

Aurora kinase A promotes epithelial-mesenchymal transition by regulating P130 and P107 molecules in thyroid cancer cells

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Received August 23, 2024; Accepted January 20, 2025

DOI: 10.3892/etm.2025.12843

Abstract. The mortality associated with thyroid cancer (THCA) has been increasing due to distant metastasis, yet the precise mechanisms remain unclear. The present study examined the role of Aurora kinase A (AURKA) in THCA cells. Reducing AURKA expression led to decreased cell proliferation and inhibited the transition of BHT101 and BCPAP cells from the G0 phase to active division. Interestingly, decreasing AURKA expression also enhanced the cells' ability to move, migrate and invade. It was found that AURKA regulates key molecules involved in cell proliferation. Specifically, reducing AURKA expression increased the levels of P130 and E2F4, while decreasing the level of P107. Furthermore, upregulating AURKA promoted epithelial-mesenchymal transition (EMT), whereas downregulating AURKA had the opposite effect. Blocking the focal adhesion kinase signaling pathway impaired the movement, migration and invasion capabilities of THCA cells, underscoring its crucial role in metastasis. In conclusion, AURKA promotes EMT by regulating P130 and P107, thereby facilitating the metastasis of THCA.

Introduction

Thyroid cancer (THCA) ranks among the most prevalent malignancies arising from the endocrine system (1), with a rising global incidence (2). Although the five-year survival rate for patients with THCA is notably high when treated with radioiodine, mortality rates escalate significantly due to lymph node and distant metastases (3). Consequently, elucidating the specific molecular mechanisms underlying THCA metastasis is of paramount importance.

Aurora kinase A (AURKA) is a member of the Aurora family of serine/threonine kinases, involved in cell division through the formation of bipolar mitotic spindles and the maintenance of chromosomal stability (4). A previous study demonstrated that AURKA promotes lymph node metastasis in papillary THCA by enhancing cofilin-1 activity (5). In our earlier research, the authors observed that AURKA reactivates dormant laryngeal squamous cell carcinoma (LSCC) cells; however, its role in THCA remains unclear (6).

Recent evidence indicated that epithelial-mesenchymal transition (EMT) serves as a primary mechanism for cancer metastasis in breast (7), gastric (8), pancreatic (9) and colorectal cancers (10), among others. EMT is the process by which epithelial cells de-differentiate into mesenchymal-like derivatives. A previous study revealed that ISL1 targeting AURKA facilitates the development of neuroblastoma (NB), promoting EMT and metastatic processes (11). Similarly, the initiation of the EMT program in thyroid epithelial cells contributes to recurrent and metastatic diseases (12). These findings suggest a link between AURKA and EMT in the context of THCA metastasis.

Therefore, in the present study, reducing AURKA expression decreased the proliferation and inhibited the transition of BHT101 and BCPAP cells from the G0 phase to active division. Furthermore, downregulating AURKA expression enhanced the movement, migration and invasion capabilities of BHT101 and BCPAP cells. Additionally, AURKA regulates proliferation-related molecules. Specifically, the knockdown of AURKA expression increased the expression of P130 and E2F4, while reducing the expression of P107. Furthermore, upregulating AURKA expression promoted EMT, whereas downregulation of AURKA expression resulted in the opposite effect. In addition, the focal adhesion kinase (FAK) signaling pathway has been shown to play a crucial role in THCA metastasis, with inhibition of FAK hindering the movement, migration and invasion of THCA cells. Therefore, AURKA promotes EMT by regulating P130 and P107 to facilitate THCA metastasis.

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Key words: thyroid cancer, epithelial mesenchymal transition, Aurora kinase A, cell cycle, signal transduction

Materials and methods

Cell culture. BCPAP and BHT101 cells were obtained from Shanghai Institutes of Biological Sciences (Shanghai, China)

and were cultured in DMEM (YOBIBIO) supplemented with 10% fetal bovine serum (YOBIBIO). The cells were maintained in a humidified incubator at 37°C. In the TAE226 treatment protocol, THCA cells (including BCPAP and BHT101) were treated with the FAK inhibitor TAE226 (2.1 µM/ml; Selleck Chemicals) for 24 h at 37°C. The control groups included THCA cells treated with DMSO and THCA cells without any treatment.

Plate colony formation assay. Cells, with or without treatment, were seeded into 6-well plates at a density of 1×10^3 cells per well. After one month of culture, the cells were washed twice with PBS and fixed with pure methanol for 15 min at 37°C. The cells were then stained with crystal violet for 30 min at 37°C. Finally, colonies consisting of >50 cells in each well were manually counted.

Flow cytometry. The cells were seeded into 6-well plates and rinsed three times with cold PBS. They were then fixed with precooled anhydrous ethanol at 4°C overnight. The fixed cells were removed from 4°C and equilibrated at room temperature for 15 min. The samples were centrifuged at $3,162 \times g$ for 5 min, and the supernatant was discarded. A total of 10 ml of PBS was added, and the mixture was well mixed before another centrifugation at $3,162 \times g$ for 5 min to remove the supernatant. After adding 2 ml of PBS and mixing, the cells were transferred to a flow tube and were centrifuged at $3,162 \times g$ for 5 min to remove the supernatant. In total, 40 µl of 50 µg/ml RNase A (Beyotime Institute of Biotechnology) was added, and the mixture was well mixed before incubation at 37°C in the dark for 30 min with intermittent shaking. A total of 20 µl of 50 µg/ml PI staining solution (BD Pharmingen) was added, and the cells were incubated at room temperature in the dark for 15 min. Cell cycle analysis was performed by flow cytometry on a FACScan instrument (Beckman Coulter, Inc.), and analyzed using FlowJo v10 (FlowJo LLC) and GraphPad Prism software v6.0 (Dotmatics).

Immunofluorescence (IF). Cells were seeded into Millicell EZ SLIDES (MilliporeSigma) and fixed with 4% paraformaldehyde for 30 min at 37°C. The slides were then rinsed three times with PBS, blocked with 5% BSA for 1 h at 37°C, and incubated overnight at 4°C with anti-AURKA (1:100; cat. no. 66757-1-Ig; Cell Signaling Technology, Inc.), anti-P130 (1:100; cat. no. 13383; Cell Signaling Technology, Inc.) and anti-P107 (1:100; cat. no. 13354-1-AP; Proteintech Group, Inc.). The slides were incubated with Alexa Fluor® 488 goat anti-rabbit IgG (1:1,000; cat. no. 4412S; Cell Signaling Technology, Inc.) and Alexa Fluor® 555 goat anti-rabbit IgG (1:1,000; cat. no. 4413S; Cell Signaling Technology, Inc.) for 1 h at room temperature in the dark. DAPI (1:1,000; Beyotime Institute of Biotechnology) was used to stain nuclei for 5 min in the dark at 37°C. The slides were rinsed three times with PBS and analyzed by fluorescent microscopy at $\times 10$ magnification.

siRNA interference. The day before transfection, cells were seeded into 6-well plates at a density of 5×10^5 cells/ml (1.5 ml per well). Transfection was performed 16 h later. For transfection, siRNA was mixed with Opti-MEM to achieve a final concentration of 100 nM, with a total volume of 250 µl, and

was left at room temperature for 5 min. In a separate tube, 5 µl of Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) was added to 250 µl of Opti-MEM and incubated at room temperature for 5 min. The diluted siRNA was then combined with the transfection reagent and incubated at room temperature for 20 min. Finally, 500 µl of the RNA transfection mixture was added to each well containing the cells. Subsequently, 6 h post-transfection, the culture medium was replaced with 1.5 ml of fresh complete medium. Cells were collected 48 h after transfection for western blot analysis. The sequences were as follows: si-AURKA forward, 5'-GAA GAGAGUUAUUCAUAGADTDT-3' and reverse, 5'-UCUAUG AAUAACUCUCUUCDTDT-3'; and siNC forward, 5'-UAC GUACUAUCGCGCGGAUTT-3' and reverse, 5'-AUCCGC GCGATAGUACGUATT-3' were purchased from Shanghai Kaiji Company.

