Original Article
Thyroid cancer prognostic biomarker ARL4A and its relationship with immune infiltration

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Abstract: Background: Thyroid cancer (THCA) is a prevalent form of cancer with high rates of morbidity and mortality. The small GTPase ADP-ribosylation factor-like 4A (ARL4A) is integral to various cellular processes, including cytoskeletal restructuring, vesicular transport, cell migration, and neuronal development. However, the role of ARL4A as a clinical predictor, particularly its relation to immune cell infiltration in THCA, remains unclear. Methods: A combination of experimental studies and analysis of online databases was employed to investigate ARL4A expression in THCA. Clinical and pathological data from THCA patients were compiled for a comprehensive subgroup analysis. The Kaplan-Meier and Cox regression methods were utilized to evaluate the prognostic significance of ARL4A in THCA patients. Finally, the "Cancer Genome Atlas" was analyzed to explore the correlation between immune cell infiltration, ARL4A expression, and their joint impact on prognosis. Results: ARL4A exhibited low expression in THCA. An elevated ARL4A was associated with poor prognosis. Moreover, the expression of ARL4A was correlated with the age, gender, and pathological stage of THCA patients. Finally, ARL4A expression was found to be negatively correlated with immune cell infiltration and influenced the prognosis of patients through changes in the immune environment. Conclusion: ARL4A may serve as a potential biomarker for the diagnosis and treatment of THCA, impacting the prognosis of patients through the modulation of the immune microenvironment.

Keywords: ADP-ribosylation factor-like 4A, thyroid cancer, prognosis, immunity

Introduction

Thyroid cancer (THCA) is one of the most common endocrine malignancies. According to statistics, in 2020, there were 449,000 new cases of thyroid cancer diagnosed globally in men and 137,000 in women, with incidence rates of 10.1 per 100,000 and 3.1 per 100,000, and mortality rates of 0.5 per 100,000 and 0.3 per 100,000, respectively [1]. Thyroid tumors exhibit diverse pathological types with unique genomic instabilities and heterogeneities. Most thyroid carcinomas are differentiated thyroid carcinoma (DTC), which generally has a favorable prognosis. Conversely, anaplastic thyroid carcinoma (ATC), which accounts for about 1% to 2% of all thyroid carcinomas, is highly malignant and associated with poorer prognosis, often presenting with distal and focal metastases [2], and a median survival of merely 3 to 4 months post-diagnosis [3]. Current conventional treatments like radiotherapy and chemotherapy offer limited survival benefits, necessitating an exploration of integrated treatment strategies combining surgery, radiotherapy, and targeted therapy [4]. This highlights the urgent need to elucidate the molecular biological mechanisms underlying THCA development and progression, and to enhance diagnostic and therapeutic protocols.

ADP-ribosylation factor-like 4A (ARL4A), a member of the ADP-ribosylation factor family of GTP-binding proteins, is implicated in various cellular events such as cytoskeletal remodeling, vesicular transport, cell migration, and neuronal development [5]. ARL4A plays a pivotal role in cell migration by facilitating cytoskeletal reorganization and cell membrane stretching, crucial in processes like neural guidance and cell migration. Its significance is underscored through its interaction with Robo1, a trans-

membrane receptor, aiding cell migration by modulating Cdc42 activation [6]. Additionally, ARL4A is integral in various signaling pathways, notably in enhancing and sustaining intracellular signaling. By interacting with specific protein complexes, it influences intracellular signaling molecules, thereby impacting cell growth, differentiation, and migration [7, 8]. Notably, elevated levels of ARL4A expression have been observed in gliomas, hinting at a potential role in tumor development and metastasis. The relevance of ARL4A in cell migration and invasiveness is particularly intriguing, as these are crucial aspects in cancer progression [9]. Despite these findings, the specific functional role of ARL4A in THCA remains unexplored, highlighting an urgent need to investigate its function and pathogenesis in THCA.

Our investigation aimed to validate the expression level of ARL4A in THCA cells through experimental techniques and bioinformatics tools. We discovered a significant association between ARL4A expression and clinicopathological characteristics, as well as the prognosis of individuals diagnosed with THCA. Moreover, our study revealed a noteworthy link between ARL4A expression and immune cell infiltration.

