Original Article

ARNTL2: a key player in promoting tumor aggressiveness in papillary thyroid cancer

Qing Zhai[1#], Han Wu[1#], Shiqiang Liu[1#], Yuehong Zhu[1#], Xi Huang[2], Junfeng Wang[3]^

[1]Department of Endocrinology, The First Affiliated Hospital of Wannan Medical College, Yijishan Hospital, Wuhu, China; [2]Department of Pathology, The First Affiliated Hospital of Wannan Medical College, Yijishan Hospital, Wuhu, China; [3]Department of Gastrointestinal Surgery, The First Affiliated Hospital of Wannan Medical College, Yijishan Hospital, Wuhu, China

Contributions: (I) Conception and design: Q Zhai, H Wu, X Huang, J Wang; (II) Administrative support: S Liu, J Wang; (III) Provision of study materials or patients: Q Zhai, S Liu; (IV) Collection and assembly of data: H Wu; (V) Data analysis and interpretation: Q Zhai, Y Zhu; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equaly to this work.

Correspondence to: Dr. Junfeng Wang, MM. Department of Gastrointestinal Surgery, The First Affiliated Hospital of Wannan Medical College, Zheshan West Road, Wuhu 241000, China. Email: yjswangjunfeng@163.com.

Background: The increasing occurrence of thyroid cancer (TC), particularly papillary thyroid cancer (PTC), highlights our need for better diagnostic indicators and new therapeutic targets. Aryl hydrocarbon receptor nuclear translocator-like 2 (ARNTL2) plays a crucial function in multiple tumor types. Accordingly, we investigated the oncogenic function and molecular pathways associated with ARNTL2 in PTC.

Methods: Our study utilized the The Cancer Genome Atlas (TCGA) database to examine ARNTL2 expression, which was subsequently confirmed in PTC tissues and cell lines employing quantitative real-time polymerase chain reaction (qRT-PCR) and western blot (WB) analysis. Herein, we evaluated PTC cell proliferation, cell cycle progression, apoptosis, migration, and invasion through Cell Counting Kit-8 (CCK-8), flow cytometry, wound-healing assay, and Transwell assay. Eventually, we determined the mechanism behind ARNTL2 in PTC via WB.

Results: In PTC, a significant ARNTL2 upregulation was observed, which exhibited a positive correlation with enhanced tumor aggressiveness. Additionally, knocking down ARNTL2 facilitated apoptosis, besides impeding cell cycle progression, cell proliferation, migration, and invasion, alongside epithelial-mesenchymal transition (EMT) in PTC. However, the outcomes were reversed when ARNTL2 was overexpressed. Enhanced expression of ARNTL2 led to an elevation in phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) phosphorylation within PTC cells, while the administration of alpelisib effectively mitigated the effects induced by upregulated ARNTL2 on EMT, PTC cell proliferation, apoptosis, and invasion.

Conclusions: Elevated ARNTL2 levels enhance PTC proliferation, migration, invasion, and EMT while inhibiting apoptosis through the cell cycle signaling, elucidating its potential as a diagnostic PTC biomarker.

Keywords: Aryl hydrocarbon receptor nuclear translocator-like 2 (ARNTL2); papillary thyroid cancer (PTC); tumorigenesis; epithelial-mesenchymal transition; phosphoinositide 3-kinase/protein kinase B (PI3K/AKT)

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^ ORCID: 0000-0001-8658-2184.

Introduction

Thyroid cancer (TC) mostly originates from the follicular epithelium of the thyroid gland, with a progressively increasing incidence in recent years. Based on epidemiological research, TC has a significant 240% increase in its incidence rate in recent decades (1,2). Moreover, TC encompasses various histologic subtypes, namely papillary, follicular, medullary, and undifferentiated cancer. Papillary thyroid cancer (PTC) constitutes about 90% of TC incidence, and the augmented PTC incidence and mortality rates contribute to an overall rise in the prevalence of thyroid malignancies (3,4). The therapeutic approaches for PTC encompass surgical intervention, radioactive iodine, thyroid-stimulating hormone (TSH) suppression, and targeted therapy. Patients with PTC generally exhibit favorable prognoses following conventional surgical intervention and radioactive iodine therapy; however, 20% to 30% of PTC patients experience recurrence, and 5% to 10% develop progressive and refractory disease (5-7). Accordingly, identifying diagnostic indicators and innovative therapeutic targets for PTC recurrence and metastasis is crucial.

Highlight box

Key findings

- This study demonstrates that aryl hydrocarbon receptor nuclear translocator-like 2 (ARNTL2) is significantly upregulated in papillary thyroid cancer (PTC) and correlates positively with tumor aggressiveness. Knockdown of ARNTL2 inhibits cell proliferation, migration, and invasion while promoting apoptosis. Conversely, overexpression leads to enhanced tumorigenic properties.

What is known and what is new?

- Previous research has indicated that ARNTL2 plays a role in various cancers, including breast and renal cancers, often linked to poor prognosis.
- This manuscript adds novel evidence of ARNTL2's oncogenic function specifically in PTC, highlighting its influence on the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) signaling pathway and epithelial-mesenchymal transition (EMT).

What is the implication, and what should change now?

- The findings suggest that ARNTL2 could serve as a valuable diagnostic biomarker for PTC and a potential therapeutic target. Future clinical strategies should incorporate ARNTL2 assessment for risk stratification in PTC patients, and further investigations into targeted therapies that inhibit ARNTL2 may improve patient outcomes.

Circadian rhythms are recurring 24-hour cyclic patterns that govern and regulate a diverse range of biological functions (8). Recent research findings suggest that disrupting circadian rhythm is pivotal in various cancer initiation and progression (9). Aryl hydrocarbon receptor nuclear translocator-like 2 (ARNTL2) protein, a Per-ARNT-Sim (PAS) superfamily member, is a crucial circadian transcription factor actively involved in the orchestration of feedback loops that contribute to the generation of nearly 24-hour rhythmicity. Moreover, its crucial involvement has been demonstrated in diverse tumor types, including triple-negative breast cancer and clear cell renal cell carcinoma, where ARNTL2 is associated with poor prognosis and immune infiltration (10-12). Moreover, the malignant progression of colorectal carcinoma is effectively impeded by the reduction in ARNTL2 levels, resulting in a decrease in secreted modular calcium-binding protein2 (SMOC2) expression and the PI3K/AKT pathway inhibition (13). Additionally, ARNTL2 oncogenic function has been extensively documented in several cancers such as breast cancer, non-small cell lung cancer, and pancreatic ductal adenocarcinoma (14-16). Nevertheless, the probable functions of ARNTL2 in PTC remain unelucidated.