Construction of overexpression plasmids. The plasmid backbone used for the overexpression of AURKA was pEX-3 (pGCMV/MCS/Neo), which was purchased from Shanghai GenePharma Co., Ltd. The AURKA gene was amplified from genomic DNA extracted from BCPAP and BHT101 cells using the Qiagen DNeasy Blood & Tissue Kit (Qiagen GmbH) according to the manufacturer's instructions. PCR amplification was performed using Taq DNA polymerase (Thermo Fisher Scientific, Inc.) with the following primers: Forward (*Eco*RI site), 5'-CCGGAATTCGCCACCATGCAT ATCACCATCACTCAACTCAGTGGAGCATTGAGATAG-3' and reverse (*Bam*HI site), 5'-CCGGGATCC TCAGTTGGA GGTATCATGAACACTG-3'. The thermocycling conditions included an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 60°C for 30 sec and extension at 72°C for 1 min per kb of template, with a final extension at 72°C for 10 min. PCR products were analyzed using 1.5% agarose gel electrophoresis and visualized with SYBR Safe staining (Invitrogen; Thermo Fisher Scientific, Inc.) under UV light using a gel imaging system. The plasmid carrying the vector was extracted from overnight bacterial cultures (3-5 ml) using the QIAGEN Plasmid Mini Kit (Qiagen GmbH). The vector was then subjected to double digestion with *Eco*RI and *Bam*HI (1.5 µl of each enzyme, 3 µl Green Buffer, 23 µl ddH₂O and 1 µg vector) at 37°C for 3 h. After digestion, the product was purified by 0.8% agarose gel electrophoresis followed by gel extraction using QG buffer and isopropanol precipitation. The target fragment was amplified using PCR with 10 ng template DNA, 2 µl of each primer (10 µM), 25 µl PCR mix and 21 µl double-distilled water. Following PCR, the product was recovered and digested under similar conditions as the vector. Both the vector and insert were ligated using T4 DNA Ligase (1 µl) in a reaction containing 2 µl T4 DNA ligation buffer, 70 ng vector, an equimolar amount of the insert (calculated based on vector concentration) and double-distilled water to a final volume of 20 µl at 22°C for 60 min. The ligation mixture (10 µl) was then used to transform *E. coli* DH5α (EC0112; Thermo Fisher Scientific, Inc.) competent BCPAP and BHT101 cells through heat shock (90 sec at 42°C), followed by incubation in SOC medium (cat. no. 15544034; Thermo Fisher Scientific, Inc.) for 45 min at $17 \times g$ at 37°C. Finally, the transformed cells were plated on selective agar plates containing the appropriate antibiotic

(ampicillin; 1 $\mu\text{g}/\text{ml}$) and incubated overnight at 37°C. Several single colonies were incubated in small quantities, which were screened and confirmed by sequencing (Sanger). The Sanger sequencing traces are shown Fig. S1.

AURKA overexpression plasmid transfection. For transfection, plasmid DNA (500 $\mu\text{g}/\mu\text{l}$) was diluted in Opti-MEM and transfected using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). The mixture was incubated at room temperature for 5–20 min to allow DNA-lipid complexes to form before being added dropwise onto the cells. Cells were incubated at 37°C with 5% CO₂ for 4–6 h before replacing the transfection mixture with fresh growth medium. For stable cell line selection, cells were cultured for 72 h post-transfection before adding selection medium containing 1 mg/ml G418. Selection was continued for 2 weeks until resistant colonies emerged. Once stable cell lines were established, the cells were maintained in selection medium containing 0.2 mg/ml G418 to preserve stable knockdown efficiency. The effect of overexpression was examined by western blotting. The time interval between transfection and subsequent experiments was 24–72 h.

Western blotting. The procedures for the western blotting have been previously described (6). Antibodies used included Anti-P130 (1:2,000; rabbit mAb; 13383), anti-E2F4 (1:2,000; rabbit mAb; 40291), anti-E-cad (1:2,000; mouse mAb; 14472), anti-N-cad (1:2,000; mouse mAb; 14215), anti-Vimentin (1:2,000; rabbit mAb; 5741), anti-Slug (1:2,000; rabbit mAb; 9585) and anti-GAPDH (1:2,000; rabbit; 2118T), which were all obtained from Cell Signaling Technology, Inc. Anti-P107 (1:2,000; rabbit; 13354-1-AP) and anti-AURKA (1:2,000; mouse; 66757-1-Ig) were sourced from Proteintech Group, Inc. The membranes were incubated with primary antibodies at 4°C overnight. Proteins (40 $\mu\text{g}/\text{lane}$) were separated by 10 or 12.5% SDS-PAGE for 2 h. Following the addition of secondary antibodies (1:2,000; anti-rabbit IgG; HRP-linked antibody; 7074; anti-mouse IgG; HRP-linked antibody; 7076; Cell Signaling Technology, Inc.), the membranes were incubated with secondary antibodies at room temperature for 1 h. The membranes were visualized using Thermo Pierce chemiluminescent (ECL) Western Blotting Substrate (Thermo Fisher Scientific, Inc.) and were detected with a Tanon 5200 system (Tanon Science and Technology Co., Ltd.).

For the detection of the proteins of interest and the loading control (GAPDH), it should be noted that the proteins were not probed for on the same membranes due to the limitations of the experimental setup. Despite this, the western blots were conducted under consistent conditions to maintain the integrity of the data. A total of 40 μg of protein was loaded into each lane to ensure sample consistency across the experiments. Separate blots were performed for each target protein to avoid cross-reactivity and ensure reliable quantification.

Wound healing assay. Cells were seeded into six-well plates at a density of 1×10^6 cells per well and cultured until they reached confluence. The monolayer was then scratched using a 200- μl pipette tip, and the medium was replaced with fresh medium containing 1% FBS. Images of the cells were captured by fluorescence microscopy at a magnification of $\times 10$ at 0 and 24 h

post-scratch. To quantify wound healing, the maximum width of the wound was measured and analyzed using GraphPad Prism software v6.0 (Dotmatics).

Migration and invasion assays. For the migration assay, 2×10^5 cells suspended in 200 μl of serum-free DMEM were added to the top chamber of a Transwell insert (pore size, 8 μm ; Corning, Inc.). The lower chamber was filled with 600 μl of DMEM supplemented with 10% FBS. After 24 h, the cells that had migrated to the lower surface of the filter were fixed with 4% paraformaldehyde for 15 min at 37°C and stained with 0.5% crystal violet solution for 20 min at 37°C, and images were captured under an Olympus BX50 light microscope (Olympus Corporation) at a magnification of $\times 10$.

For the invasion assay, the top chamber membrane was first coated with Matrigel (Becton, Dickinson and Company) at 4°C overnight. After 24 h of culture at 37°C, tumor cells remaining on the upper side of the inserts were removed with cotton swabs. The remaining steps were identical to those of the migration assay.

