Original Article

Patchouli alcohol inhibits GPBAR1-mediated cell proliferation, apoptosis, migration, and invasion in prostate cancer

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Background: G protein-coupled bile acid receptor 1 (GPBAR1) is a G protein-coupled receptor for bile acids, which is widely expressed in many human tissues. Patchouli alcohol (PA) has been shown to have an anti-cancer effect, including in prostate cancer (PCa). This study sought to confirm the regulatory mechanism of GPBAR1 in the anti-cancer activity of PA in PCa.

Methods: The SwissTargetPrediction website (Pro >0) was used to predict the target of PA. The UALCAN and The Cancer Genome Atlas-Prostate cohort was used to examine the differentially expressed genes and PCa recurrence. A gene set enrichment analysis (GSEA) was conducted to analyze the relationship between the expression of GPBAR1 and PCa proliferation, migration, and invasion. Cell proliferation, migration, and invasion were assessed by colony formation, 5-Ethynyl-2'-deoxyuridine staining, cell scratch assays, and Transwell invasion assays, respectively. A xenograft animal model was established to assess the effect of PA on tumor growth *in vivo*. GPBAR1 protein and apoptosis related protein expression was measured by western blot.

Results: GPBAR1 was a PA target predicted by the SwissTargetPrediction website. PA inhibited the expression of GPBAR1 in PCa cells in a time- and dose-dependent manner. The abnormal expression of GPBAR1 was related to cell proliferation, migration, and invasion. Additionally, GPBAR1 overexpression promoted the cell proliferation, migration, and invasion, and inhibited the apoptosis of PCa cells. GPBAR1 silencing inhibited the cell proliferation, migration, and invasion, and promoted the apoptosis of PCa cells. High expressions of GPBAR1 suppressed tumor growth in tumor-bearing mice. Further, GPBAR1 promoted the activation of nuclear factor kappa B (NF-κB) signaling, and PA regulated the malignant phenotypes of PCa cells via the NF-κB signaling pathway mediated by GPBAR1.

Conclusions: GPBAR1 is a promising drug target of PA, and was shown to regulate the proliferation, apoptosis, migration, and invasion of PCa cells through GPBAR1/NF-κB inhibition.

Keywords: Patchouli alcohol; G protein-coupled bile acid receptor 1 (GPBAR1); prostate cancer (PCa); tumorigenesis; nuclear factor kappa B (NF-κB)

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Introduction

Prostate cancer (PCa) is a genitourinary systemic malignancy tumor, and the 2nd most common cancer in men (1,2). The mortality and morbidity rates of PCa continue to increase each year (2). In 2021, PCa accounted for 10% of male cancer-related deaths; thus, PCa represents a major challenge for men's health (3). Metastasis is the most lethal clinical manifestation of PCa, and PCa has a

5-year overall survival rate of only 30% (1). PCa has no obvious symptoms at its early stages, which makes early detection and prevention difficult (4). Due to the high sensitivity of PCa cells to the androgen pathway, androgen deprivation therapy (ADT) is the most common treatment for PCa (5). However, PCa patients face the survival burden of metastasis and recurrence. Thus, it is essential that the underlying mechanisms of PCa be elucidated to enable the development of new therapeutic strategies, including the identification of key biomarkers.

G protein-coupled receptors (GPCRs) are the largest superfamily of receptors and are involved in almost all signaling processes in human physiology. Currently, GPCRs are drug-based therapeutic targets for multiple diseases, as they represent approximately 30% of all drug targets (6). Bile acids (BAs) are closely related to the tumor development process. The G protein-coupled bile acid receptor 1 (GPBAR1) is a GPCR that was first discovered in 2002 (7) and is activated by both primary and secondary BAs (7,8). GPBAR1 is expressed not only in the gallbladder and intestinal epithelium but also in other tissues of the human body. For example, GPBAR1 is expressed in cardiomyocytes, and GPBAR1 activation significantly improves cardiac hypertrophy and heart failure (9,10). Further, GPBAR1 alleviates the associated inflammatory response caused by hepatic ischemia-reperfusion injury and inhibits hepatocyte apoptosis (11). GPBAR1 is involved in metabolic regulation, cell survival, proliferation, and apoptosis *in vitro* (12,13). GPBAR1 is overexpressed in multiple types of cancer (14-17), but has not yet been examined in PCa.

Previous research has shown that patchouli alcohol (PA) inhibits the cell malignant progression of PCa (18). The gene set enrichment analysis (GSEA) predicted that GPBAR1 affects the proliferation, migration, and invasion of PCa, and the network pharmacology analysis showed that GPBAR1 might be a potential target of PA. We hypothesized that PA could restrain the proliferation, migration, and invasion of PCa by regulating GPBAR1. In this study, we sought to test this hypothesis and explore what is the mechanism of action. We present the following article in accordance with the ARRIVE reporting checklist (available at https://tau.amegroups.com/article/view/10.21037/tau-22-667/rc).

Highlight box

Key findings

- GPBAR1 promoted PCa progression, and PA restrained PCa progression through GPBAR1/NF-κB inhibition.

What is known and what is new?

- PA inhibits the cell malignant progression of PCa.
- GPBAR1 might be a potential target of PA.

What is the implication, and what should change now?

- Biomarkers and mechanisms of PCa need to be further explored.

Methods

TargetNet and SwissTarget analysis

The PA spatial data format (SDF) file from the Pubchem database was loaded into TargetNet (http://targetnet.scbdd.com/) and SwissTargetPrediction (http://www.swisstargetprediction.ch/) websites. All the parameters were set to the default values, and the protein targets identified by the 2 software programs were allowed to overlap. Consequently, the overlap can be regarded as a pre-selection target for further studies.

UALCAN online analysis

The UALCAN (http://ualcan.path.uab.edu/analysis.html) website was used to analyze the upregulated genes in The Cancer Genome Atlas-Prostate Adenocarcinoma (TCGA-PRAD). Based on the TCGA-PRAD cohort and clinical information, the target genes were screened. The study was conducted in accordance with the Declaration of Helsinki (2013 revision).

Gene set enrichment analysis (GSEA)

A GSEA was conducted on the messenger ribonucleic acid (RNA) data in TCGA-PRAD. The relationship between GPBAR1 expression and the malignant cell phenotypes of PCa was analyzed.

Cell culture

The human PCa cell lines PC-3 and DU145 were obtained from ATCC (Maryland, USA). PC-3 and DU145 were cultured in Roswell Park Memorial Institute medium-1640 (Gibco, NY, USA) with 10% fetal bovine serum (FBS; HyClone, UT, USA) in a humidity control incubator with 5% carbon dioxide (CO_2) at 37 ℃.