Our study explored the role of ARNTL2 and elucidated its underlying molecular mechanisms in PTC, thereby providing novel evidence on the oncogenic function and molecular pathways associated with ARNTL2 in PTC. We present this article in accordance with the MDAR reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1205/rc).

Methods

Bioinformatics analysis

Our study acquired the gene expression data and medical records through The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/), extracting the data in the level 3 HTSeq fragments per kilobase per million (FPKM) format. ARNTL2 expression levels were observed in 33 human cancer types using the TCGA database, along with 512 cases of TC and 59 non-malignant tissues. Herein, we generated the receiver operating characteristic (ROC) curve by employing the pROC package in R and conducted enrichment analyses utilizing the clusterProfiler package for Kyoto Encyclopedia of Genes and Genomes (KEGG), Gene Ontology (GO), and gene set enrichment analysis (GSEA).

Tissue samples

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by institutional ethics committee of The First Affiliated Hospital of Wannan Medical College, Yijishan Hospital (No. 2024-76) and informed consent was obtained from all individual participants. Between 2022 and 2023, we collected a total of 32 tumor tissues along with the corresponding adjacent noncancerous tissues from PTC patients who received thyroidectomy at Yijishan Hospital hospital (11 males and 21 females, aged 20–69 years, median age: 41 years). All clinical samples underwent histopathological diagnosis performed by a panel of 2-3 expert pathologists.

Cell culture and reagents

PTC cell lines TPC-1 (Catalog No. SNL-231) and IHH4 (Catalog No. SNL-507), along with the normal human thyroid cell line Nthy-ori3-1 (Catalog No. SNL-213), were procured from Sunncell Bioscience (Wuhan, China). The PTC cell line K1 (Catalog No. E0447) was obtained from Boke Biotechnology (Shanghai, China). The culture was performed in RPMI 1640 medium (Gibco, USA) that contained 10% fetal bovine serum (FBS; Gibco, USA) under a controlled environment of 37 ℃ and 5% CO_2. Alpelisib was ordered from MCE (CAS No. 1217486-61-7, Shanghai, China).

Lentivirus-mediated ARNTL2 overexpression and knockdown

The design and synthesis of ARNTL2 overexpression and knockdown lentivirus were conducted by Genechem (Shanghai, China). The lentivirus was diluted with OPTI-MEM to achieve a multiplicity of infection (MOI) of 10 for transfecting TPC-1 cells in 24-well plates. Incubation was conducted at 5% CO_2 and 37 ℃ using OPTI-MEM for a duration of 24 hours. Afterward, we replaced the medium with RPMI 1640 with 10% FBS supplements, and culturing continued for a further 48 hours under the same conditions. Transfected cells were obtained through selective culturing with puromycin dihydrochloride (Beyotime, China). The overexpression and knockdown efficiencies were assessed using the western blot (WB) assay.

WB assay

Protein extraction was conducted through radioimmunoprecipitation assay (RIPA) buffer containing a mixture of phosphatase and protease inhibitors (Beyotime, China), quantifying the protein with the enhanced bicinchoninic acid (BCA) Protein Assay Kit (Beyotime, China). The protein samples underwent electrophoresis on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and subsequent transfer onto a polyvinylidene difluoride membrane (Merck-Millipore, USA), blocking the blots with 5% milk for a duration of 1 hour and incubated at 4 ℃ for a period of 12 hours with these primary antibodies: anti-ANRTL2 antibody (1:1,000, ab221557), anti-p-PI3K antibody (1:500, ab182651), anti-PI3K antibody (1:1,000, ab302958), anti-p-AKT antibody (1:500, ab38449), anti-AKT antibody (1:500, ab8933), anti-E-cadherin antibody (1:1,000, ab231303), anti-Vimentin antibody (1:1,000, ab16700), anti-N-cadherin antibody (1:5,000, ab76011), anti-GADPH antibody (1:1,000, ab8245); all sourced from Abcam. This was followed by a subsequent incubation with the corresponding secondary antibodies for 1 hour at 37 ℃. Eventually, we visualized the band via enhanced Luminol/Enhancer chemiluminescence (Bio-Rad, USA).

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Initially, total RNA extraction was conducted through TRIZOL reagent (Invitrogen, USA) and then reversely transcripted to generate cDNA. Subsequently, we conducted qRT-PCR analysis utilizing the ABI system (Applied Biosystems, USA). The primer sequence was: ARNTL2 (F: 5'-TCCAGTACACAAACGTGGTAAG-3', R: 5'-TCTACGCACTGTCCCTCTAATA-3'), GAPDH (F: 5'-GTGGACCTGACCTGCGTCT-3', R: 5'-GTGTCGCTGTTGAAGTCAGAGGAG-3'). The target gene mRNA expression level was normalized relative to the reference gene GADPH.

Apoptosis and cell cycle analysis

Our study employed flow cytometry to analyze apoptosis and cell cycle by following standardized procedures. The cell culture was conducted in 6-well plates and harvested at a 1×10^6 cells/well density. The Annexin V-FITC/PI

apoptosis detection kit (YEASEN, China) was employed in flow cytometry analysis to evaluate cell apoptosis following two cycles of PBS washing. In addition, propidium iodide (YEASEN, China) was employed for conducting cell cycle analysis. The data obtained were analyzed using FlowJo 10.6.2.

Cell proliferation assay

Employing the Cell Counting Kit-8 (CCK-8) Assay Kit (Biosharp, China), we assessed cell viability. The culture of cell suspensions containing 1×10^4 cells per well (100 µL) was performed in 96-well plates for 24, 48, and 72 hours before being treated with an additional 2-hour incubation using a CCK-8 reagent (10 µL). Our study utilized a microplate reader (YUNKE, China) for absorbance measurement at 450 nm.

Transwell assay

To investigate the invasion of cells, we employed 24-well Transwell invasion chambers with Matrigel-coated membranes (Corning, USA). A free-medium serum was used to introduce 5×10^4 cells into the upper chambers while adding media that contained 10% FBS into the lower chamber. Post-24-hour incubating, we employed a solution containing 0.5% crystal violet to immobilize and color the cells that effectively traversed the membrane filter. Following this, microscopic observation was conducted to count these cells.