Statistical analysis. All experiments were repeated at least three times and the data are presented as the mean $\pm$ SD. Significant differences between groups were determined using an unpaired Student's t-test (two groups) and one-way ANOVA (multiple groups; GraphPad Prism software v6.0; Dotmatics), and Tukey's Honestly Significant Difference test was used for post hoc comparisons. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Decreasing AURKA expression reduces the proliferation of BHT101 and BCPAP cells. To investigate the specific regulatory mechanism by which AURKA promotes THCA metastasis, AURKA expression was silenced by transfecting AURKA-siRNA into BHT101 and BCPAP cells (designated as BHT101/AURKA-siRNA and BCPAP/AURKA-siRNA, respectively) and were compared with untreated controls (BHT101/AURKA-NC and BCPAP/AURKA-NC). The silencing effect was confirmed by results shown in Fig. 1A and B ($P < 0.01$). The plate colony formation assay demonstrated a reduction in the number of colonies formed by BHT101/AURKA-siRNA (207 ± 26.8) and BCPAP/AURKA-siRNA cells (184 ± 16.2) compared with BHT101/AURKA-NC (118 ± 14.9) and BCPAP/AURKA-NC cells (101 ± 12.8 ; Fig. 1C and D; $P < 0.01$). Flow cytometric analysis revealed that AURKA inactivation induced G2/M phase arrest in BHT101/AURKA-siRNA and BCPAP/AURKA-siRNA cells (Fig. 1E–H; $P < 0.01$). These findings suggest that AURKA promotes proliferation by inhibiting G2/M phase arrest in BHT101 and BCPAP cells.

Decreasing AURKA expression inhibits the migration and invasion of BHT101 and BCPAP cells. To further explore the true role of AURKA in THCA metastasis, wound healing, migration and invasion assays were conducted to assess the capabilities of BHT101 and BCPAP cells. The results of the wound healing assay indicated that BHT101/AURKA-siRNA and BCPAP/AURKA-siRNA cells exhibited reduced

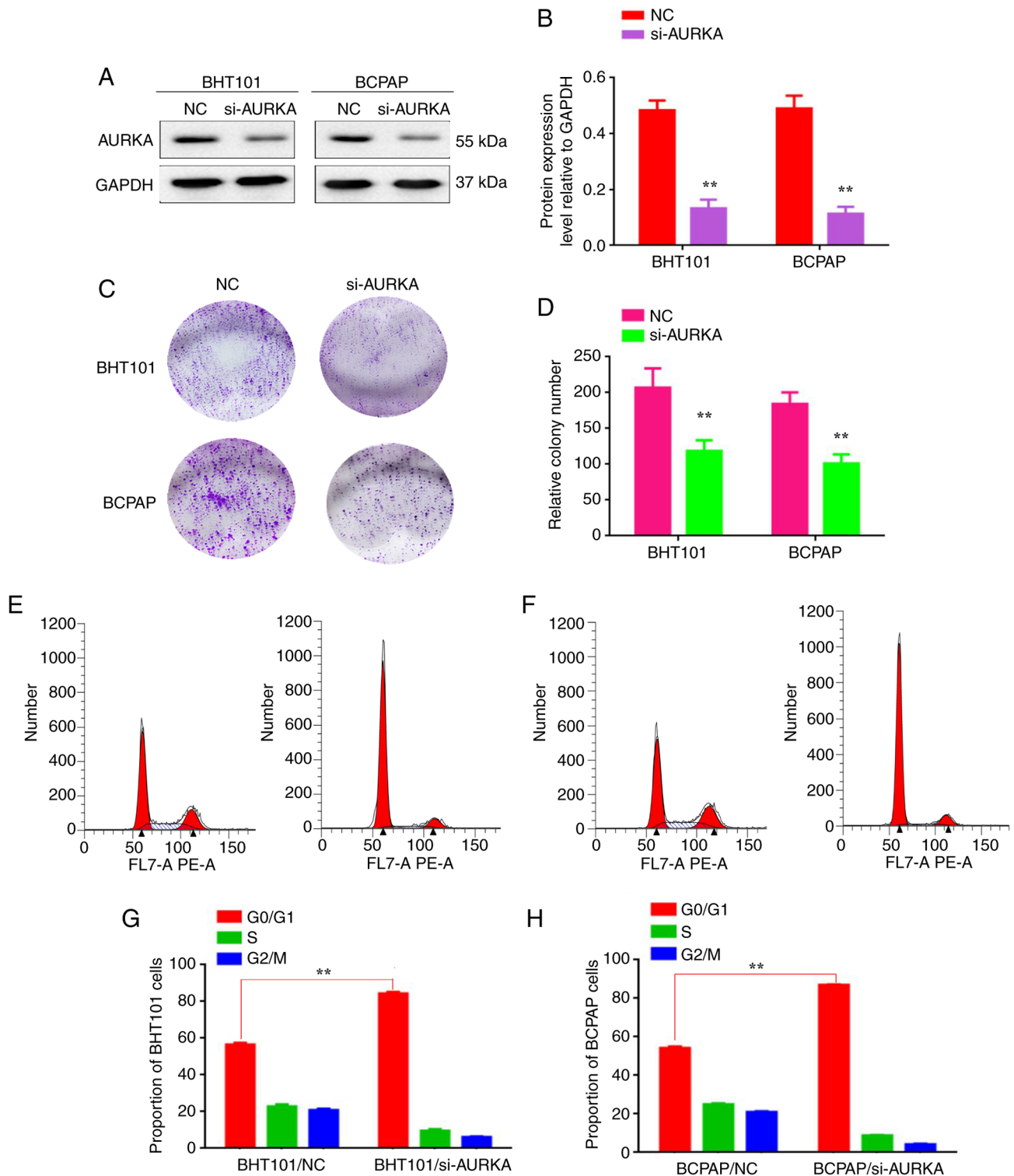


Figure 1. Decreasing AURKA expression reduces the proliferation of BHT101 and BCPAP cells. (A) Western blot analysis was used to detect the expression of AURKA in BHT101/si-NC, BHT101/si-AURKA, BCPAP/si-NC and BCPAP/si-AURKA cells. (B) The protein expression level relative to GAPDH in BHT101/si-NC, BHT101/si-AURKA, BCPAP/si-NC and BCPAP/si-AURKA cells. (C) Plate colony formation assay was used to detect the proliferation of BHT101/si-NC, BHT101/si-AURKA, BCPAP/si-NC and BCPAP/si-AURKA cells. (D) The relative colony number of BHT101/si-NC, BHT101/si-AURKA, BCPAP/si-NC and BCPAP/si-AURKA cells. (E-H) The proportion of G₀/G₁ cells of BHT101/si-NC, BHT101/si-AURKA, BCPAP/si-NC and BCPAP/si-AURKA cells. **P<0.01. AURKA, Aurora kinase A; siRNA, small interfering RNA; NC, negative control.

motility at 24 h compared with BHT101/AURKA-NC and BCPAP/AURKA-NC cells (Fig. 2A-D; P<0.01). Additionally, it was observed that BHT101/AURKA-siRNA (64±2.6) and BCPAP/AURKA-siRNA (70±1.76) migrated less than BHT101/AURKA-NC cells (109±2.6) and

BCPAP/AURKA-NC cells (129±2.6; Fig. 2E-H; P<0.01). Furthermore, BHT101/AURKA-siRNA cells (17±1.1) and BCPAP/AURKA-siRNA cells (9±1.45) invaded less than BHT101/AURKA-NC cells (56±3.5) and BCPAP/AURKA-NC cells (47±3.48; Fig. 2E-H; P<0.01). These results suggest that