Cell transfection

The GPBAR1 open reading frame was cloned into recombinant plasmid. pFLAG-CMV (pFLAG-GPBAR1) was constructed from Genepharma (Shanghai, China). Lentivirus-mediated short-hairpin RNA (shRNA) targeted GPBAR1 (shRNA1# or shRNA2#) were synthesized from GeneChem (Shanghai, China). pFLAG-GPBAR1, shGPBAR11#, or shGPBAR1# were transfected into the PC-3 or DU145 cells using Lipofectamine™ 3000 (Thermo Fisher, DE, USA).

CCK-8 assays

PC-3 or DU145 cell suspension (100 µL) was seeded in a 96-well plate and incubated for 24 h with 5% CO_2 at 37 ℃. Next, 10 µL of Cell Counting Kit-8 (CCK-8) (Beyotime, Shanghai, China) solution was added to each well (avoiding bubbles). The plate was incubated in an incubator for 1–6 days. The absorbance at 450 nm was detected using a microplate reader (Thermo Fisher Scientific, USA).

Colony formation assays

The basic agar supplemented with 0.6% agarose was prepared in a 6-well plate (NEST, Wuxi, China). The PC-3 and DU145 cells were transfected with pFLAG-GPBAR1, shRNA1#, or shRNA1# for 24 h each. The cell medium was replaced with fresh medium every 2 days for 14 days with 5% CO_2 at 37 ℃. The cells were fixed with 4% formaldehyde for 20 min and stained with 0.1% crystal violet (Macklin, Shanghai, China). The colony (≥25 cells) images were obtained using a light microscopes (Nikon, Tokyo, Japan).

5-EdU staining

The PC-3 and DU145 cells were added to a 24-well plate (1×10^5 cells/well) for 12 h. The cells were fixed with 4% paraformaldehyde at room temperature for 15 min. Next, 100 µL of ethynyl-2'-deoxyuridine (EdU) solution was added to the corresponding well and incubated for 30 min at room temperature in darkness. Images were captured with an inverted fluorescence microscope (Olympus, Tokyo, Japan).

Cell scratch assays

The PC-3 and DU145 cells transfected with pFLAG-GPBAR1 or shRNA were seeded into a 6-well plate (NEST, Wuxi, China). When the cells reached about 90% fusion, the cells were scraped directly using a pipette tip at the center of the plate bottom. The scratches at 0 h or 24 h were measured, and cell mobility was examined.

Transwell invasion assays

The serum-free medium was added to the upper chamber coated with Matrigel (Corning, NY, USA) and then left for 30 min at room temperature. After 24 h, the cell suspension (1×10^5 cells/well) was transferred to the upper chambers, and 600 µL of medium supplemented with 10% FBS (HyClone, UT, USA) was added to the lower chamber for 48 h at 37 ℃. The non-invasive cells were wiped with a sterile cotton swab. Finally, the cells were fixed with 95% ethanol and then stained with 0.1% crystal violet (Macklin, Shanghai, China). The cell invasion number was counted by a light microscope (Nikon, Tokyo, Japan).

Xenograft model

All the animal studies were authorized by the Ethics Committee of Chengdu University of Traditional Chinese Medicine (No. 2022DL-009) and were conducted according to the declaration of the Guide for the Care and Use of Laboratory Animals of the National Research Council (8th Edition, 2011). The PC-3 cells (1×10^6 cells) that had been transfected with pFLAG-GPBAR1 or the vector were then subcutaneously injected into the female BALB/c nude mice (20.63±5.37 g, 4-week-old), and the mice were randomly assigned to the pFLAG-GPBAR1 and vector groups (n=6). Tumor size and weight were measured every 2 days for 3 weeks. A protocol was prepared before the study without registration.

Immunohistochemistry

The tumor tissues of the nude mice were fixed with 10% formalin and embedded in paraffin, and the 4 µm-thick slices were dewaxed and rehydrated. Next, the slices were placed in phosphate buffered solution containing 30% hydrogen peroxide for 30 min and repaired in heated citric acid buffer for 10 min. Subsequently, the samples were incubated with anti-Ki67 and anti-GPBAR1 at 4 ℃ overnight. Afterwards, the samples were incubated with the corresponding secondary antibodies at 37 ℃ for 1 h. Finally, the slices were visualized using a 3,3'-Diaminobenzidine (DAB) Kit (Solarbio, Beijing, China), and the number

of positive cells was observed and counted by an optical microscope (Nikon, Tokyo, Japan).

Western blot

The total protein of the PC-3 or DU145 cells were extracted with ice-cold radioimmunoprecipitation assay buffer (Sigma, Missouri, USA). Next, the sample concentration was quantified using a bicinchoninic acid assay kit (Beyotime, Shanghai, China). The steps followed were described previously (12). Finally, the enhanced chemiluminescence reagent (Beyotime, Shanghai, China) was used to visualize the target band.

Statistical analysis

An unpaired t-test with Welch's correction was employed to evaluate the endpoints using GraphPad Prism 5.0 (GraphPad Software, CA, USA). All the statistical tests were 2-sided, and a P value <0.05 was considered significant.

Results

Network pharmacology analysis of in PCa

We used the TargetNet and SwissTargetPrediction websites to predict the targets of PA and obtained 20 PA targets (see *Figure 1A*). The UALCAN website was used to assess the upregulated genes in TCGA-PRAD, and the results revealed the upregulation of 3 genes, including GPBAR1 (see *Figure 1B*). We observed that the high expression of GPBAR1 was associated with the recurrence of PCa (see *Figure 1C*). The PCa patients were grouped into high and low expression groups based on the median expression of GPBAR1. The GSEA showed that the expression of GPBAR1 was associated with cell proliferation, migration, and invasion (see *Figure 1D-1F*). Further, we observed that PA inhibited the expression of GPBAR1 protein in a time- and dose-dependent manner (18) in the PC-3 and DU145 cells (see *Figure 1G*,*1H*).

Effect of GPBAR1 overexpression on cell proliferation, apoptosis, migration, and invasion

Next, we transfected the PC-3 and DU145 cells with pFLAG-GPBAR1 or the vector. As *Figure 2A* shows, the protein expression of GPBAR1 was significantly increased after pFLAG-GPBAR1 transfection, which confirmed that GPBAR1 overexpression was successfully established. As *Figure 2B*,*2C* show, GPBAR1 overexpression promoted the cell viability of the PC-3 and DU145 cells. The colony formation and EdU results indicated that GPBAR1 overexpression increased the cell proliferation of the PC-3 and DU145 cells (see *Figure 2D*,*2E*). As *Figure 2F* shows, GPBAR1 overexpression reduced the expression of cleaved-caspase-3 and Bax, and promoted Bcl-2 expression. Further, GPBAR1 overexpression stimulated the migration (see *Figure 3A*,*3B*) and invasion (see *Figure 3C*) of the PC-3 and DU145 cells.