Materials and methods

Cell culture

The Institute of Cell and Bio-Biochemistry at the Shanghai Academy of Life Sciences provided the THCA cell lines (TPC1, BCPAP) and normal cell line (Nthy-ori3-1). These cells were cultured in DMEM (Gibco, Carlsbad, CA) medium, supplemented with 10% fetal bovine serum (FBS) (Gibco, Carlsbad, CA). When reaching approximately 80% confluence, cells were subcultured in a cell incubator maintained at 37°C, with saturated humidity and 5% CO_2.

Western blotting

Cells were lysed using chilled RIPA buffer (Beyotime), followed by centrifugation at high speed for 10-15 minutes at 4°C for protein extraction. The supernatant was carefully transferred to a new tube. Protein quantification was performed using the BCA kit (Beyotime) according to the manufacturer's instructions, involving the mixture of the sample with the BCA working solution and incubation at 37°C

for 30 minutes. Protein concentrations were determined from the standard curve. Proteins were separated via SDS-PAGE electrophoresis and transferred onto a PVDF membrane. The membrane was blocked using 5% skimmed milk at room temperature for 1 hour. Primary antibodies targeting β-actin (Beyotime) and ARL4A (Absin Biotechnology) were incubated overnight at 4°C. Following this, the membrane was washed three times with PBST, incubated with the secondary antibody at room temperature for 1 hour, and washed three more times with PBST. Luminescence was detected employing the ECL method. Protein band intensities were quantified using Image J (National Institutes of Health, Bethesda, MD), and the data normalized against the intensity of the β-actin bands.

Resource for estimating tumor immunity

The tumor immune estimating resource (TIMER) online service (https://timer.cistrome.org/) [10] contains 10,897 samples from "The Cancer Genome Atlas" (TCGA) and estimates tumor-infiltrating immune cells across 32 different cancer types by re-analyzing gene expression data [11]. We utilized the "TIMER-Gene" module to explore the relationship between ARL4A expression and immune infiltration in patients with THCA. The "Immune-gene" module of TCGA was also employed to evaluate the association between ARL4A expression and immunological infiltration.

XIANTAO academic platform

The XIANTAO platform (https://www.xiantao.love/) provided data on ARL4A expression and its prognostic implications. Expression levels of ARL4A and clinicopathological factors from XIANTAO platform were used to develop a nomogram. The association between ARL4A expression and immune cell markers was examined using the "Immune Infiltration" panel.

Analysis of mutations in genetics

Features within the TCGA pan-cancer atlas were evaluated using the cBioPortal site (https://www.cbioportal.org/) [12], enabling the investigation of the ARL4A alteration frequency, copy number alteration, and mutation type.

GEPIA2

Data on the top 100 ARL4A-correlated genes were gathered, and the expression correlation between ARL4A and the top three genes was examined. This data was obtained through GEPIA2 [13], a platform for analyzing RNA sequencing data from TCGA and GTEx studies (https://gepia2.cancer-pku.cn/).

String analysis

The STRING website (https://string-db.org) [14] was used to construct the network of ARL4A-binding proteins. The "Search" module was initiated to locate the protein "ARL4A" in "Homo sapiens". The following parameters were set: active interaction source ("Textmining and experiment"), minimum needed interaction score ("low confidence (0.150)"), and the maximum number of interactors to show ("no more than 50 interactors" in the first shell), along with others as "default".

Kaplan Meier Plotter database analysis

The Kaplan-Meier (KM) Plotter (https://kmplot.com) [15] was utilized to determine ARL4A's prognostic ability in THCA. Patients were categorized into high and low expression groups based on median expression for studying overall survival (OS) and recurrence-free survival (RFS).

Statistical analysis

For the statistical analysis, we utilized GraphPad Prism (version 8.0.1). The Student's t-test was employed to compare measurement data between two groups that exhibited normal distribution and equal variance. For analyses involving more than two groups, the ANOVA was utilized. For datasets not adhering to normal distribution or when the distribution type was unknown, the nonparametric rank-sum test was applied, and for comparisons across multiple groups, the Kruskal-Wallis test was used. The impact of gene expression differences on patient survival was assessed using Kaplan-Meier survival analysis. Additionally, COX regression analysis was conducted to investigate the relationship between gene expression variability and patient survival, aiming to identify potential risk factors affecting the survival of THCA patients. A *p*-value of less than 0.05 was considered indicative of statistical significance.