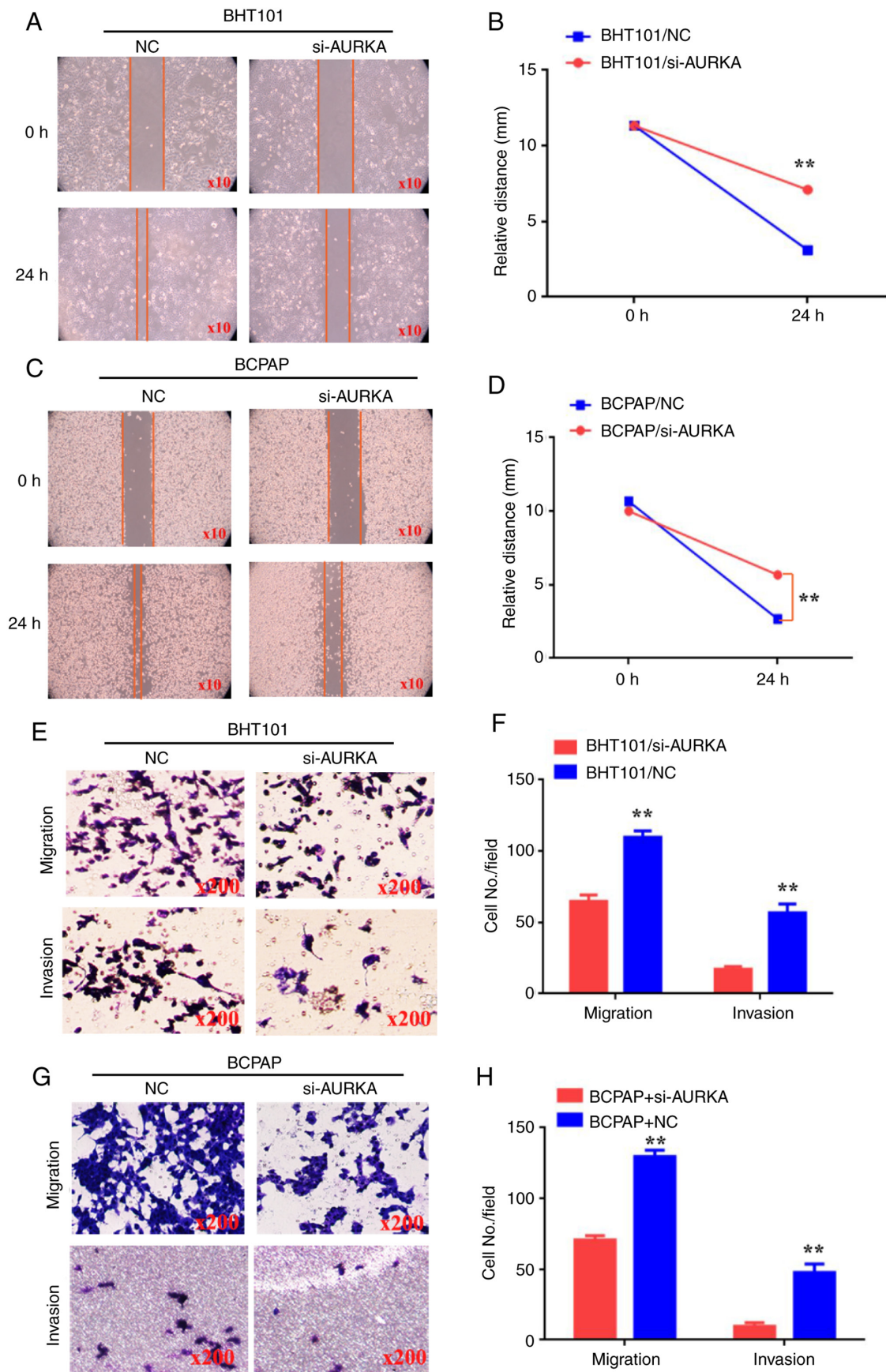


Figure 2. Decreasing AURKA expression inhibits the migration and invasion of BHT101 and BCPAP cells. (A and C) Wound healing assay was used to detect the ability of movement in BHT101/si-NC, BHT101/si-AURKA, BCPAP/si-NC and BCPAP/si-AURKA cells. (B and D) The relative distance (mm) of BHT101/si-NC, BHT101/si-AURKA, BCPAP/si-NC and BCPAP/si-AURKA cells. (E and G) Transwell and Matrigel assays were used to detect the ability of migration and invasion in BHT101/si-NC, BHT101/si-AURKA, BCPAP/si-NC and BCPAP/si-AURKA cells. (F and H) The cell number in every field in BHT101/si-NC, BHT101/si-AURKA, BCPAP/si-NC and BCPAP/si-AURKA cells. ** $P < 0.01$. AURKA, Aurora kinase A; siRNA, small interfering RNA; NC, negative control.