Wound healing assay

The cells went through culture in six-well plates until reaching 90% cellular density. A sterile 200 µL pipette tip was utilized for the creation of a straight scratch. Subsequently, the wound was rinsed with PBS, culturing the cells in serum-free 1640 medium for a duration of 12 hours. The microscope was employed to capture images of the wound area, which were subsequently measured using ImageJ software.

Statistical analysis

The SPSS 24.0 software was carried out for the statistical analysis, expressing the results as mean ± standard deviation (SD). The bioinformatics analysis was conducted using R version 4.2.1. The One-way analysis of variance (ANOVA) and the Tukey Post Hoc Test were deployed to conduct comparisons among different groups, while utilizing the Student's t-test to compare two groups. All experiments were performed in triplicate, and the data were obtained from technical replicates. $P<0.05$ indicated statistically significant differences.

Results

ARNTL2 shows overexpression in TC

According to a comprehensive TCGA database analysis, a significant ARNTL2 upregulation was observed across multiple cancers compared to their respective normal tissues, including TC (*Figure 1A*,*1B*). Additionally, a paired line graph of 59 pairs of TC tissues and matched nontumorous samples revealed higher ARNTL2 mRNA expression in most TC tissues compared to adjacent normal tissues (*Figure 1C*). The predictive capability of ARNTL2 in identifying TC was assessed via ROC analysis, revealing an area under the curve (AUC) value of 0.736 (*Figure 1D*). The association between heightened levels of ARNTL2 and advanced T stage, N stage, as well as extrathyroidal extension demonstrated statistically significant correlations (*Figure 1E-1G*). The WB and qRT-PCR analysis results showcased that ARNTL2 experienced a significant overexpression in PTC tissues compared to healthy tissues (*Figure 1H*,*1I*).

ARNTL2 inhibits PTC cell apoptosis, enhances cell proliferation, and induces cell cycle progression

The ARNTL2 expression levels were evaluated in the Nthy-ori3-1 normal human thyroid cell line as well as in the TPC-1, K1, and IHH PTC cell lines. Remarkably, significant ARNTL2 upregulation was observed across all PTC cells, particularly in TPC-1 cells (*Figure 2A*). To investigate ARNTL2's functional importance in enhancing PTC cell development *in vitro*, stable TPC-1 cells were generated with increased or decreased ARNTL2 expression levels through lentiviral transduction. The efficacy of overexpression and knockdown was validated through WB analysis (*Figure 2B*). The results of flow cytometry manifested that ARNTL2 overexpression significantly mitigated TPC-1 cell apoptosis, whereas suppression of ARNTL2 expression induced apoptosis (*Figure 2C*). CCK-8 analysis manifested that ARNTL2 overexpression significantly promoted TPC-1 cell proliferation, whereas