Results

Expression analysis of ARL4A in THCA

Figure 1A displays the expression level of ARL4A mRNA in cancer cells, derived from TCGA (33 cancer types) and GETx data, as analyzed using the XIANTAO tool. The combined results indicated differential expression of ARL4A in 15 cancer types, with notably lower expression in 7 cancers, including THCA ($P < 0.001$, **Figure 1B**). Similarly, the expression of ARL4A was also reduced in paired samples of THCA ($P < 0.001$, **Figure 1C**). Next, to further explore the diagnostic potential of ARL4A in THCA, a receiver operating characteristic curve was plotted, revealing a 1-year area under the curve of 0.879 (**Figure 1D**), thus suggesting a positive diagnostic effect. Western blotting experiments indicated that ARL4A protein levels were lower in TPC1 and BCPAP cell lines compared to Nthy-ori3-1 (**Figure 1E**).

Correlation between ARL4A expression and clinical parameters in THCA patients

To further determine the significance of ARL4A expression in THCA, the correlation between ARL4A expression and clinical parameters was analyzed using the XIANTAO online tool. Reduced expression of ARL4A was observed in both male and female patients compared to normal levels (**Figure 2A**). Moreover, ARL4A expression levels were significantly lower in individuals of all ages relative to healthy individuals (**Figure 2B**). Furthermore, decreased ARL4A expression was significantly associated with advanced TNM stage (**Figure 2C-F**).