Effect of GPBAR1 silencing on cell proliferation, apoptosis, migration, and invasion

Additionally, we transfected the PC-3 and DU145 cells with shGPBAR11#, shGPBAR1# or NC. As *Figure 4A* shows, the protein expression of GPBAR1 was significantly decreased after shRNA transfection, which confirmed that GPBAR1 silencing was successfully established. As *Figure 4B*,*4C* show, GPBAR1 silencing inhibited the cell viability of the PC-3 and DU145 cells. The colony formation and EdU assays indicated that GPBAR1 silencing also reduced the cell proliferation of the PC-3 and DU145 cells (see *Figure 4D*,*4E*). As *Figure 4F* shows, GPBAR1 silencing increased the expression of cleaved-caspase-3 and Bax, and decreased Bcl-2 expression. Further, GPBAR1 silencing restrained the migration (see *Figure 5A*,*5B*) and invasion (*Figure 5C*) of the PC-3 and DU145 cells.

GPBAR1 overexpression increased tumor growth in the tumor-bearing mice

As *Figure 6A-6C* shows, compared to the vector group, GPBAR1 overexpression significantly promoted the volume and size of the xenograft tumors. Additionally, GPBAR1 overexpression significantly increased the weight of the xenograft tumors (see *Figure 6D*). The positive cell number of Ki67 (see *Figure 6E*) and GPBAR1 (see *Figure 6F*) was significantly increased in the pFLAG-GPBAR1 group. These findings indicated that the overexpression of GPBAR1 promoted the tumor growth of PCa *in vivo*.

GPBAR1 regulated the NF-κB signaling pathway

The PCa patients were grouped into high and low

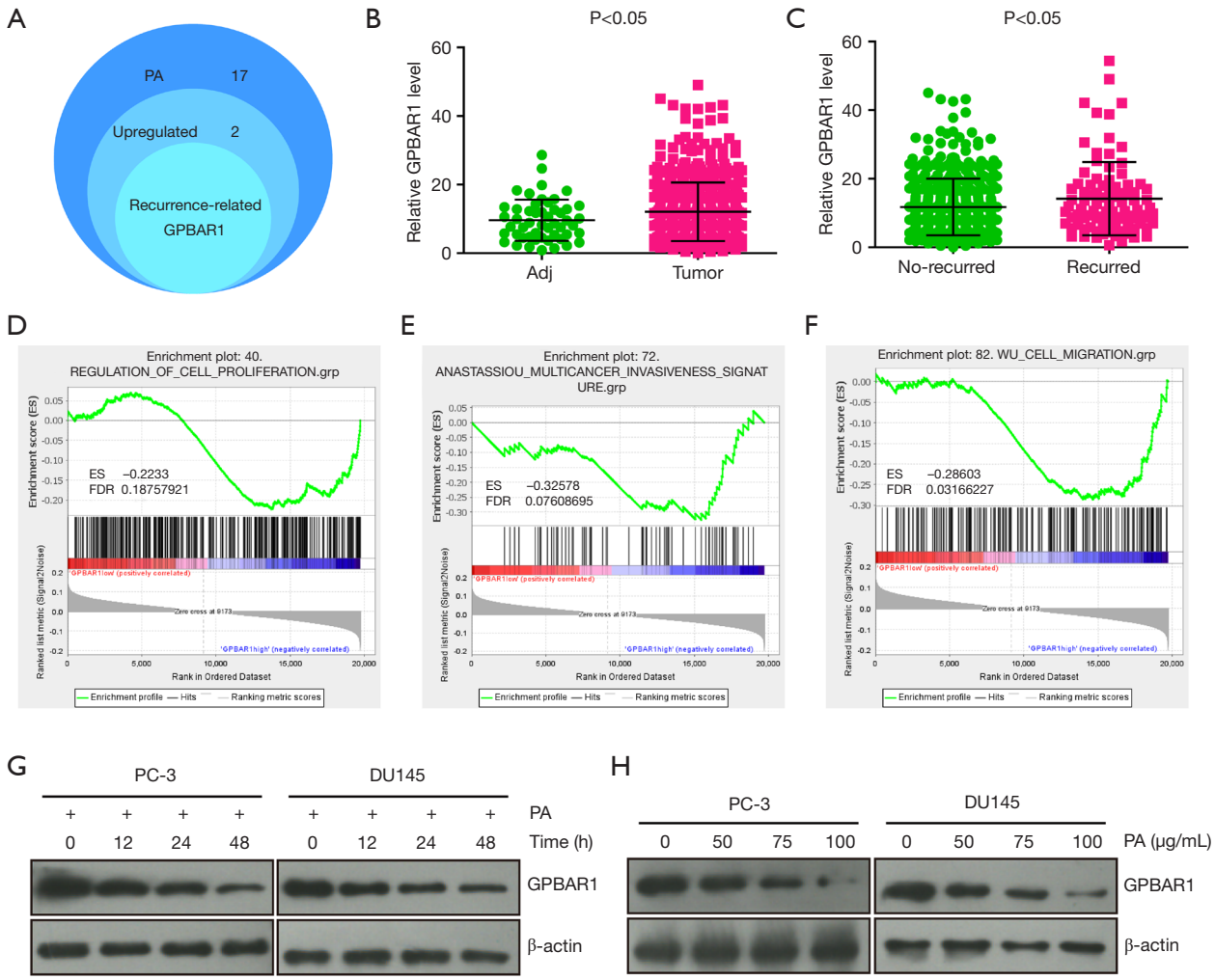

Figure 1 Network pharmacology analysis of PA targets in PCa. (A) The SwissTargetPrediction (pro >0) and TargetNet (pro >0) websites were employed to predict the targets of PA. (B) The expression of GPBAR1 in TCGA-PRAD. (C) The expression of GPBAR1 was correlated with the recurrence of PCa. (D-F) A GSEA was conducted to evaluate the relationship between GPBAR1 expression and PCa cell proliferation, migration, and invasion. (G-H) The relative protein expression of GPBAR1 in the PC-3 and DU145 cells were examined using western blot. P<0.05 was considered statistically significant. PA, patchouli alcohol; PCa, prostate cancer; TCGA-PRAD, The Cancer Genome Atlas Prostate Adenocarcinoma ; GPBAR1, G protein-coupled bile acid receptor 1; ES, enrichment score; FDR, false discovery rate.

expression groups based on the median expression of GPBAR1. The GSEA showed that the expression of GPBAR1 was associated with nuclear factor kappa B (NF-κB) activation (see *Figure 7A*). As *Figure 7B* shows, the overexpression of GPBAR1 significantly increased the phosphorylation of inhibitor of NF-κBα (IkBα) and NF-κB p65. Conversely, the silencing of GPBAR1 significantly reduced the phosphorylation of IkBα and p65 (see *Figure 7C*).

