ZSTK474 reversed the role of AC245100.4 overexpression on increasing PCa cell viability, the numbers of colony and p-AKT protein levels (Fig. 4E, F and G). These data manifested that AC245100.4 has the promotion of PCa cell proliferation by activating PI3K/AKT pathway. We also added ZSTK474 into PC3 cells with PAR2 overexpression, and then detected the cell viability in PC3 cells. We found that ZSTK474 reversed the role of PAR2 overexpression on increasing PCa cell viability (Fig. S1). These data manifested that PAR2 also has the promotion of PCa cell proliferation by activating PI3K/AKT pathway. We next transfected pcDNA3.1-*AC245100.4* and si-*PAR2* into PCa cells and performed Western blot in these cells. The results showed PAR2 knockdown reversed effects of AC245100.4 overexpression on increasing p-AKT protein levels (Fig. 4H). Altogether, we found that AC245100.4 regulates the PAR2/PI3K/AKT axis in prostate cancer cells.

4. Discussion

Long non coding RNAs (lncRNAs) are a series of RNAs that do not have the protein-coding ability and participate in regulating gene expression [17]. Recently, numerous studies have indicated that the dysregulated expression of numerous lncRNAs have close connections to many cancers, including prostate cancer [18,19]. At the moment, there is an urgent need for new methods to treat PCa, and regulating the intracellular abundance of PCa-associated lncRNAs could be one of them. Our previous study predicted AC245100.4 , a novel lncRNA, closely related to the development of prostate cancer, and confirmed that AC245100.4 can combine with HSP90 to affect cell proliferation [12], but how it affects cell proliferation through regulation of downstream target genes and the related mechanisms are unclear. Further studies had also shown that AC245100.4 can affect the migration of prostate cancer cells by regulating PAR2 [11]. However, the effect and mechanism of AC245100.4/PAR2 axis on the proliferation of prostate cancer cells have not been explored. The present study demonstrated that AC245100.4 affects PCa cell proliferation, and PAR2 as a downstream target of AC245100.4 also affects PCa cell proliferation. Mechanically, AC245100.4 activates the PI3K/AKT pathway via elevating PAR2.

Tumorigenesis is a biologically complex process. In the process of malignant transformation, cells and their microenvironment must acquire a variety of biological characteristics that are conducive to the malignant growth of cells, and maintenance of cell proliferation is one of the crucial biological characteristics [20]. Previous research has found that numerous lncRNAs are involved in the regulation of cancer cell proliferation. For instance, lncRNA-422 suppresses the proliferation and growth of colorectal cancer cells by targeting SFPQ [21]; lncRNA RPSAP52 promotes cell proliferation via modulating miR-665/STAT3 in gastric cancer [22]; lncRNA UBE2R2-AS1, as prognostic marker, promotes cell proliferation and EMT in prostate cancer [23]. In this study, we found that AC245100.4 is highly expressed in PCa tissues and cells, and affects cell proliferation.

Previous reports have demonstrated that PAR2 was related to carcinogenesis and has been promoted as a potential therapeutic target [9]. For instance, PAR2 promotes colorectal cancer self-renewal and metastasis via β-catenin and periostin [24]; PAR2 enhances PCa cell proliferation [25]; PAR2 promotes HCC cell proliferation and metastasis by inducing EMT [26]. In our study, differentially expression of PAR2 has been observed in numerous cancers, including PCa. We analyzed the data from two databases (GEPIA2 and GEO), and found that PAR2 is highly expressed in PCa tissues. Then, we also found that PAR2 is highly expressed in prostate cancer cells. In addition, we further discovered that PAR2 affects PCa cell proliferation, and PAR2 expression is positively correlated with Cyclin D1 and PCNA.

By regulating gene expression through various mechanisms, lncRNAs play a vital role in cancer progression. For instance, lncRNA SNHG17 could facilitate ESCC tumor growth by promoting the expression of c-Myc [27]; lncRNA ZFPM2-AS1 promotes the proliferation of gastric cancer cells by regulating MIF [28]. Previous research has shown that PAR2 is the downstream target of AC245100.4 [11]. In the present study, we used Western blot to re-verified the target relationship between AC245100.4 and PAR2, and the results are in agreement with the previous findings. Furthermore, the data of CCK8 assay and clonal formation assay showed that PAR2 knockdown reversed effects of AC245100.4 overexpression on increasing PCa cell viability and the numbers of colony. The results showed that AC245100.4 enhanced PCa cell proliferation by regulating PAR2.