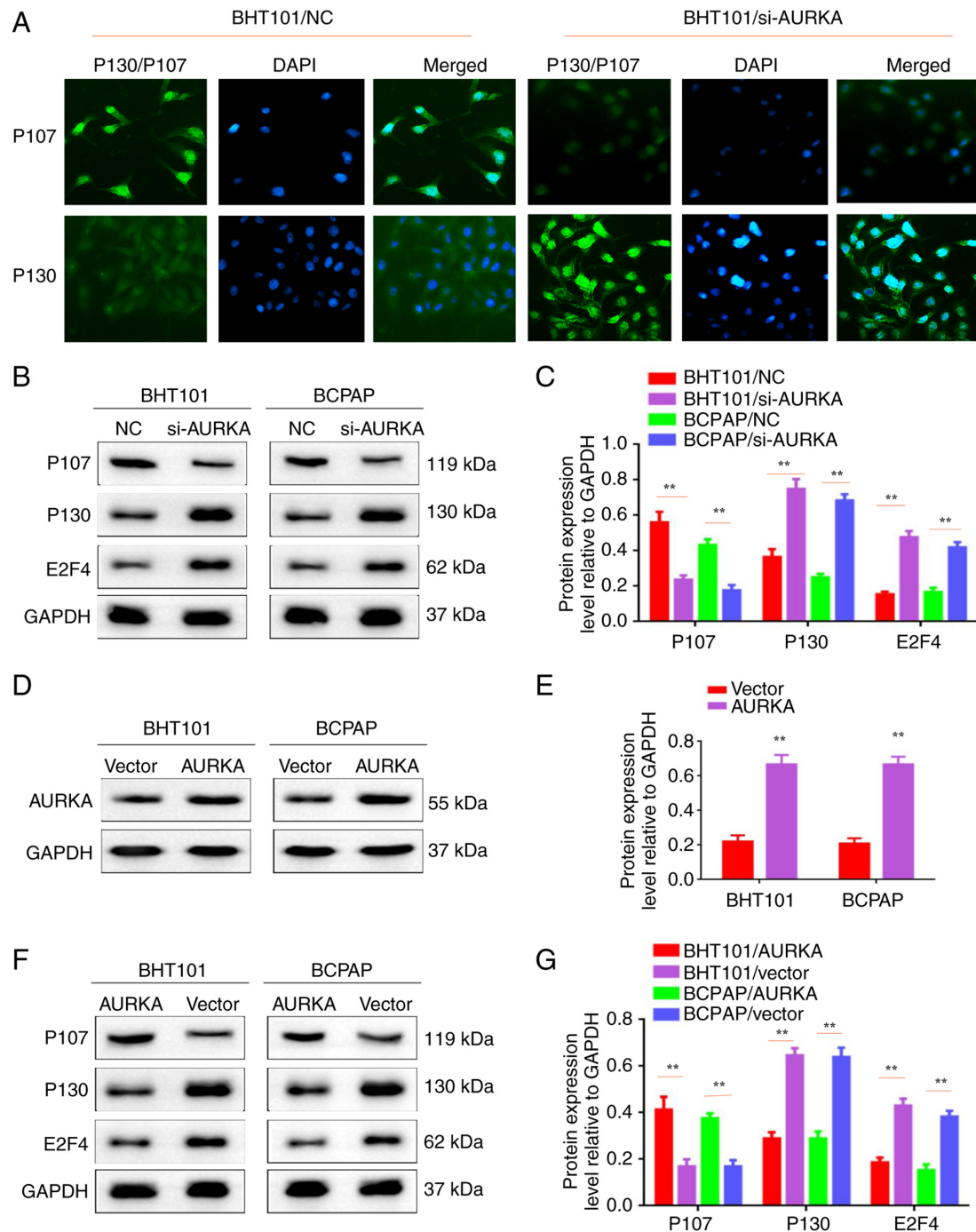


Figure 3. AURKA inhibition can reduce the proliferation by regulating P130 and P107 molecules. (A) Immunofluorescent staining assay was used to detect the expression levels of P130 and P107 in BHT101/si-NC, BHT101/si-AURKA, BCPAP/si-NC and BCPAP/si-AURKA cells. (B) Western blot analysis was used to detect the expression levels of P107, P130, E2F4 and GAPDH in BHT101/si-NC, BHT101/si-AURKA, BCPAP/si-NC and BCPAP/si-AURKA cells. (C) The protein expression level relative to GAPDH in BHT101/si-NC, BHT101/si-AURKA, BCPAP/si-NC and BCPAP/si-AURKA cells. (D) Western blot analysis was used to detect the expression of AURKA in BHT101/vector, BHT101/AURKA, BCPAP/vector and BCPAP/AURKA cells. (E) The protein expression level relative to GAPDH in BHT101/vector, BHT101/AURKA, BCPAP/vector and BCPAP/AURKA cells. (F) Western blot analysis was used to detect the expression levels of P107, P130, E2F4 and GAPDH in BHT101/vector, BHT101/AURKA, BCPAP/vector and BCPAP/AURKA cells. (G) The protein expression level relative to GAPDH in BHT101/vector, BHT101/AURKA, BCPAP/vector and BCPAP/AURKA cells. ** $P < 0.01$. AURKA, Aurora kinase A; siRNA, small interfering RNA; NC, negative control.

reducing AURKA expression inhibited the migration and invasion of BHT101 and BCPAP cells.

AURKA inhibition could reduce the proliferation by regulating P130 and P107 molecules. Our previous study indicated that P130 and P107 molecules played crucial roles in cancer cell cycle

and proliferation (6). In the present study, IF staining showed that P107 and P130 were localized in the nucleus. P107 levels decreased, while P130 increased in BHT101/AURKA-siRNA and BCPAP/AURKA-siRNA cells compared with controls (BHT101/AURKA-NC cells and BCPAP/AURKA-NC cells; Fig. 3A). Similarly, western blotting showed that

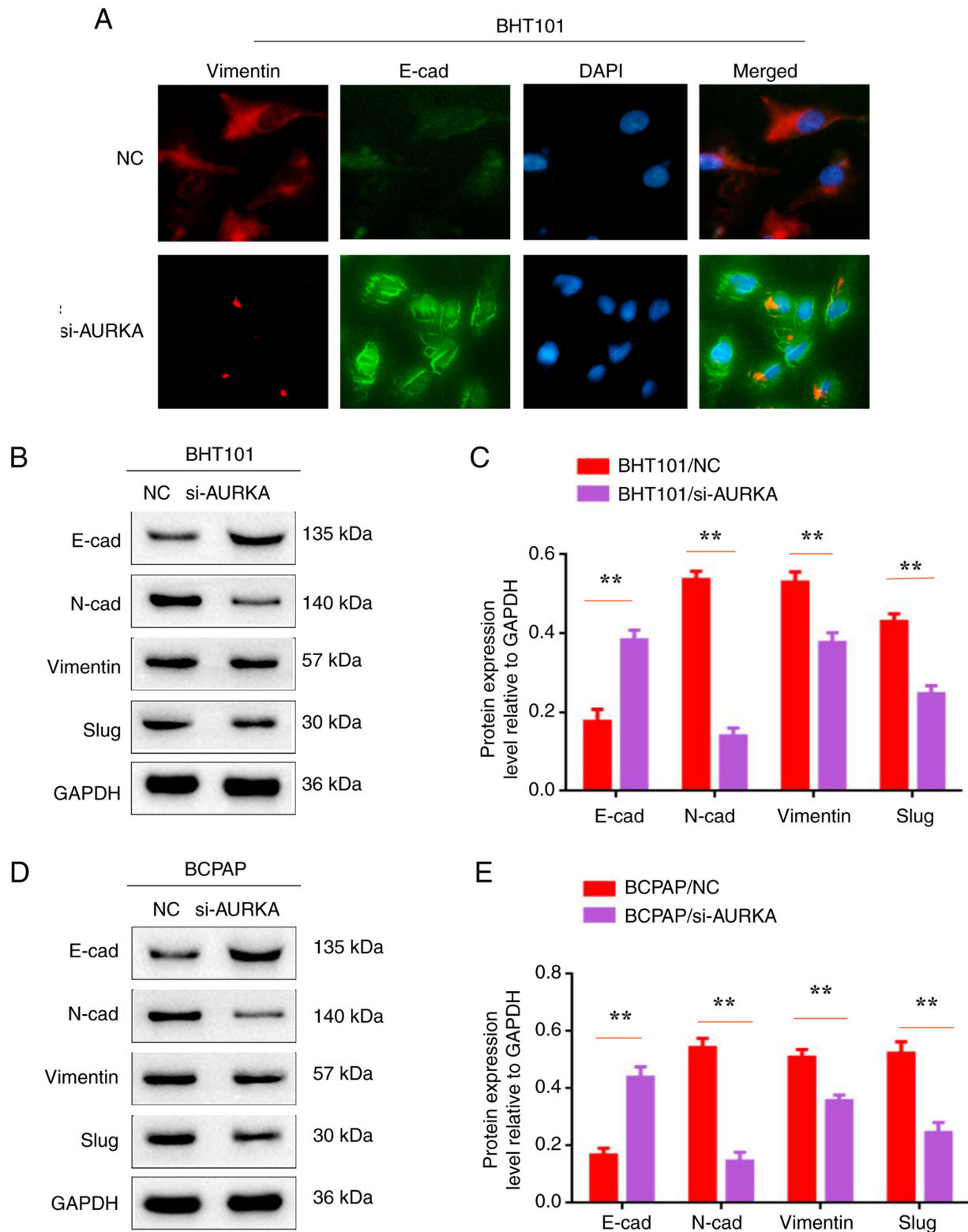


Figure 4. Inactivation of AURKA inhibits epithelial-mesenchymal transition in BHT101 and BCPAP cells. (A) Immunofluorescent staining assay was used to detect the expression levels of Vimentin and E-cad in BHT101/si-NC, BHT101/si-AURKA, BCPAP/si-NC and BCPAP/si-AURKA cells. (B and D) Western blot analysis was used to detect the expression levels of E-cad, N-cad, Vimentin, Slug and GAPDH in BHT101/si-NC, BHT101/si-AURKA, BCPAP/si-NC and BCPAP/si-AURKA cells. (C and E) The protein expression level relative to GAPDH in BHT101/si-NC, BHT101/si-AURKA, BCPAP/si-NC and BCPAP/si-AURKA cells. ** $P < 0.01$. AURKA, Aurora kinase A; siRNA, small interfering RNA; NC, negative control; E-cad, E-cadherin; N-cad, N-cadherin.

proliferation-related molecule (P107) expression decreased in BHT101/AURKA-siRNA and BCPAP/AURKA-siRNA cells. Proliferation-related molecules (P130 and E2F4) increased in BHT101/AURKA-siRNA and BCPAP/AURKA-siRNA cells

(Fig. 3B and C; $P < 0.01$). Furthermore, AURKA was efficiently upregulated in BHT101 and BCPAP cells (Fig. 3D and E; $P < 0.01$). Conversely, the proliferation-related molecule (P107) increased in BHT101/AURKA and BCPAP/AURKA cells.