PA inhibited PCa progression through the GPBAR1/NF-κB pathway

As *Figure 8A* shows, the overexpression of GPBAR1 significantly reduced the phosphorylation of IkBα and increased p65 phosphorylation, and PA attenuated the effect of GPBAR1 on the phosphorylation of IkBα and p65. Additionally, GPBAR1 overexpression increased the cell proliferation of the PC-3 and DU145 cells, and PA rescued

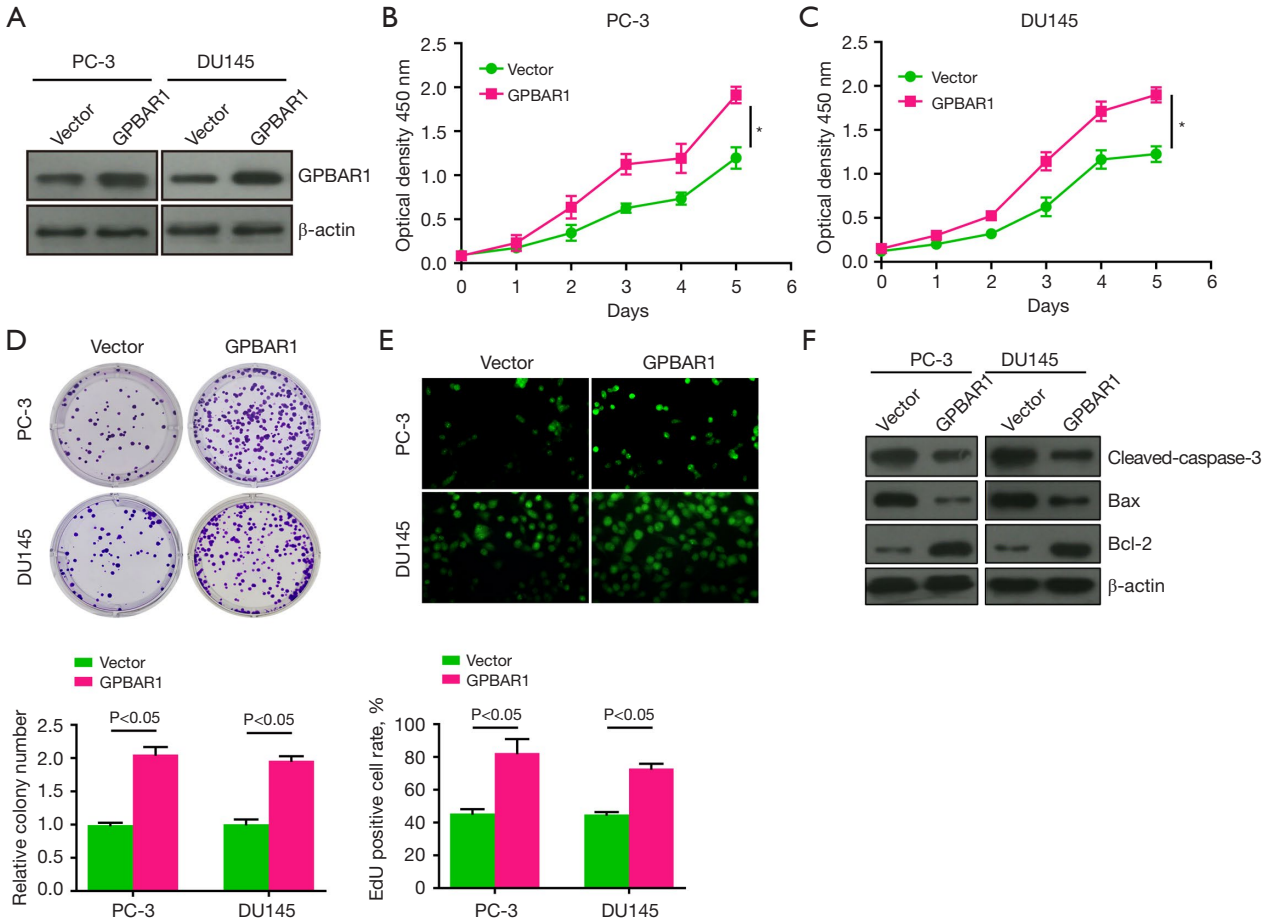

Figure 2 The effect of GPBAR1 overexpression on cell proliferation and apoptosis of PCa. (A) The relative protein expression of GPBAR1 in the PC-3 and DU145 cells was examined using western blot. (B,C) The cell viability of the PC-3 and DU145 cells was assessed using a CCK-8 kit. (D,E) The cell proliferation capacity of the PC-3 and DU145 cells was assessed using clone formation assays (40× magnification) and EdU staining. (F) The relative protein expression of cleaved-caspase-3, Bax, and Bcl-2 in the PC-3 and DU145 cells were examined using western blot. *P<0.05 *vs.* vector. PCa, prostate cancer; GPBAR1, G protein-coupled bile acid receptor 1; CCK-8, cell counting kit-8; EdU, 5-Ethynyl-2'-deoxyuridine.

GPBAR1-induced PCa cell proliferation (see *Figure 8B,8C*). As *Figure 8D* shows, GPBAR1 overexpression inhibited the apoptosis of the PC-3 and DU145 cells, which was rescued by PA treatment. Further, GPBAR1 overexpression promoted the migration and invasion ability of the PC-3 and DU145 cells, but PA prevented GPBAR1-induced PCa cell migration and invasion (see *Figure 8E-8G*).

Discussion

PCa is a heterogeneous malignancy that represents a serious threat to men's health. A variety of treatment methods exist, but the prevention and control of PCa progress is not ideal. The identification of potential biomarkers is important in risk identification and prediction in adjuvant therapy (19). BA is a cholesterol-derived atypical steroid that is considered a potential carcinogen. As a BA-specific cell membrane G protein-coupled receptor of BAs, the high expression of GPBAR1 may be a risk factor for cancers (16,17). Our GSEA showed that GPBAR1 expression was associated with PCa cell proliferation, migration, and invasion, and the high expression of GPBAR1 was associated with the recurrence of PCa. We hypothesized that abnormal GPBAR1 expression might be responsible for the occurrence of PCa, and that its expression may affect the prognosis of PCa patients.