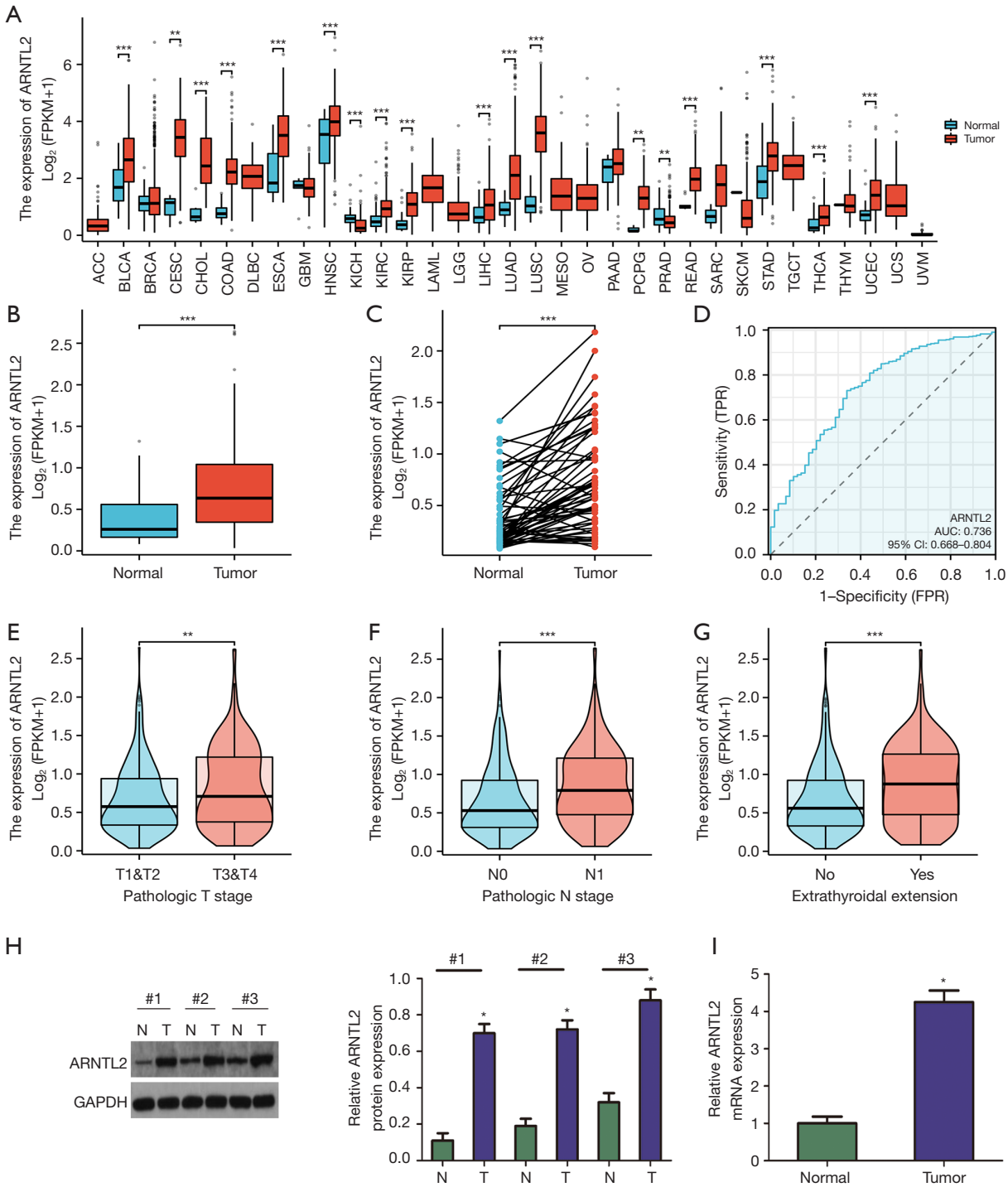

Figure 1 ARNTL2 overexpression in TC. (A) ARNTL2 levels across various human tumor types were compared utilizing the TCGA database. (B,C) The ARNTL2 significant overexpression in TC was further corroborated through the utilization of the TCGA database. (D) Diagnostic ROC curve for discriminating between TC tissues and normal tissues. (E-G) The ARNTL2 overexpression was significantly related to advanced T/N stages and extrathyroidal extension. (H,I) Assessment of ARNTL2 expression in both PTC and adjacent non-tumor tissues through Western blotting and qRT-PCR analysis. *, P<0.05; **, P<0.01; ***, P<0.001. FPKM, fragments per kilobase per million; AUC, area under the curve; TPR, true positive rate; FPR, false positive rate; N, normal; T, tumor; TC, thyroid cancer; TCGA, The Cancer Genome Atlas; ROC, receiver operating characteristic; qRT-PCR, quantitative real-time polymerase chain reaction.

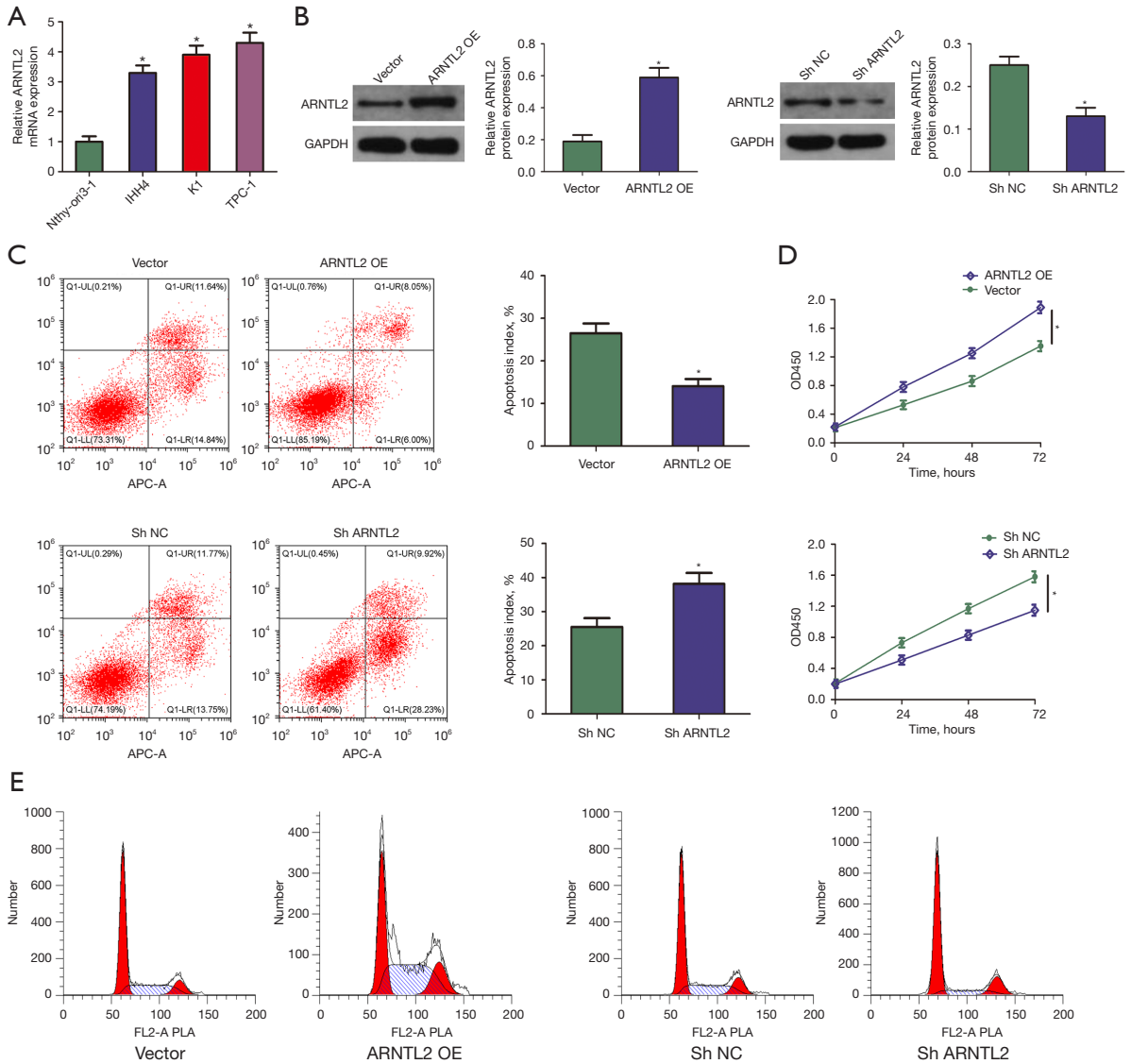

Figure 2 ARNTL2 inhibits apoptosis, promotes PTC proliferation, and induces cell cycle progression. (A) ARNTL2 expression assessment in TPC-1, K1, and IHH4 human thyroid cancer cell lines and in the Nthy-ori3-1 normal human thyroid cell line through RNA analysis. (B) Assessment of transfection efficiency via Western blot analysis. (C) Assessment of cell apoptosis extent through flow cytometry technique. (D) Assessment of TPC-1 cell proliferation via the CCK-8 assay. (E) The cell cycle analysis of TPC-1 cells through flow cytometry. *, P<0.05. Sh, short hairpin RNA; NC, normal control; OE, overexpression; PTC, papillary thyroid cancer; CCK-8, Cell Counting Kit-8.

silencing ARNTL2 notably suppressed TPC-1 cell proliferation (*Figure 2D*). The results of flow cytometry revealed that TPC-1 cells overexpressing ARNTL2 had reduced G0/G1 phase and elevated G2/M phase distribution, while knocking down ARNTL2 resulted in the opposite effect (*Figure 2E*).