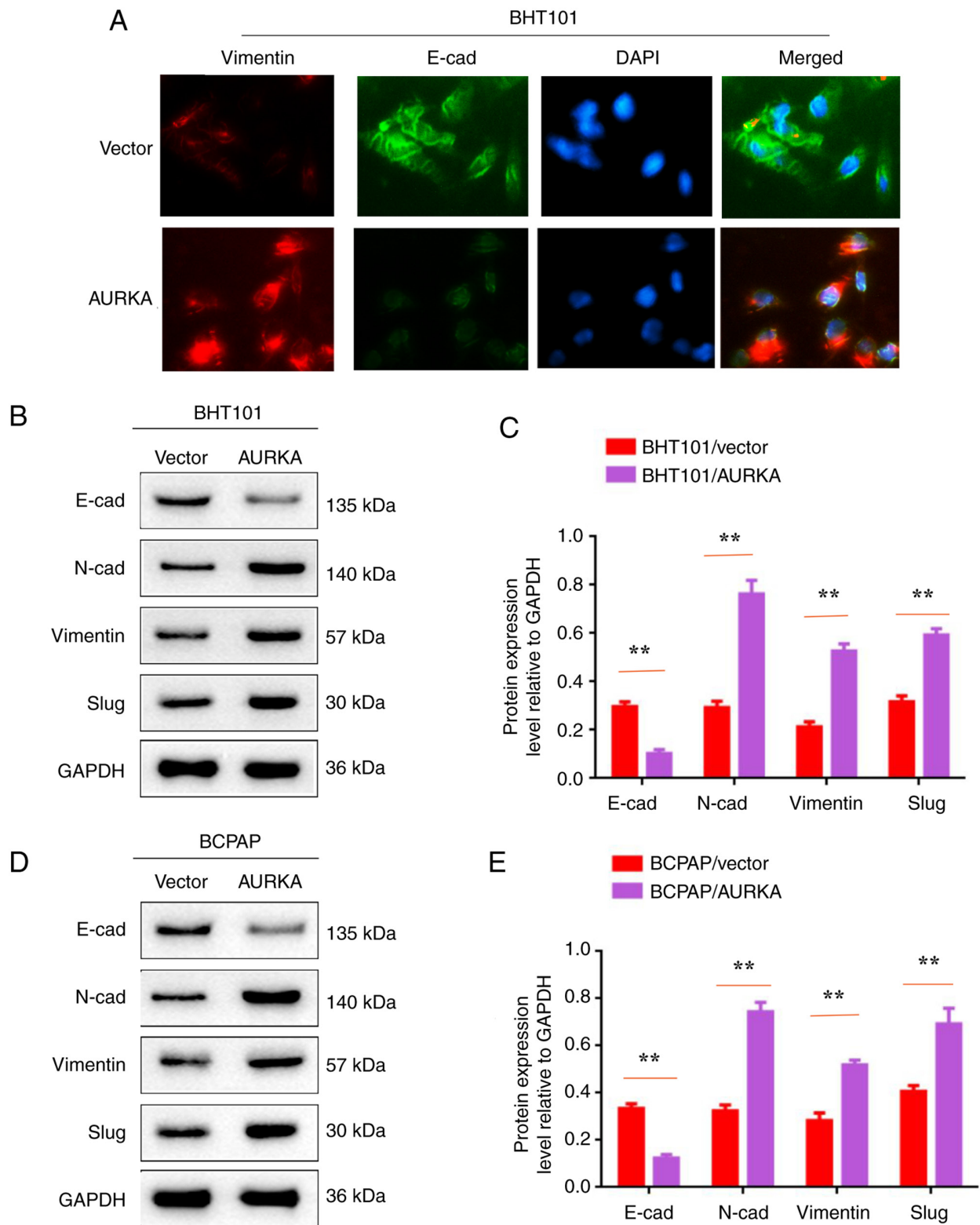


Figure 5. Activation of AURKA promotes epithelial-mesenchymal transition in BHT101 and BCPAP cells. (A) Immunofluorescent staining assay was used to detect the expression levels of Vimentin and E-cad in BHT101/vector, BHT101/AURKA, BCPAP/vector and BCPAP/AURKA cells. (B and D) Western blot analysis was used to detect the expression levels of E-cad, N-cad, Vimentin, Slug and GAPDH in BHT101/vector, BHT101/AURKA, BCPAP/vector and BCPAP/AURKA cells. (C and E) The protein expression level relative to GAPDH in BHT101/vector, BHT101/AURKA, BCPAP/vector and BCPAP/AURKA cells. **P<0.01. AURKA, Aurora kinase A; E-cad, E-cadherin; N-cad, N-cadherin.

Proliferation-related molecules (P130 and E2F4) decreased in BHT101/AURKA and BCPAP/AURKA cells (Fig. 3F and G; P<0.01). Therefore, it was concluded that AURKA inhibition reduced proliferation by regulating P130 and P107 molecules.

Inactivation of AURKA inhibits EMT in BHT101 and BCPAP cells. EMT has been recognized to play a crucial role in metastasis. In the present study, EMT-related proteins E-cadherin, N-cadherin, vimentin and slug were assessed