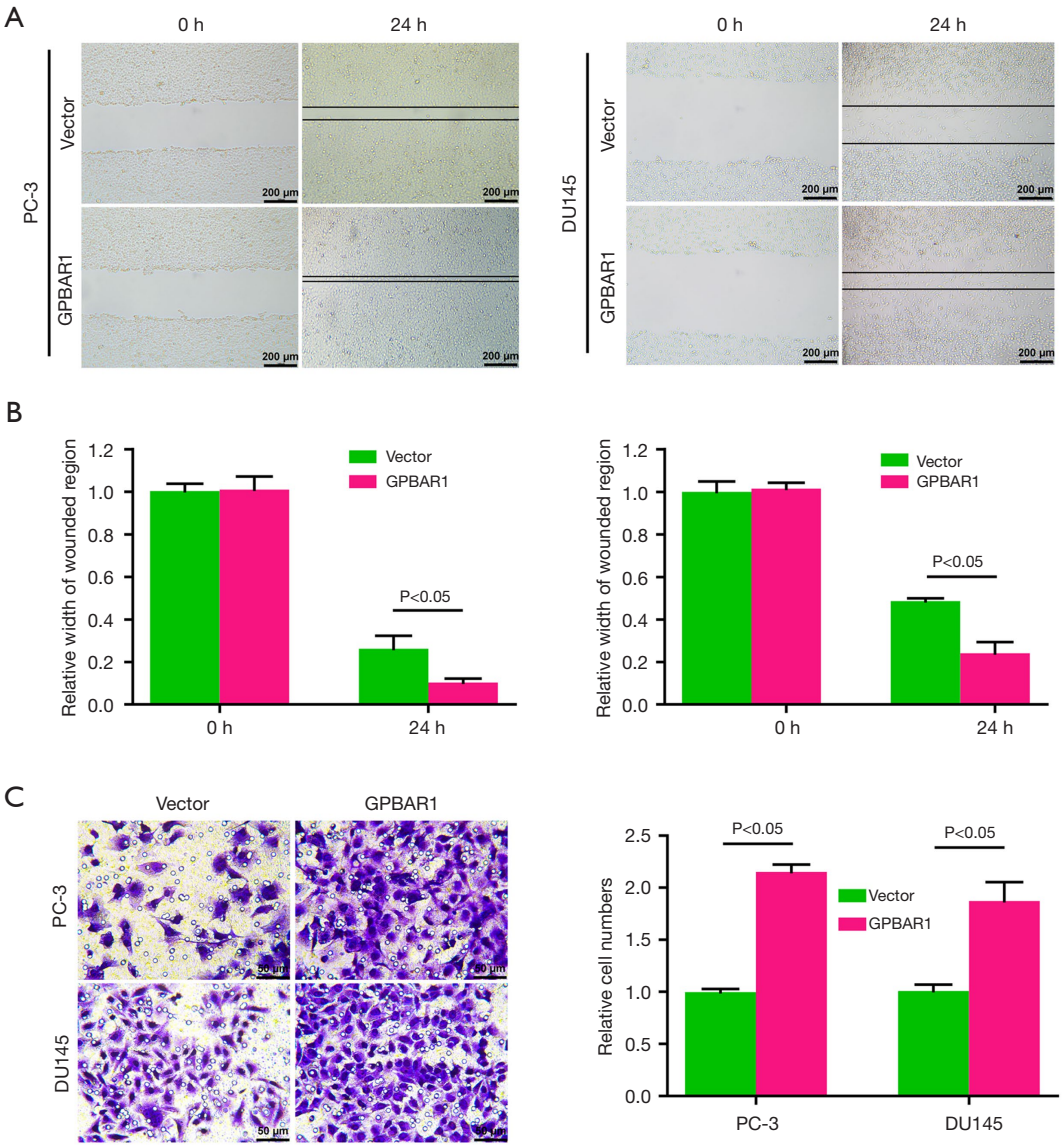

Figure 3 The effect of GPBAR1 overexpression on cell migration and invasion of PCa. (A,B) The migration capacity of the PC-3 and DU145 cells was assessed by cell scratch assays. Scale, 200 μm. (C) The invasion capacity of the PC-3 and DU145 cells was detected by Transwell assays and stained by 0.1% crystal violet. Scale, 50 μm. *P<0.05 vs. vector. PCa, prostate cancer; GPBAR1, G protein-coupled bile acid receptor 1.

Cancer cells are variable cells characterized by lethality, migration, and a loss of contact inhibition. *In vitro*, GPBAR1 is involved in the signaling pathways for cell metabolism, survival, and apoptosis, which suggests that GPBAR1 may play a role in tumor development (13). There is increasing experimental evidence that GPBAR1 promotes the cell proliferation and tumor growth of cancer cells (14,20), and is associated with a low survival rate (21).

In this study, we focused on the relationship between GPBAR1 and PCa. We overexpressed or silenced GPBAR1 in the human PCa cell lines PC-3 and DU145 to observe the effect of GPBAR1 on proliferation, apoptosis, migration, and invasion. We found that GPBAR1 overexpression significantly increased the cell proliferation, migration, and invasion, and inhibited the apoptosis of PCa cells, which further demonstrated that GPBAR1 accelerated PCa occurrence *in vitro*. Further, we established an animal xenograft tumor model *in vivo* and confirmed that GPBAR1