Prognostic significance of ARL4A expression in THCA

To evaluate the prognostic value of ARL4A expression in THCA patients, we stratified the patients into high and low-expression groups based on their ARL4A levels. As shown in **Figure 3**, ARL4A overexpression in THCA patients correlated with poor overall survival (OS), as indicated by analyses from KM Plotter, GEPIA2, and XIANTAO online tool. Subsequently, we investigated the association between ARL4A expression, tumor characteristics, and progno-
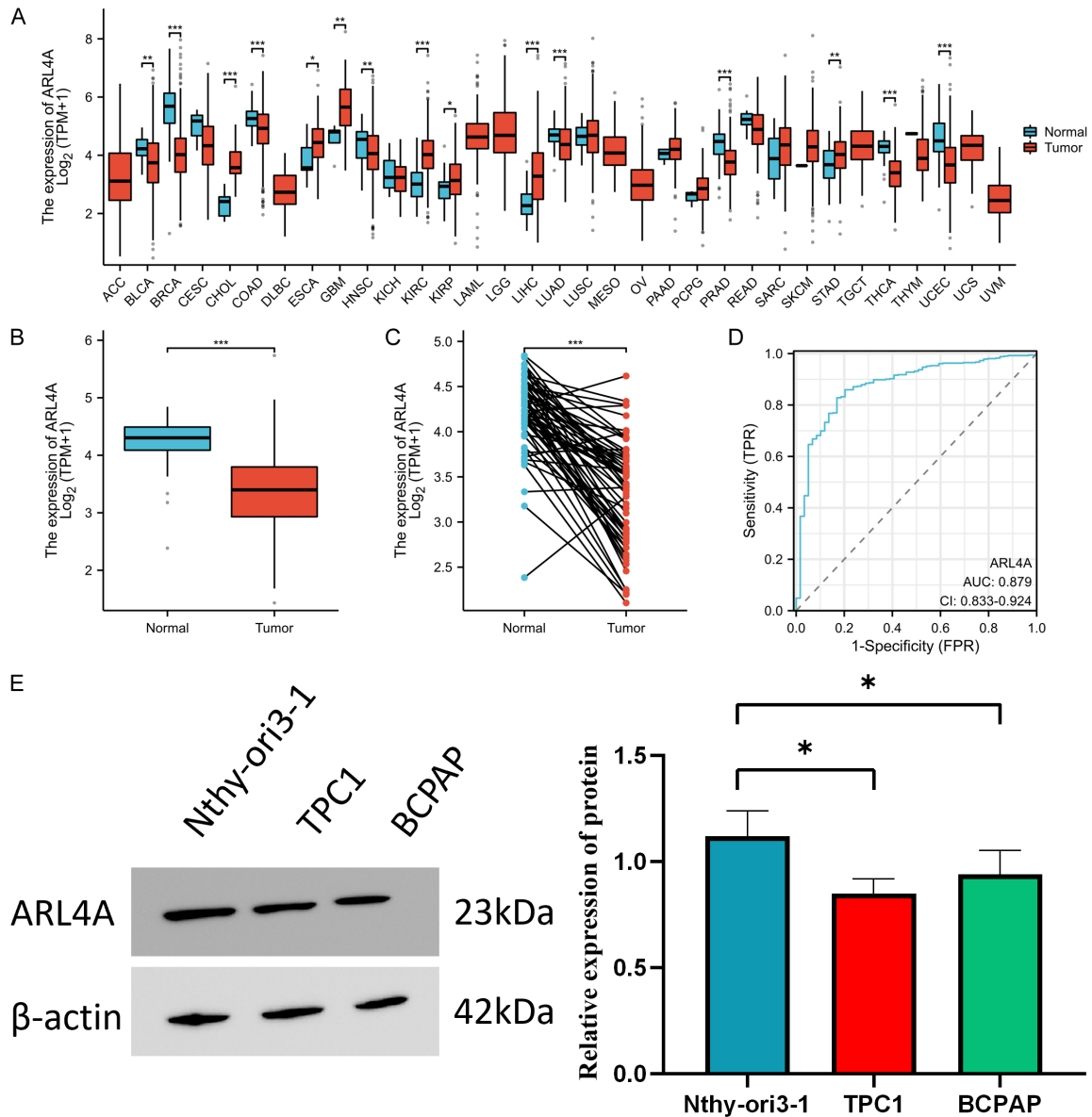
Figure 1. Expression of ARL4A in THCA. A. Analysis of ARL4A expression in TCGA and GETx databases. B. Comparison of ARL4A expression levels in cancer and normal tissues using TCGA data. C. Differential expression of ARL4A mRNA in THCA tissue and adjacent normal tissue in TCGA. D. Diagnostic value of the ARL4A gene in THCA. E. Expression levels of ARL4A in TPC1, BCPAP, and Nthy-ori3-1 cell lines, as analyzed by Western blotting. *, P < 0.05; **, P < 0.01; ***, P < 0.001. LAML: acute myeloid leukemia, ACC: adrenocortical carcinoma, BLCA: bladder urothelial carcinoma, LGG: brain lower grade glioma, BRCA: breast invasive carcinoma, CESC: cervical squamous cell carcinoma and endocervical adenocarcinoma, CHOL: cholangiocarcinoma, ESCA: esophageal carcinoma, GBM: glioblastoma multiforme, HNSC: head and neck squamous cell carcinoma, KICH: kidney chromophobe, KIRC: kidney renal clear cell carcinoma, KIRP: kidney renal papillary cell carcinoma, LIHC: liver hepatocellular carcinoma, LUAD: lung adenocarcinoma, LUSC: lung squamous cell carcinoma, DLBC: lymphoid neoplasm diffuse large B-cell lymphoma, MESO: mesothelioma, OV: ovarian serous cystadenocarcinoma, PAAD: pancreatic adenocarcinoma, PCPG: pheochromocytoma and paraganglioma, PRAD: prostate adenocarcinoma, READ: rectum adenocarcinoma, SARC: sarcoma, SKCM: skin cutaneous melanoma, STAD: stomach adenocarcinoma, TGCT: testicular germ cell tumors, THYM: thymoma, THCA: thyroid carcinoma, UCS: uterine carcinosarcoma, UCEC: uterine corpus endometrial carcinoma, UVM: uveal melanoma.

sis. Higher ARL4A expression was associated with poorer OS in stage 3 and 4 THCA patients,