The dysregulation of lncRNAs greatly contributes to the progression of numerous tumors via specific signaling pathways [29]. The RAP-MS was performed to screen out proteins that interact with AC245100.4 and we found that these proteins were enriched in 28 signaling pathways, including PI3K/AKT pathway [12]. The PI3K/AKT pathway plays a vital regulatory function in carcinogenesis by regulating cell proliferation and other processes [30]. In our current study, p-AKT expression is found to be positively correlated with AC245100.4 level. These results showed that AC245100.4 can activate PI3K/AKT pathway, which are in accord with the result of RAP-MS. Since above results confirmed that AC245100.4, which activated PI3K/AKT pathway, also promoted PCa cell proliferation, it is rational to investigate whether AC245100.4 affects the proliferation of PCa cells by regulating the PI3K/AKT pathway. ZSTK474 is a PI3K inhibitor that inhibits PI3K/AKT pathway activation [31]. To validate this conjecture, we added ZSTK474 into PC3 cells with AC245100.4 overexpression and detected the cell proliferation and phosphorylation level of PI3K/AKT pathway. The rescue experiment showed that AC245100.4 has promotion on cell proliferation via activating PI3K/AKT pathway in PCa, revealing a novel regulatory mechanism for AC245100.4 on PCa cell proliferation.

According to the report, PAR2 could promote 3T3-L1 preadipocyte differentiation through activation of the PI3K/AKT pathway

[16]. However, whether PAR2 activates PI3K/AKT in prostate cancer has not been reported. In this study, we found that ZSTK474 has a better effect on prostate cancer patients with high PAR2 expression, and observed the significant positive correlation between PAR2 and PI3K/AKT pathway. Next, Western blot was used to verify the relationship between PAR2 and PI3K/AKT pathway. We also added ZSTK474 into PC3 cells with PAR2 overexpression and detected the cell proliferation. The rescue experiment showed that PAR2 has promotion on cell proliferation via activating PI3K/AKT pathway in PCa, and it was the first time that PAR2 is found to promote PCa cell proliferation via activating PI3K/AKT pathway. In the above study, we have found that the functions of AC245100.4 in PCa cells include regulating the expression of PAR2 and activating the PI3K/AKT pathway, and we also found that PAR2, as a downstream target gene of AC245100.4, can promote PCa cell proliferation via activating PI3K/AKT pathway. Based on the above conclusions, we are of the opinion that PAR2 may act as a key node in the process of AC245100.4 activating PI3K/AKT pathway. That is, AC245100.4 may activate PI3K/AKT pathway by regulating PAR2. Here, we found that PAR2 knockdown reversed effects of AC245100.4 overexpression on increasing p-AKT protein levels. These results showed that AC245100.4 activates the PI3K/AKT pathway by regulating PAR2.

It has been found that PAR2 is usually activated by PAR2 agonists such as serine protease, and thus plays a role in activating PI3K/AKT pathway [32]. However, our initial observation when starting this study was that PAR2 is highly expressed in prostate cancer tissues compared to normal tissues. Therefore, we designed the experiment by altering the expression of PAR2 to explore its role in prostate cancer cell proliferation，and we think it will be more appropriate to explore the role of PAR2 expression in PCa in our study. In the study, we found that AC245100.4 can affect the proliferation of prostate cancer cells by activating the PI3K/AKT pathway by increasing the expression of PAR2. Mechanistically, we think that the activation of the PI3K/AKT pathway by AC245100.4 may be partially achieved by indirectly increasing the activation level of PAR2, and the increase in PAR2 activation level is due to the overall increase in PAR2 expression. The role of PAR2 activation in PCa cell proliferation is likely only a part of its functional contribution, and other mechanisms that have not been elucidated yet may also be involved. One limitation of our study is that we have not explored whether AC245100.4 directly promotes PAR2 activation. Because we did not consider the role of a wide range of potential PAR2 agonists in PCa in our study, in future studies we plan to the investigate potential relationship of PAR2 agonists to the AC245100.4/PAR2/PI3K/AKT axis in vitro and in vivo. We think that these future studies will provide a more complete understanding of the specific mechanism of AC245100.4 regulating PAR2 in PCa. In conclusion, although we did not investigate the effect of AC245100.4 on PAR2 activation, we have demonstrated that AC245100.4 can exert its effects by elevating the expression of PAR2.

5. Conclusion

In summary, the present study showed a novel mechanism by which AC245100.4 regulates the proliferation of prostate cancer cells, and that is AC245100.4 promotes the proliferation of prostate cancer cells by positively regulating PAR2 to activate PI3K/AKT pathway. Thus, we provide the innovative evidence of the possibility of AC245100.4 and PAR2 as the potential therapeutic targets for PCa.

Declarations

Data availability

On reasonable request, the corresponding author will provide the data that support the conclusions of this study.

Author contributions

Ke Zhang: Performed the experiments, Wrote the paper.
Chi Liu: Analyzed and interpreted the data, Performed the experiments.
Changbin Hu; Ping Lin: Analyzed and interpreted the data, Wrote the paper.
Huizhen Jia: Performed the experiments, Wrote the paper.
Qi Qi: Performed the experiments.
Jiebing Tang: Conceived and designed the experiments, Performed the experiments, Wrote the paper.
Xiaoguang Yu: Wrote the paper, Contributed reagents, materials, analysis tools or data.

Ethical approval

N/A.

Data availability

On reasonable request, the corresponding author will provide the data that support the conclusions of this study.

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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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e16870.

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