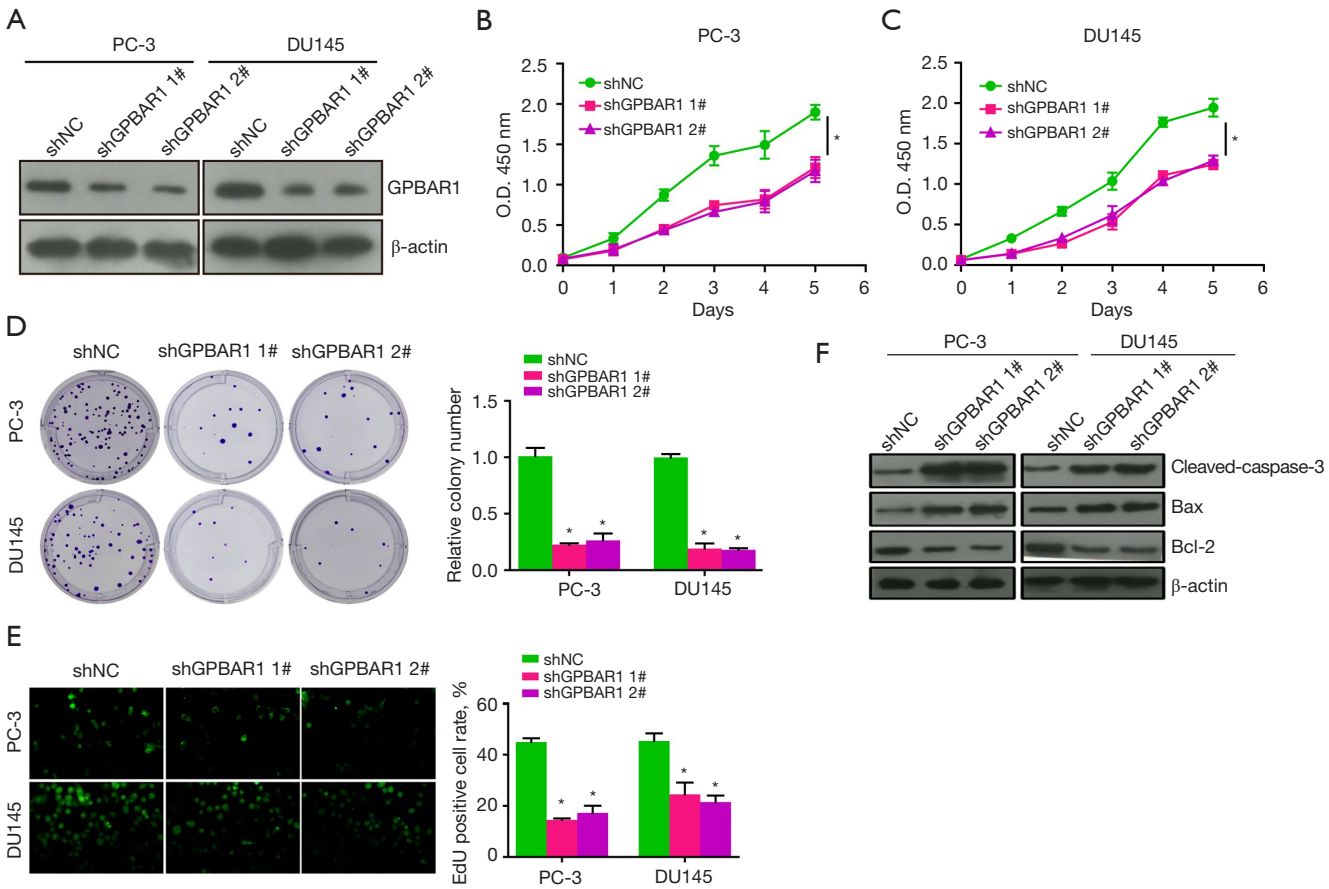

Figure 4 The effect of GPBAR1 silencing on cell proliferation and apoptosis of PCa. (A) The relative protein expression of GPBAR1 in the PC-3 and DU145 cells was examined using western blot. (B,C) The cell viability of the PC-3 and DU145 cells was assessed using a CCK-8 kit. (D,E) The cell proliferation capacity of the PC-3 and DU145 cells was assessed using clone formation assays (40× magnification) and EdU staining. (F) The relative protein expression of cleaved-caspase-3, Bax, and Bcl-2 in the PC-3 and DU145 cells were examined using western blot. *P<0.05 *vs.* vector. PCa, prostate cancer; GPBAR1, G protein-coupled bile acid receptor 1; CCK-8, cell counting kit-8; EdU, 5-Ethynyl-2'-deoxyuridine.

overexpression significantly promoted the xenograft tumor growth. These results indicated that GPBAR1 has a significant tumor-promoting effect on PCa, which is a novel marker for the treatment and prognosis of PCa. To the best of our knowledge, this study is the first to reveal an association between GPBAR1 protein expression and PCa progression.

PA is a natural bioactive ingredient isolated from patchouli, and it has been proven to have anti-oxidant (22), anti-inflammatory (23), and anti-tumor (24,25) activities. However, very few reports have examined the anti-cancer activity of PA and potential mechanisms of PCa. Our previous research demonstrated that PA inhibited the cell proliferation, migration, and invasion, and induced the apoptosis of PCa cells (18). In the present study, network pharmacology predicted that GPBAR1 was a PA target. PA inhibited the expression of GPBAR1 in PCa cells in a time- and dose-dependent manner. Further, GPBAR1 overexpression increased the cell proliferation, migration, and invasion of PCa cells. These findings suggested that PA suppressed PCa progression by regulating GPBAR1 expression.

NF-κB signaling is present in almost all animal cells that are involved in the cellular response to external stimuli. The dysregulation of NF-κB can cause autoimmune diseases, chronic inflammation, and a variety of cancers. Studies have shown that the inhibition of NF-κB activity reduces the proliferation and metastatic capacity of cancer cells