ARNTL2 promotes TPC-1 cell invasiveness and migratory capacity

We subsequently evaluated the impact of ARNTL2 on PTC cell invasion and migration abilities. Transwell invasion experiments showcased that ARNTL2

upregulation significantly promoted cell invasion, while its downregulation resulted in its inhibition (*Figure 3A*). The overexpression of ARNTL2 was observed to enhance TPC-1 cell migration in wound healing assays, whereas a significant attenuation of this effect was observed upon the knockdown of ARNTL2 (*Figure 3B*).

Identification and functional enrichment analysis of ARNTL2-associated genes using the TCGA-THCA dataset

TCGA-THCA dataset was utilized to identify genes that exhibited a robust correlation with ARNTL2, employing a screening criterion of |log2FC|>1.5 to ascertain significant up-regulation and down-regulation (*Figure 4A*). The genes were subsequently subjected to a comprehensive examination using the KEGG and GO for an in-depth analysis. The KEGG analysis elucidated a significant enrichment of up-regulated genes primarily within the PI3K/AKT pathway (*Figure 4B*). Furthermore, the GO analysis (*Figure 4C*) demonstrated that up-regulated genes exhibited a predominant enrichment in processes associated with leukocyte cell-cell adhesion and the external side of the plasma membrane. In *Figure 4D*,*4E*, the KEGG analysis unveiled a notable enrichment of down-regulated genes primarily linked to the neuroactive ligand-receptor interaction, whereas the GO analysis represented that these down-regulated genes experienced a main enrichment in processes pertaining to signaling receptor activator activity and receptor-ligand activity.

ARNTL2 promotes proliferation invasion and EMT while inhibiting apoptosis in PTC cells via the PI3K/AKT pathway modulation

GSEA analysis via the TCGA-THCA dataset showcased a significantly enriched PI3K/AKT pathway and EMT in TC samples with elevated ARNTL2 expression levels (*Figure 5A*,*5B*). Based on the results obtained from our investigations utilizing KEGG and GSEA analyses, we proceeded to conduct WB analysis in PTC, aiming at determining ARNTL2's impact on the PI3K/AKT pathway and EMT. Enhanced ARNTL2 expression upregulated p-PI3K, p-AKT, Vimentin, and N-cadherin levels and simultaneously downregulated E-cadherin expression. Furthermore, alpelisib treatment, a potent PI3K inhibitor, effectively attenuated ARNTL2-induced EMT and the phosphorylation of PI3K and AKT (*Figure 5C*). WB findings provide evidence that the downregulation of ARNTL2 leads to a decrease in p-PI3K, p-AKT, Vimentin, and N-cadherin levels, simultaneously enhancing the expression of E-cadherin (*Figure 5D*). In addition, the findings revealed a notably reduced TPC-1 cell proliferation and invasion when treated with alpelisib, effectively counteracting the impact of ARNTL2 overexpression (*Figure 6A*,*6B*). Consistent with expectations, overexpression of ARNTL2 suppressed apoptosis, and subsequent treatment with alpelisib effectively reversed this phenotype in TPC-1 cells (*Figure 6C*).

Discussion

While partially ascribed to overdiagnosis, the global incidence of TC, particularly PTC, has risen significantly in recent years (17,18). Risk stratification and tailored management, along with the identification of novel prognostic markers, are crucial, yet unmet clinical goals in TC management (19). The current classification of benign and malignant thyroid nodules primarily relies on conventional ultrasonography as well as needle aspiration biopsy. However, the misdiagnosis rate in clinical practice remains a concern due to variations in operator expertise and discrepancies in sampling locations (20). Hence, the identification of molecular biomarkers for PTC is of utmost importance in facilitating early detection, management, and mitigation of postoperative relapse associated with PTC.

ARNTL2, also referred to as BMAL2, is a paralogous counterpart of BMAL1 and has the ability to form dimers with Clock protein, thereby inducing E-box-dependent trans-activation and effectively regulating biological rhythms (21). ARNTL2 is widely recognized as a prominent regulator, with numerous reports highlighting its oncogenic effects in various human malignancies. Recent investigation has unveiled the susceptibility of ARNTL2 as a metastasis-associated gene in estrogen receptor-negative BC (15). Brady *et al.* (22) manifested that ARNTL2 exhibited the ability to facilitate autonomous metastasis, with heightened levels of ARNTL2 serving as an indicator of unfavorable prognoses in patients diagnosed with lung adenocarcinoma. Wang *et al.* (14) observed that ARNTL2 constituted an independent adverse prognostic factor for clear cell renal cell carcinoma patient survival, and its overexpression was related to immune infiltration. In this study, bioinformatics analysis showcased a significantly upregulated ARNTL2 in human TC tissues compared to healthy tissues, which was further confirmed through examination of clinical specimens. Our findings further demonstrated a positive