across varying tumor stages. This presents a paradoxical observation where ARLA4 is less
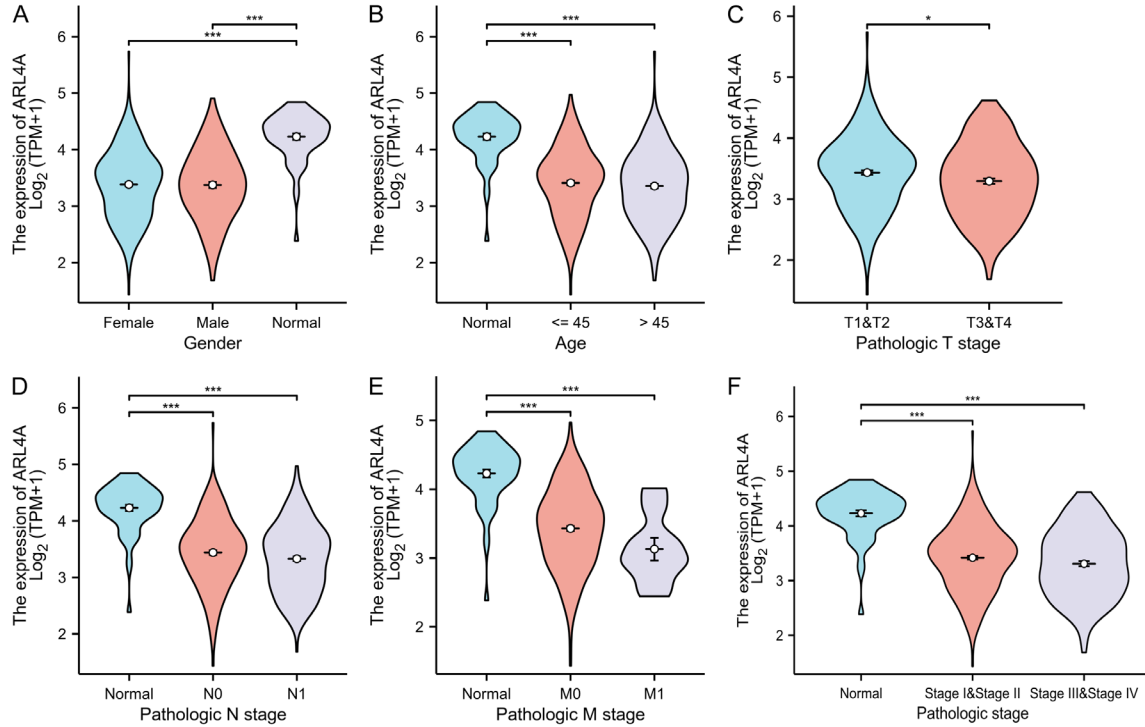
Figure 2. ARL4A expression in THCA patients according to different clinical characteristics (TCGA Data). A. Gender -based expression. B. Age -related expression. C. T classification comparison. D. N classification comparison. E. M classification comparison. F. Pathologic stage comparison. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

expressed in cancerous tissues compared to adjacent normal tissues. However, lower expression in cancer tissue corresponded with improved prognosis, suggesting that while ARL4A may act as an oncogene, its pathway could be generally suppressed or regulated, leading to its low expression. Nonetheless, it still possesses the potential to influence tumor growth, albeit not as a primary driver gene [16].

Analysis of ARL4A interaction-related genes and their genetic variations

To investigate the interactions between ARL4A and other proteins in THCA, we utilized the STRING online tool to create a protein-protein association (PPI) network (**Figure 4A**). Employing the GEPIA2 tool, we identified the top 100 genes associated with ARL4A expression. As shown in **Figure 4B**, ARL4A expression demonstrated positive correlations with NUAK family kinase 1 (NUAK1) (R = 0.62), connector enhancer of kinase suppressor of Ras 3 (CNKSR3) (R = 0.6), and Kruppel-like factor 9 (KLF9) (R = 0.6). A heatmap effectively illustrated the positive association between ARL4A and these genes across various cancer types (**Figure 4D**). The intersection of the predicted

genes from the two datasets yielded two groups, two common genes: FERM domain containing 4A (FRMD4A) and Jun dimerization protein 2 (JDP2) (**Figure 4C**).

An enrichment analysis of 52 genes associated with ARL4A was performed to predict their functional roles (**Figure 5A-F**). Gene Ontology (GO) enrichment analysis identified highly enriched GO terms such as "Ras protein signal transduction", "regulation of small GTPase mediated signal transduction", "tight junction", and "GTPase regulator activity". The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis revealed that the majority of these genes were involved in "endocytosis" and the "phospholipases D signaling pathway". Using the cBioPortal tool in the TCGA dataset, we further investigated the mutational characteristics of ARL4A in THCA, finding that the mutant frequency of ARL4A was particularly low in THCA (Figure S1).