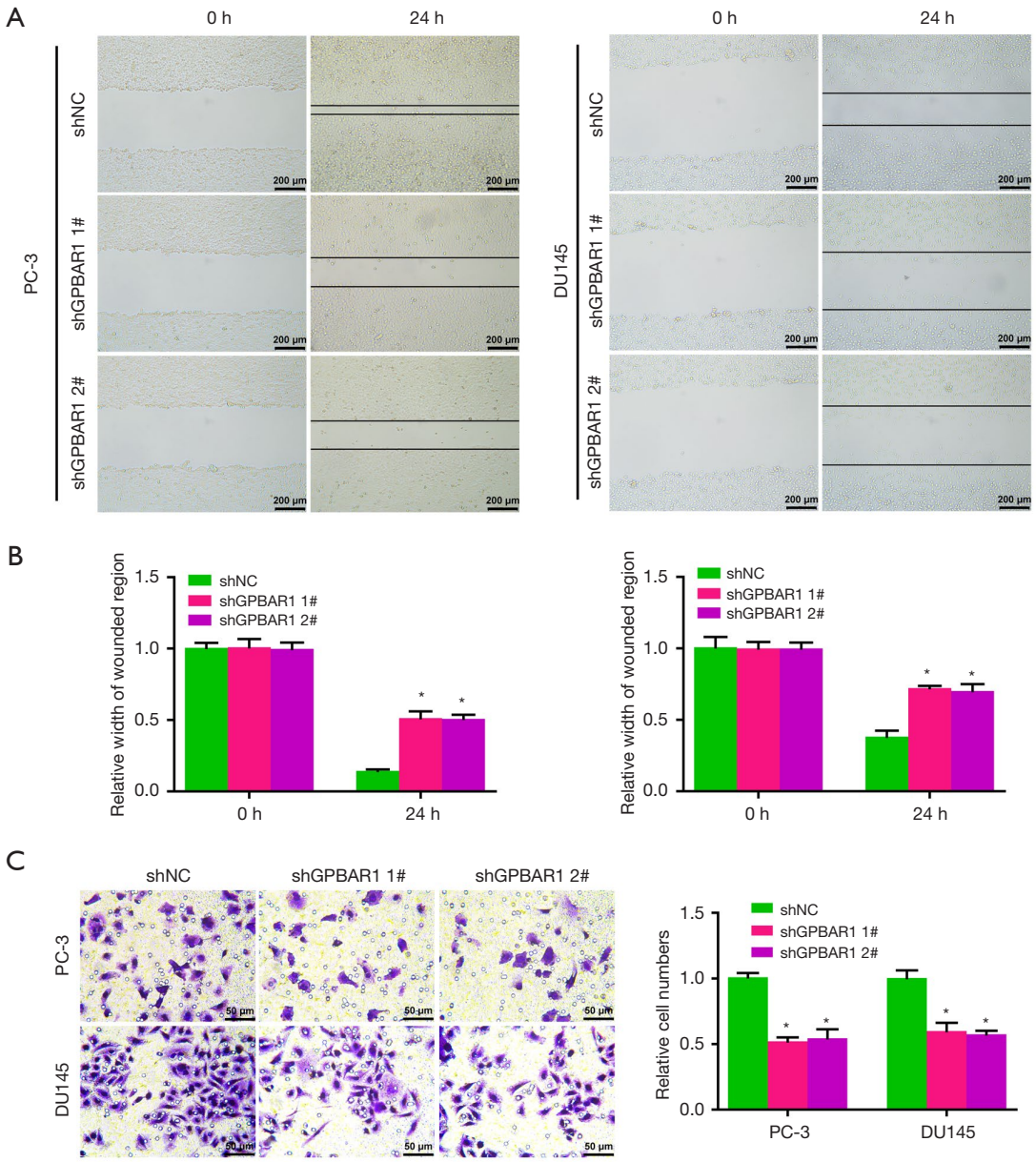

Figure 5 The effect of GPBAR1 silencing on cell migration and invasion of PCa. (A,B) The migration capacity of the PC-3 and DU145 cells was assessed by cell scratch assays. Scale, 200 μm. (C) The invasion capacity of the PC-3 and DU145 cells was detected by Transwell assays and stained by 0.1% crystal violet. Scale, 50 μm. *P<0.05 vs. vector. PCa, prostate cancer; GPBAR1, G protein-coupled bile acid receptor 1; CCK-8, cell counting kit-8; EdU, 5-Ethynyl-2'-deoxyuridine.

in vivo (26). During the development and progression of PCa, NF-κB/p65 is constitutively activated, and the mechanism involves an increased phosphorylation of inhibitor of NF-κB (IκB) protein (27,28). In this study, we found GPBAR1 inhibited the phosphorylation of IkBα, which promoted p65 phosphorylation release *in vivo* and *in vitro* (29). We previously showed that PA induced PCa cell apoptosis by inhibiting NF-κB activity (18). Together, these findings indicate that PA inhibits PCa progression through the GPBAR1/NF-κB pathway.

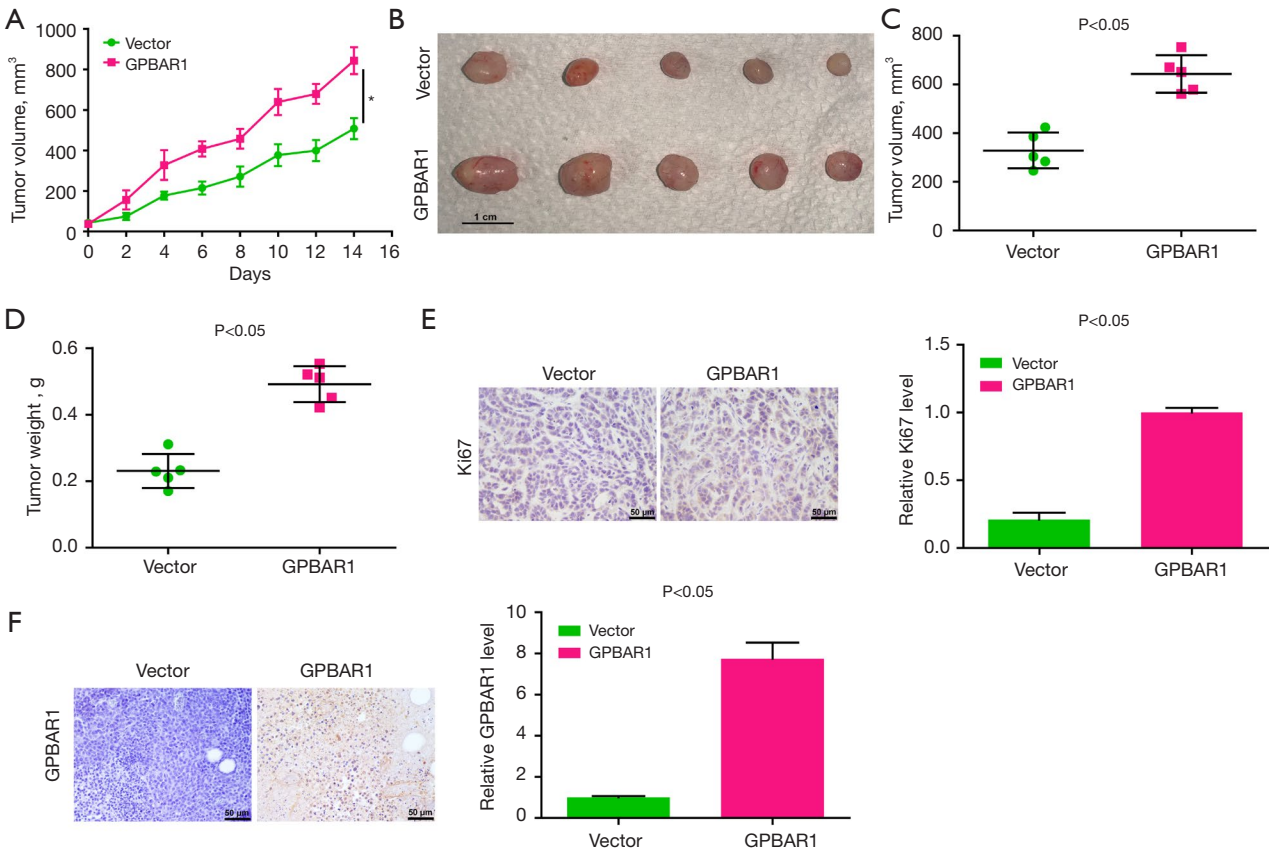

Figure 6 The effect of GPBAR1 overexpression on tumor growth in tumor-bearing mice. (A-C) The volumes of the xenograft tumors were calculated. (D) The weights of the transplanted tumors were calculated. (E,F) The positive number of Ki67 and GPBAR1 were calculated by immunohistochemistry assays. Scale, 50 μm. *P<0.05 *vs.* vector. GPBAR1, G protein-coupled bile acid receptor 1.