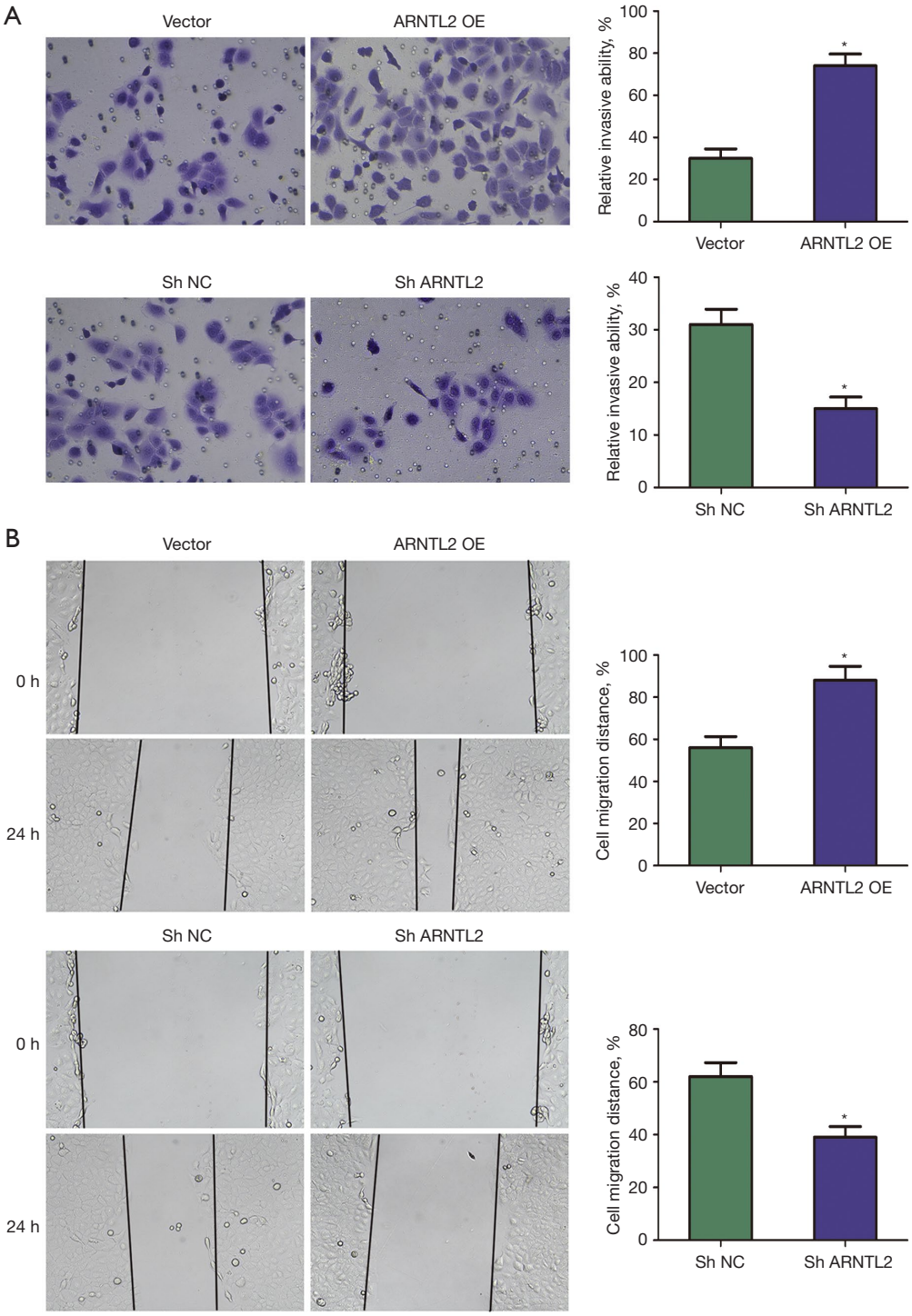

Figure 3 ARNTL2 promotes TPC-1 cell invasiveness and migratory capacity. (A) Cell invasion assessment in TPC-1 cells via the Transwell assay. Cells were stained with crystal violet. Images were taken at 200× magnification. (B) Migratory potential evaluation in TPC-1 cells using the wound-healing assay. Images were taken at 100× magnification. *, P<0.05. OE, overexpression; Sh, short hairpin RNA; NC, normal control.