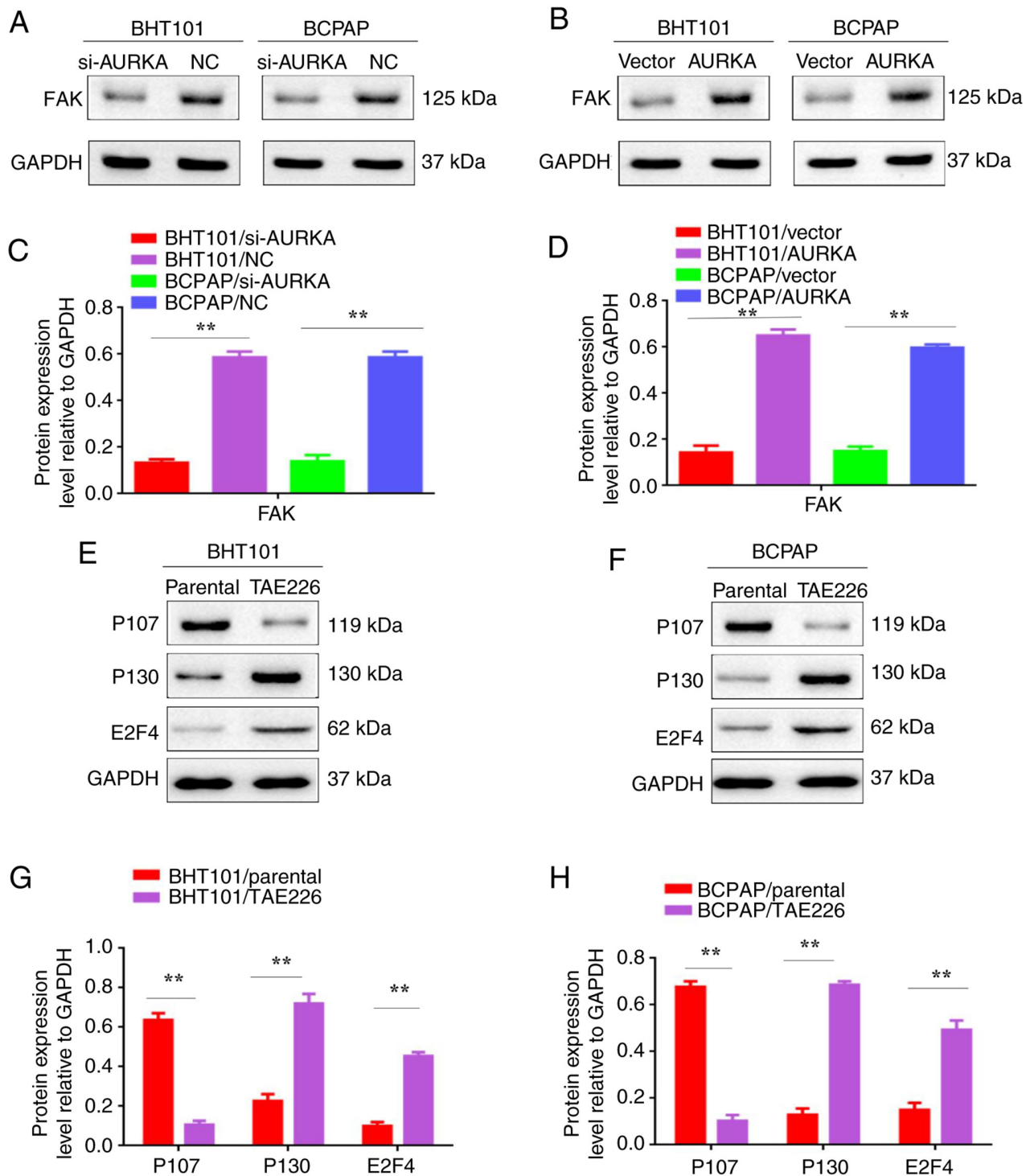


Figure 6. AURKA promotes epithelial-mesenchymal transition through the FAK signaling pathway and FAK pathway inhibition impacts proliferation-related proteins. (A) Western blot analysis was used to detect the expression of FAK in BHT101/si-NC, BHT101/si-AURKA, BCPAP/si-NC and BCPAP/si-AURKA cells. (B) Western blot analysis was used to detect the expression of FAK in BHT101/vector, BHT101/AURKA, BCPAP/vector and BCPAP/AURKA cells. (C) The protein expression level relative to GAPDH in BHT101/si-NC, BHT101/si-AURKA, BCPAP/si-NC and BCPAP/si-AURKA cells. (D) The protein expression level relative to GAPDH in BHT101/vector, BHT101/AURKA, BCPAP/vector and BCPAP/AURKA cells. (E) Western blot analysis was used to detect the expression levels of P107, P130, E2F4 and GAPDH in BHT101/si-NC, BHT101/si-AURKA, BCPAP/si-NC and BCPAP/si-AURKA cells. (F) Western blot analysis was used to detect the expression levels of P107, P130, E2F4 and GAPDH in BHT101/vector, BHT101/AURKA, BCPAP/vector and BCPAP/AURKA cells. (G) The protein expression level relative to GAPDH in BHT101/si-NC, BHT101/si-AURKA, BCPAP/si-NC and BCPAP/si-AURKA cells. (H) The protein expression level relative to GAPDH in BHT101/vector, BHT101/AURKA, BCPAP/vector and BCPAP/AURKA cells. **P<0.01. AURKA, Aurora kinase A; FAK, focal adhesion kinase; siRNA, small interfering RNA; NC, negative control.

in BHT101/AURKA-siRNA, BCPAP/AURKA-siRNA, BHT101/AURKA-NC and BCPAP/AURKA-NC cells. IF staining results showed that E-cadherin expression was higher

in BHT101/AURKA-siRNA and BCPAP/AURKA-siRNA cells, while vimentin expression was lower compared with controls (BHT101/AURKA-NC and BCPAP/AURKA-NC

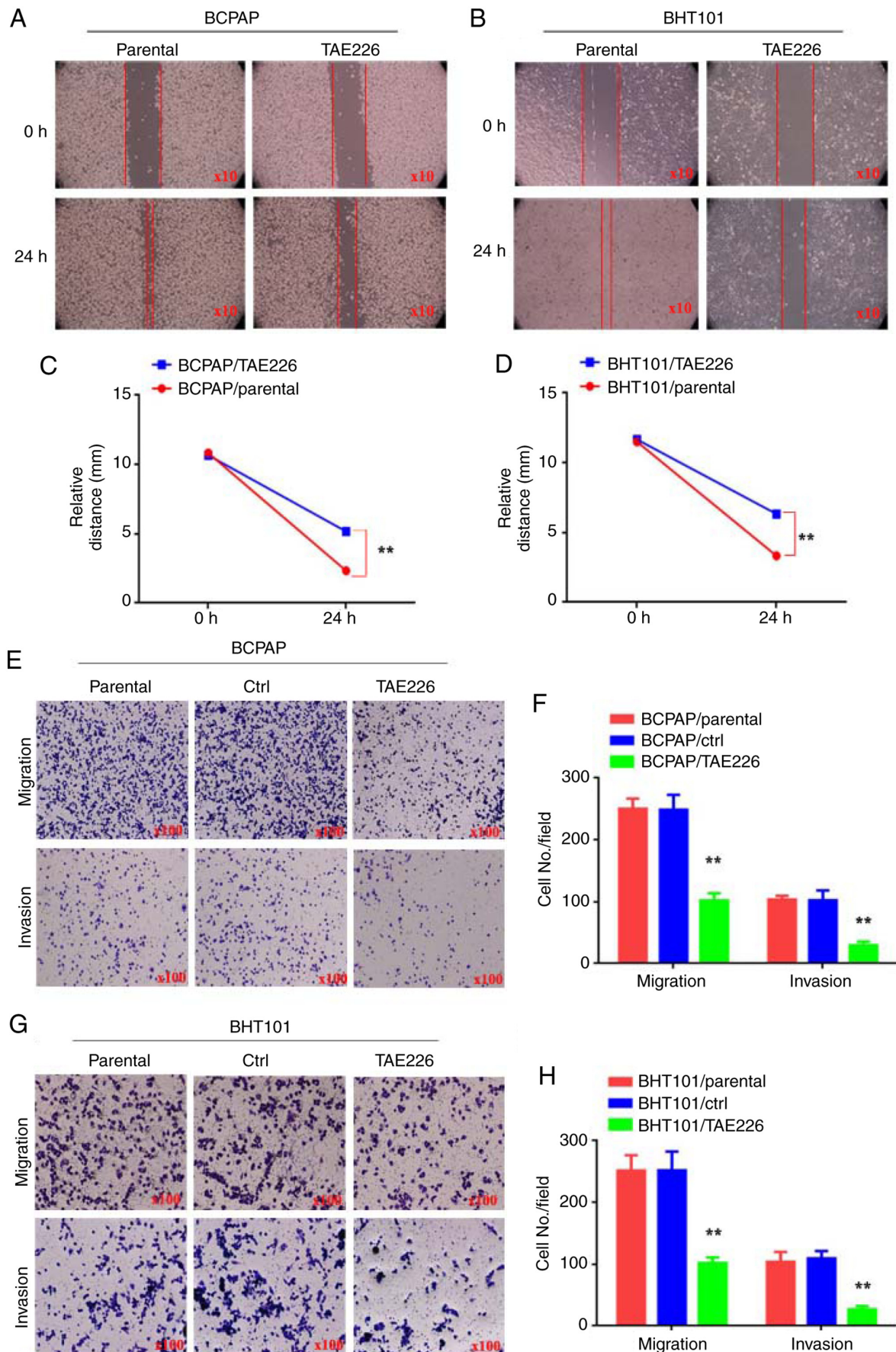


Figure 7. Blocking FAK impairs the movement, migration and invasion of THCA cells. (A-D) The relative distance (mm) of BTH101/parental, BTH101/TAE226, BCPAP/parental and BCPAP/TAE226 cells. (E and G) Transwell assay was used to detect the ability of migration and invasion in BTH101/parental, BTH101/ctrl, BTH101/TAE226, BCPAP/parental, BCPAP/ctrl and BCPAP/TAE226 cells. (F and H) The cell number in every field in BTH101/parental, BTH101/ctrl, BTH101/TAE226, BCPAP/parental, BCPAP/ctrl and BCPAP/TAE226 cells. ** $P < 0.01$. THCA, thyroid cancer; FAK, focal adhesion kinase; ctrl, control.

cells). Western blot results similarly revealed increased E-cadherin levels. However, other EMT-related molecules (N-cadherin, vimentin and slug) were decreased (Fig. 4B-E; $P<0.01$). Thus, it was concluded that AURKA inactivation inhibits EMT in BHT101 and BCPAP cells.