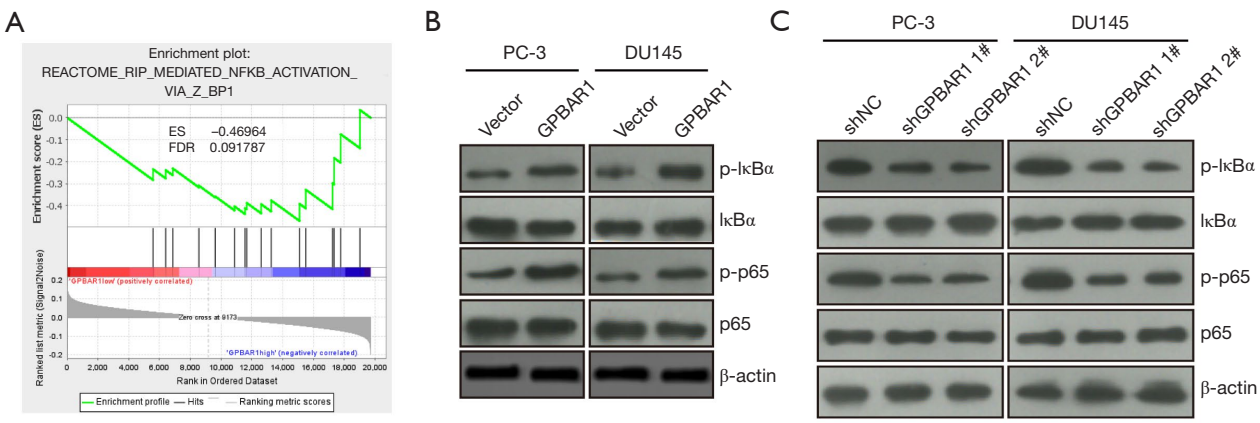

Figure 7 The effect of GPBAR1 overexpression on the NF-κB signaling pathway. (A) A GSEA was conducted to evaluate the relationship between GPBAR1 expression and NF-κB activation. (B) The relative protein expression of IkBα and p65 in the PC-3 and DU145 cells transfected with pFLAG-GPBAR1. (C) The relative protein expression of GPBAR1 in the PC-3 and DU145 cells transfected with shGPBAR1# or shGPBAR2#. GPBAR1, G protein-coupled bile acid receptor 1; GSEA, gene set enrichment analysis; IkBα, inhibitor of NF-κBα; ES, enrichment fraction; FDR, false discovery rate.

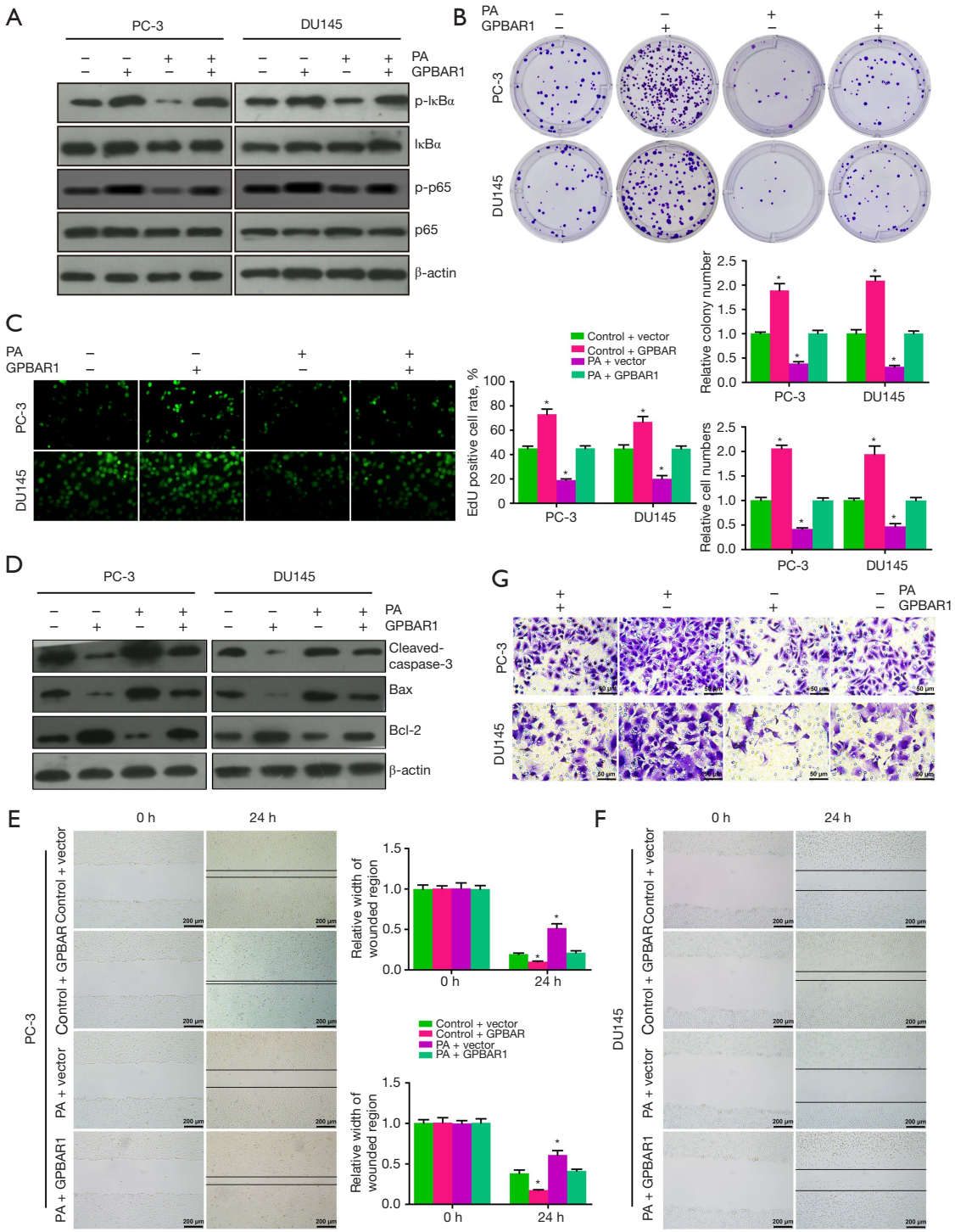

Figure 8 PA regulated the GPBAR1/NF-κB pathway. (A) The relative protein expression of IkBα and p65 in the PC-3 and DU145 cells was examine using western blot. (B,C) The cell proliferation of the PC-3 and DU145 cells was assessed using clone formation assays (40× magnification) and EdU staining. (D) The relative protein expression of cleaved-caspase-3, Bax, and Bcl-2 in the PC-3 and DU145 cells were examined using western blot. (E,F) The migration capacity of the PC-3 and DU145 cells was assessed by cell scratch assays. Scale, 200 μm. (G) The invasion capacity of the PC-3 and DU145 cells was assessed using Transwell assays. Scale, 50 μm. *P<0.05 *vs.* control + vector. GPBAR1, G protein-coupled bile acid receptor 1; IkBα; inhibitor of NF-κBα; EdU, 5-Ethynyl-2'-deoxyuridine.

Conclusions

The present study showed that GPBAR1 promoted the cell proliferation, migration, and invasion, and inhibited the apoptosis of DU145 and PC-3 cells, and GPBAR1 promoted p65 phosphorylation by inhibiting IkBα *in vivo* and *in vitro*. Further, PA restrained PCa progression through GPBAR1/NF-κB inhibition. Our findings provide a novel biomarker for PA in the treatment of PCa and enrich pharmacological data.

Acknowledgments

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Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at https://tau.amegroups.com/article/view/10.21037/tau-22-667/rc

Data Sharing Statement: Available at https://tau.amegroups.com/article/view/10.21037/tau-22-667/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tau.amegroups.com/article/view/10.21037/tau-22-667/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (2013 revision). All the animal studies were authorized by the Ethics Committee of Chengdu University of Traditional Chinese Medicine (No. 2022DL-009) and were conducted according to the declaration of the Guide for the Care and Use of Laboratory Animals of the National Research Council (8th Edition, 2011).

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