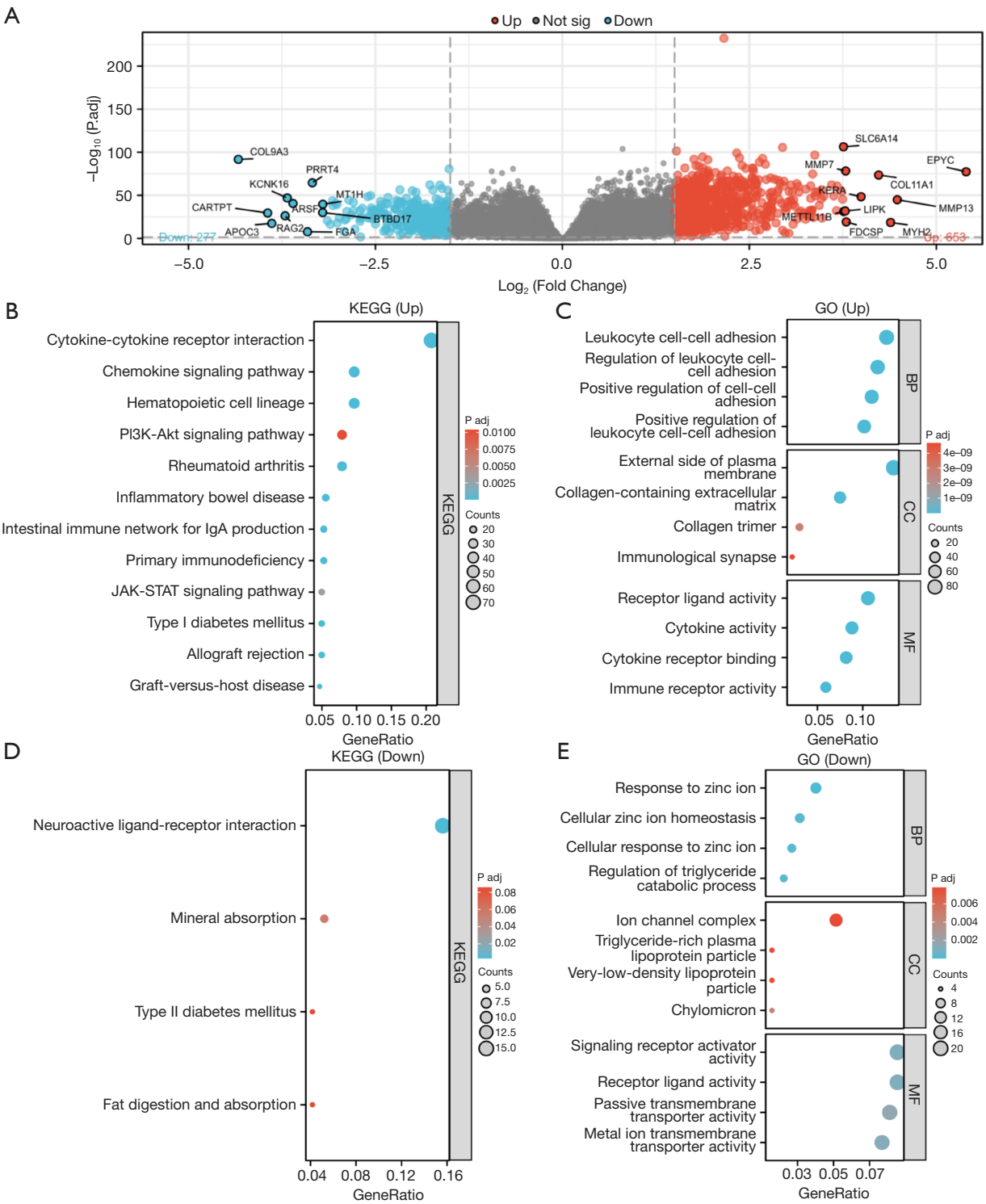

Figure 4 Identification and functional enrichment analysis of ARNTL2-associated genes using the TCGA-THCA dataset. (A) Employing the Volcano plot analysis to identify genes associated with ARNTL2 in the TCGA-THCA dataset. (B,C) The genes positively associated with ARNTL2 in PTC were subjected to the KEGG and GO analyses. (D,E) The ARNTL2-negatively-correlated genes in PTC were subjected to KEGG and GO analyses. KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology; BP, biological process; CC, cellular component; MF, molecular function; TCGA-THCA, The Cancer Genome Atlas–Thyroid Cancer; PTC, papillary thyroid cancer.

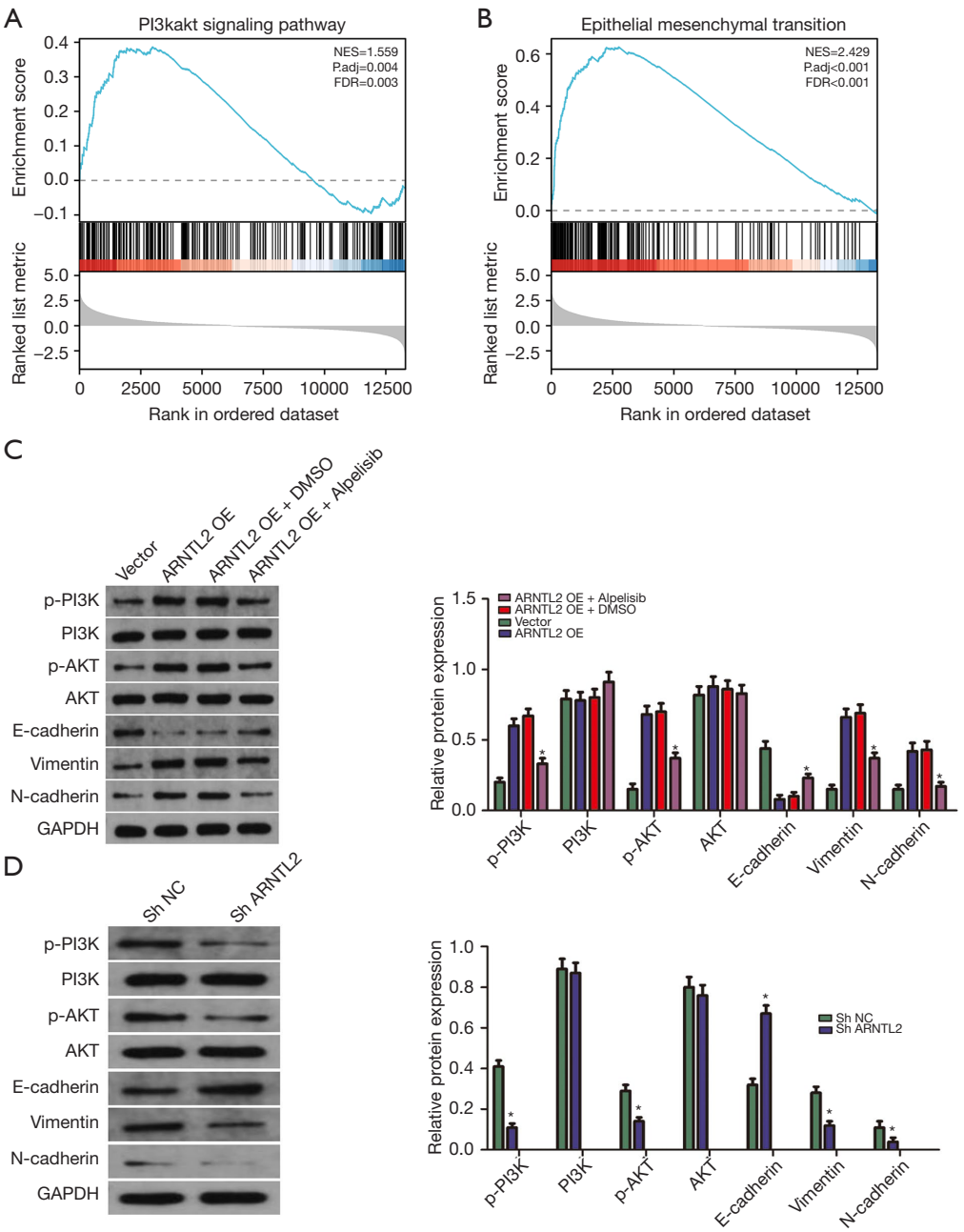

Figure 5 ARNTL2 regulates the EMT of PTC via the PI3K/AKT pathway. (A,B) The GSEA analysis identified notable differences in the enrichment of the PI3K/AKT pathway and EMT between samples with varying levels of ARNTL2 expression. (C,D) Assessment of PI3K, p-PI3K, AKT, and p-AKT protein expression levels in TPC-1 cells. Alpelisib treatment (10 μM) of TPC-1 cells of the ARNTL2 OE + alpelisib group. *, P<0.05. NES, normalize enrichment score; FDR, false discovery rate; OE, overexpression; DMSO, dimethyl sulfoxide; Sh, short hairpin RNA; NC, normal control; EMT, epithelial-mesenchymal transition; PTC, papillary thyroid cancer; PI3K/AKT; phosphoinositide 3-kinase/protein kinase B; GSEA, gene set enrichment analysis.