Activation of AURKA promotes EMT in BHT101 and BCPAP cells. To further explore AURKA-mediated EMT in THCA metastasis, EMT-related proteins E-cadherin, N-cadherin, vimentin and slug were assessed in BHT101/AURKA, BCPAP/AURKA, BHT101/vector and BCPAP/vector cells. IF staining revealed that E-cadherin expression was decreased and vimentin expression was increased in BHT101/AURKA and BCPAP/AURKA cells compared with controls (BHT101/vector and BCPAP/vector cells; Fig. 5A). Western blot analysis demonstrated decreased E-cadherin levels and increased expression of other EMT-related molecules (N-cadherin, vimentin and slug) in AURKA-activated BHT101 and BCPAP cells (Fig. 5B-E; $P<0.01$). Therefore, it was concluded that AURKA activation promotes EMT in BHT101 and BCPAP cells.

AURKA promotes EMT through the FAK signaling pathway and FAK pathway inhibition impacts proliferation-related proteins. The FAK signaling pathway has been recognized to play a crucial role in cancer metastasis (13-16). To explore the importance of FAK in THCA metastasis, protein expression levels were determined using western blot analysis. FAK protein levels decreased in BHT101/si-AURKA and BCPAP/si-AURKA cells (Fig. 6A-D; $P<0.01$). To further demonstrate that AURKA promotes EMT by regulating P130 and P107 molecules through FAK signaling in THCA cells, P107, P130 and E2F4 expression levels were explored in THCA cells treated with the FAK inhibitor TAE226 (17). Western blot results revealed that P130 and E2F4 were increased and P107 decreased in BHT101/TAE226 and BCPAP/TAE226 cells (Fig. 6E-H, $P<0.01$). These findings indicate that AURKA promotes EMT by regulating P130 and P107 molecules through the FAK signaling pathway in THCA cells.

Blocking FAK impairs the movement, migration and invasion of THCA cells. To further investigate the potential of inhibiting the tumor-promoting effects of THCA by blocking FAK expression, wound-healing, migration and invasion assays were used to examine the movement, migration and invasion abilities of THCA cells. THCA cells were treated with the FAK inhibitor TAE226 (BCPAP/TAE226 and BHT101/TAE226 cells). Control groups included THCA cells treated with DMSO (BCPAP/parental and BHT101/parental cells) and THCA cells without any treatment (BCPAP/ctrl and BHT101/ctrl cells). In the wound-healing assay, BCPAP/TAE226 and BHT101/TAE226 cells demonstrated reduced motility at 24 h compared with BCPAP/parental and BHT101/parental cells (Fig. 7A-D; $P<0.01$). Moreover, the migration assay results demonstrated that BCPAP/TAE226 (102 ± 6.8) and BHT101/TAE226 (101 ± 5.23) cells showed decreased movement through Matrigel compared with BCPAP/parental (250 ± 9.64), BCPAP/ctrl (248 ± 13.96), BHT101/parental (251 ± 14.34) and BHT101/ctrl cells (252 ± 17.61 ; Fig. 7E and F; $P<0.01$). For the invasion assay, the results identified that BCPAP/TAE226 (29 ± 3.48) and BHT101/TAE226 (26 ± 3.18) cells exhibited decreased

movement through Matrigel compared with BCPAP/parental (103 ± 3.48), BCPAP/ctrl (102 ± 9.20), BHT101/parental (103 ± 9.33) and BHT101/ctrl cells (109 ± 6.93 ; Fig. 7G and H; $P<0.01$). These findings indicated that blocking FAK impairs the movement, migration and invasion of THCA cells.

Discussion

THCA is a malignancy originating from thyroid epithelial cells, characterized by a high incidence rate. Metastasis remains the leading cause of mortality among patients with THCA (18). However, the precise molecular and cellular mechanisms governing THCA metastasis are still not fully understood. Clarifying these mechanisms is critical for identifying potential molecular targets aimed at improving patient survival rates and quality of life.

Years following initial treatment, patients with THCA might experience the development of local remnants or disseminated tumors. This phenomenon can be attributed to tumor dormancy—a phase where residual disease exists but remains asymptomatic. The reactivation of dormant tumor cells could lead to recurrence and metastasis. Therefore, exploring the specific molecular mechanisms behind the activation of THCA dormancy holds significant theoretical and practical value.

Building upon findings of previous studies that highlighted the pivotal role of AURKA in LSCC tumorigenesis and metastasis both *in vitro* (19) and *in vivo* (20), the authors examined the impact of AURKA on cell proliferation, cell cycle progression and mobility in LSCC cell lines. AURKA, an evolutionarily conserved Aurora serine/threonine kinase, regulates the cell cycle (21) through processes such as centrosome maturation, mitotic entry, centrosome separation, bipolar spindle assembly, chromosome alignment, cytokinesis and mitotic exit (22). Disruption during mitosis can result in genetic instability and tumorigenesis. Colony formation and flow cytometry assays demonstrated that AURKA promoted proliferation, whereas the downregulation of AURKA inhibited the transition from the G0 phase to active division in BHT101 and BCPAP cells. Increased AURKA expression also enhanced movement, migration and invasion capabilities in BHT101 and BCPAP cells.

Research increasingly indicates that the proliferation-associated proteins P130 and E2F4 are abundant in quiescent cells (23,24), while P107 proteins are less prevalent (25). E2F4, an E2F transcription factor, mediates the expression of cell cycle proteins (26). In the present study, the inhibition of AURKA reduced proliferation by regulating P130 and P107 molecules.

There is increasing evidence suggesting that EMT plays a role in tumor invasion and metastasis (27). During tumor metastasis, cancer cells acquire a mesenchymal phenotype and increased invasiveness through EMT, allowing them to penetrate surrounding tissues. At implantation sites, cancer cells eventually form metastases that resemble the primary tumor through EMT (28), which is closely linked to tumor biological characteristics and involves multiple regulatory mechanisms. As research advances, various mechanisms have been identified, including those involved in tumor growth, evasion and dissemination, which hold significant clinical implications and may provide new approaches for diagnosing and treating tumor metastasis.

However, the molecular basis and regulatory mechanisms of THCA EMT remain largely unexplored, presenting a major challenge for research. In the present study, the relationship between AURKA and EMT was explored in the context of THCA metastasis. To the best of the authors' knowledge, no prior studies have examined this aspect within THCA. Additionally, upregulated AURKA expression promoted EMT, whereas downregulated AURKA expression inhibited EMT.

While the present study provided valuable insights, several limitations should be acknowledged. Primarily, the research focuses on *in vitro* cell models without conducting *in vivo* experiments to validate the observed phenomena, limiting the applicability of the findings to clinical settings. Additionally, the relatively small sample size may impact the generalizability and statistical power of the results. The use of a single or limited number of cell lines might not fully capture the variability between different cell types. Long-term effects and complex molecular mechanisms were also not thoroughly investigated, and the techniques employed each have inherent limitations. Future studies should aim to address these constraints to provide more comprehensive and accurate information.

In conclusion, based on the aforementioned findings, it was demonstrated that AURKA promotes EMT by regulating P130 and P107 genes, thereby facilitating the metastasis of THCA. Consequently, AURKA may represent a viable therapeutic target in the treatment of THCA.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Shanghai Healthy Youth Project (grant no. 20234Y0055), the Natural Science Foundation of Fujian (grant no. 2023J011342), Pudong the New Area Clinical Characteristic Discipline (grant no. PWYts2021-15), the Subject Construction Project of Pudong Health Commission of Shanghai (grant no. PWZy2020-06), the Gongli Hospital National Fund Cultivation Project (grant no. 2022GPY-B04), the Key Specialty Construction Project of Health Bureau of Shanghai (grant no. ZX2019C06) and the Pudong New Area Clinical Characteristic Discipline (grant no. PWYts2021-15).

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

LY and YG performed the experiments. LY and JL analyzed the data. LY wrote the manuscript. GW revised the manuscript. LY and GW designed the study. LY and GW interpreted the data. LY and GW confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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