Analyzing the relationship between ARL4A expression and immune cell infiltration

We proceeded to examine correlation between ARL4A and immune cells in THCA. As shown in
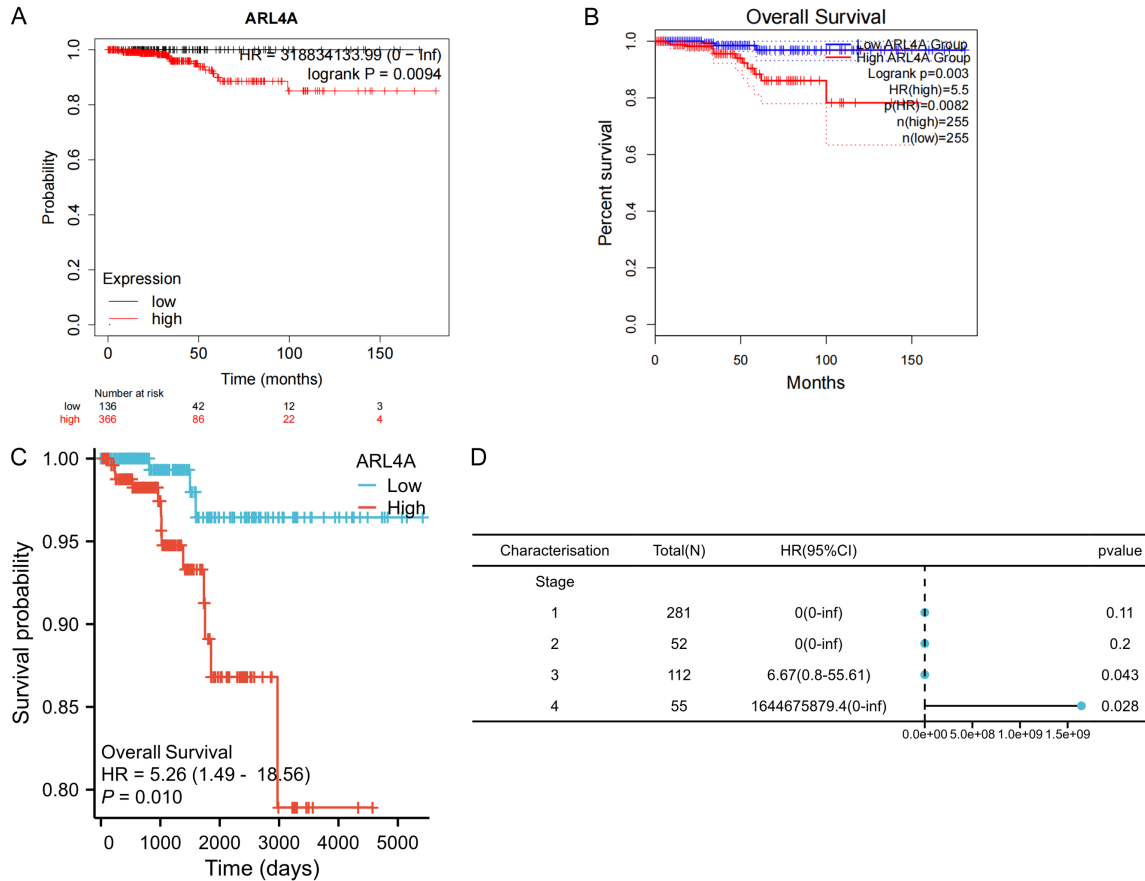
Figure 3. The survival distributions in THCA were estimated using Kaplan-Meier curves. A-C. The OS was expressed using the Kaplan-Meier survival curves (KM Plotter, GEPIA2, and XIANTAO). D. Forest plot summarizing the correlation between ARL4A expression and pathological stage in patients with THCA.

Figure 5A, ARL4A expression was found to have a negative correlation with various immune cells, including CD4+ T cells, macrophages, neutrophils, CD8+ T, and dendritic cells (**Figure 6A**). Next, we delved deeper into the relationship between the immune microenvironment and ARL4A expression. In this regard, the expression of ARL4A in 571 THCA patients from the TCGA database was first classified into high- and low-expression groups. We then assessed the differences in the expression levels of 24 immune cell subtypes between these groups. In the high expression group, there was a discernible reduction trend in activated dendritic cells (aDC), B cells, cytotoxic cells, dendritic cells (DC), immature dendritic cells (iDC), macrophages, T cells, and other cells (**Figure 6B**). Concurrently, a negative association was observed between ARL4A expression and 14 of the 24 immune cell types analyzed (**Figure 6C**).

Finally, we further investigated the impact of different immune cell subpopulations on ARL4A

expression and, consequently, on the prognosis of THCA patients. The findings revealed that an enrichment of high immune cells, including macrophages, natural killer T-cells, and regulatory T-cells, as well as a scarcity of low immune cells, such as mesenchymal and type 1 T-helper cells, both contributed to poor prognosis in THCA patients, mediated through ARL4A expression. On the other hand, the levels of CD4+ memory T-cells, CD8+ T-cells, and eosinophils, irrespective of being high or low, were found to be poorly related to prognosis (**Figure 7**).