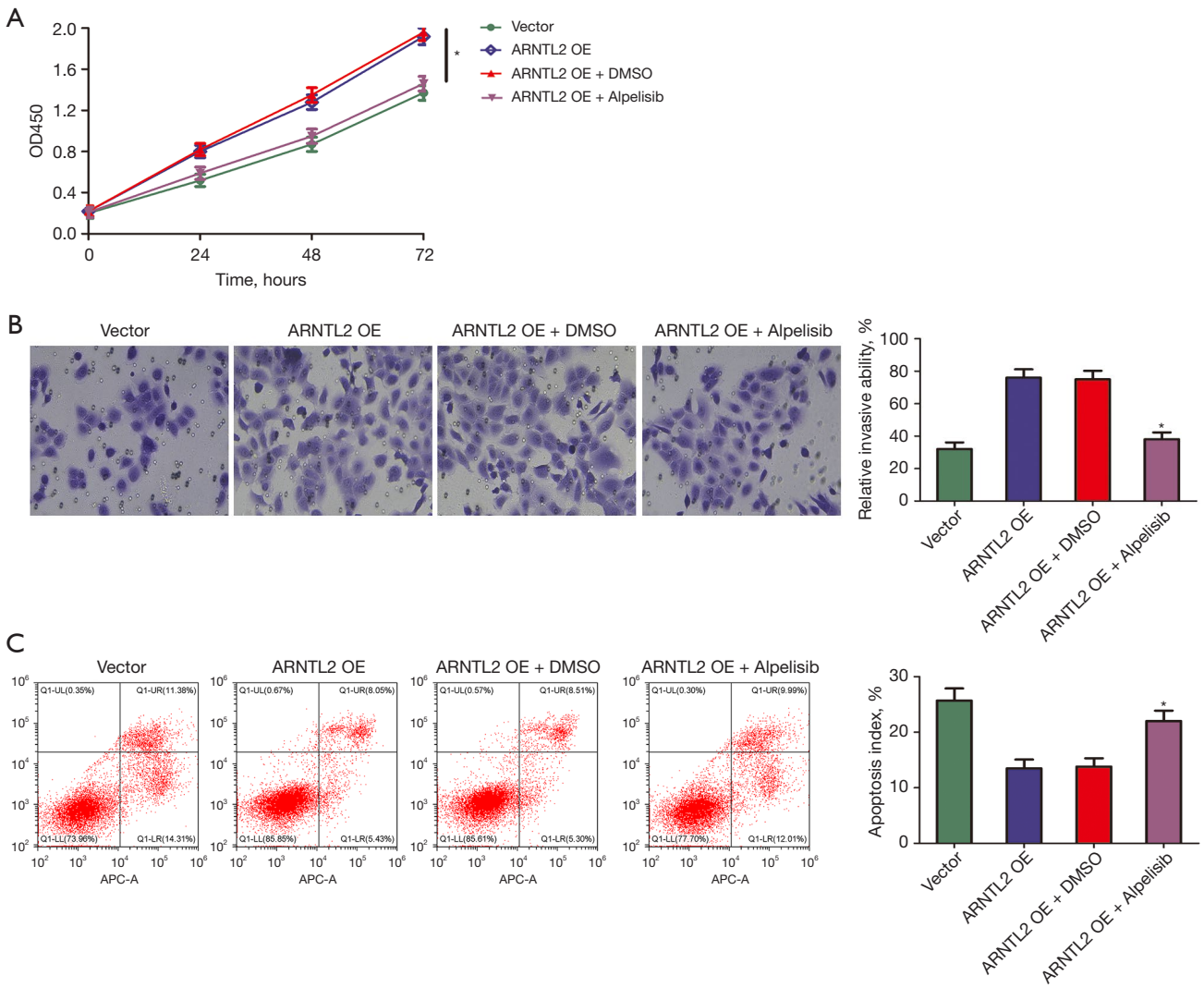

Figure 6 ARNTL2 promotes PTC proliferation and invasion while inhibiting apoptosis through the PI3K/AKT pathway modulation. (A,B) The significant attenuation of TPC-1 proliferation and invasion triggered by ARNTL2 overexpression upon treatment with alpelisib, as evidenced by CCK-8 and Transwell assays. Cells were stained with crystal violet. Images were taken at 200× magnification. (C) The significant mitigation of the inhibitory impact of ARNTL2 overexpression on TPC-1 apoptosis upon treatment with alpelisib, as evidenced by flow cytometry analysis. *, P<0.05. OE, overexpression; DMSO, dimethyl sulfoxide; PTC, papillary thyroid cancer; PI3K/AKT, phosphoinositide 3-kinase/protein kinase B; CCK-8, Cell Counting Kit-8.

correlation between elevated levels of ARNTL2 and augmented cell cycle progression, proliferation, invasion, and migration in PTC cells. Simultaneously, it was observed that overexpression of ARNTL2 suppressed apoptosis. Conversely, the downregulation of ARNTL2 exhibited contrasting effects.

EMT is crucial in facilitating cancer dissemination by endowing cancer cells with augmented motility and invasiveness, and it also has a close association with PTC metastatic progression (23,24). The PI3K/AKT pathway is an intracellular cascade triggered by receptor tyrosine kinases and contributes to governing distinct pathophysiological processes, including cell proliferation, DNA replication, and apoptosis, among others (25). PI3K/AKT pathway is initiated by PI3K activation, thereby phosphorylating phosphatidylinositol 4,5-bisphosphate

and leading to phosphatidylinositol 3,4,5-triphosphate generation. This subsequently triggers activating downstream effector molecules, including AKT, thereby facilitating PTC progression (26-28). Alpelisib (BYL719) is an effective inhibitor of PI3K, showing promising antitumor activity both *in vitro* and *in vivo* (29). Our study conducted GO, KEGG, and GSEA analyses, demonstrating that ARNTL2 overexpression was significantly related to both EMT as well as the PI3K/AKT pathway. The knocked-down of ARNTL2 hindered the PI3K/AKT pathway and EMT in PTC cells, with its overexpression having the opposite effects that can be counteracted by alpelisib administration. Therefore, our findings substantiate the pivotal function of ARNTL2 in augmenting the PI3K/AKT pathway and EMT, thereby facilitating the acquisition of a malignant phenotype in PTC cells.

Conclusions

Our results indicate that increased ARNTL2 levels can enhance PTC proliferation, migration, invasion, cell cycle progression, and EMT while inhibiting apoptosis by activating the PI3K/AKT pathway. Accordingly, we offer innovative perspectives on ARNTL2 function and mechanism in PTC, as well as its possible application as a diagnostic PTC biomarker.

Acknowledgments

None.

Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1205/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1205/dss

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1205/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 (as revised in 2013). The study was approved by institutional ethics committee of The First Affiliated Hospital of Wannan Medical College, Yijishan Hospital (No. 2024-76) and informed consent was obtained from all individual participants.

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