Discussion

THCA is a prevalent malignant tumor with an increasing incidence rate. Despite some therapeutic advancements, the pathogenesis of thyroid cancer remains incompletely understood. THCA can be classified into four types: mesenchymal, follicular, medullary, and papillary [17]. Most patients with thyroid cancer cases are
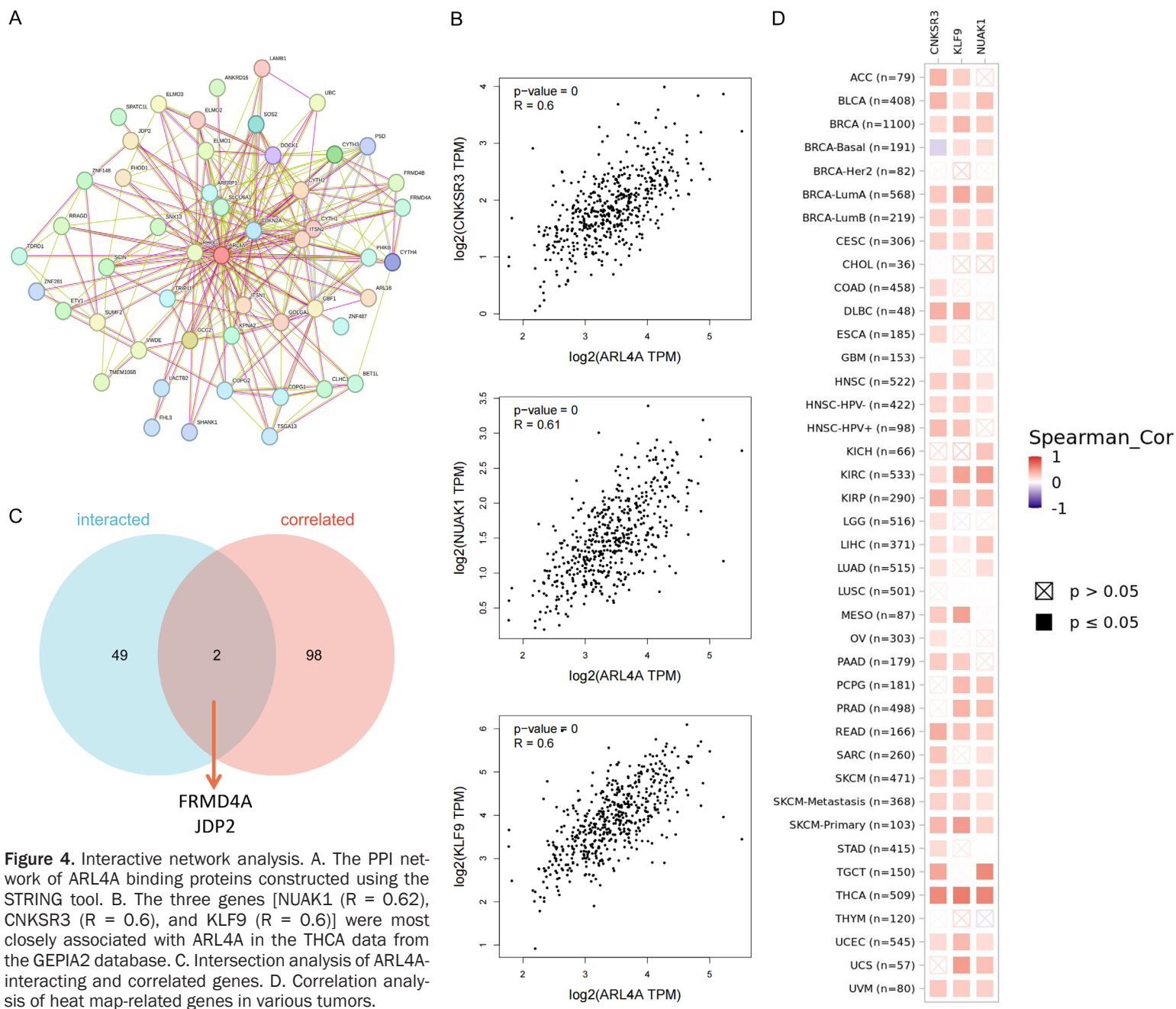
Figure 4. Interactive network analysis. A. The PPI network of ARL4A binding proteins constructed using the STRING tool. B. The three genes [NUAK1 (R = 0.62), CNKSR3 (R = 0.6), and KLF9 (R = 0.6)] were most closely associated with ARL4A in the THCA data from the GEPIA2 database. C. Intersection analysis of ARL4A-interacting and correlated genes. D. Correlation analysis of heat map-related genes in various tumors.
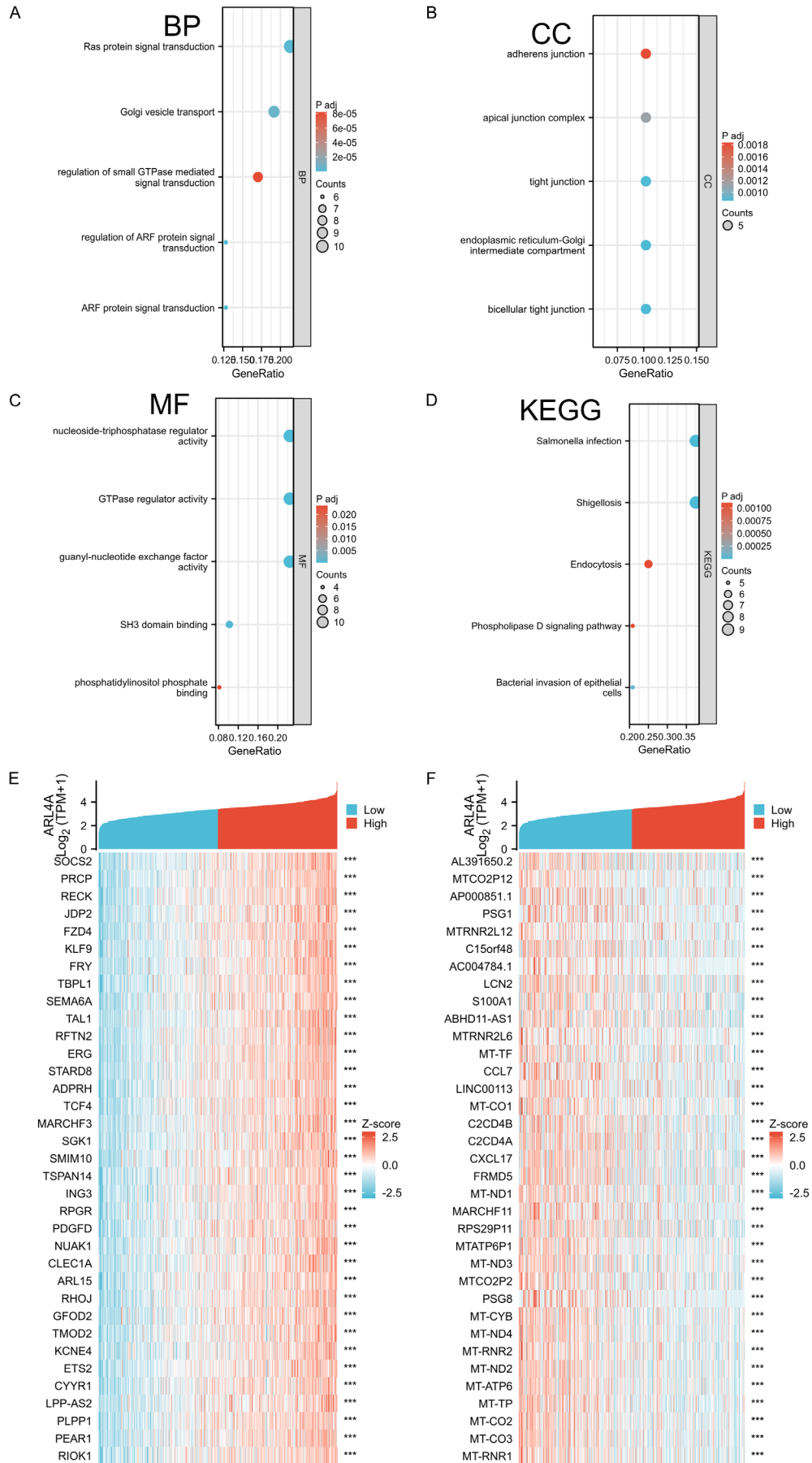
Figure 5. Functional analysis of ARL4A-interacting and correlated genes. (A-D) GO and KEGG enrichment analyses were performed on these 53 ARL4A-interacting and correlated genes. GO, Gene Ontology; BP, biological process; CC, cellular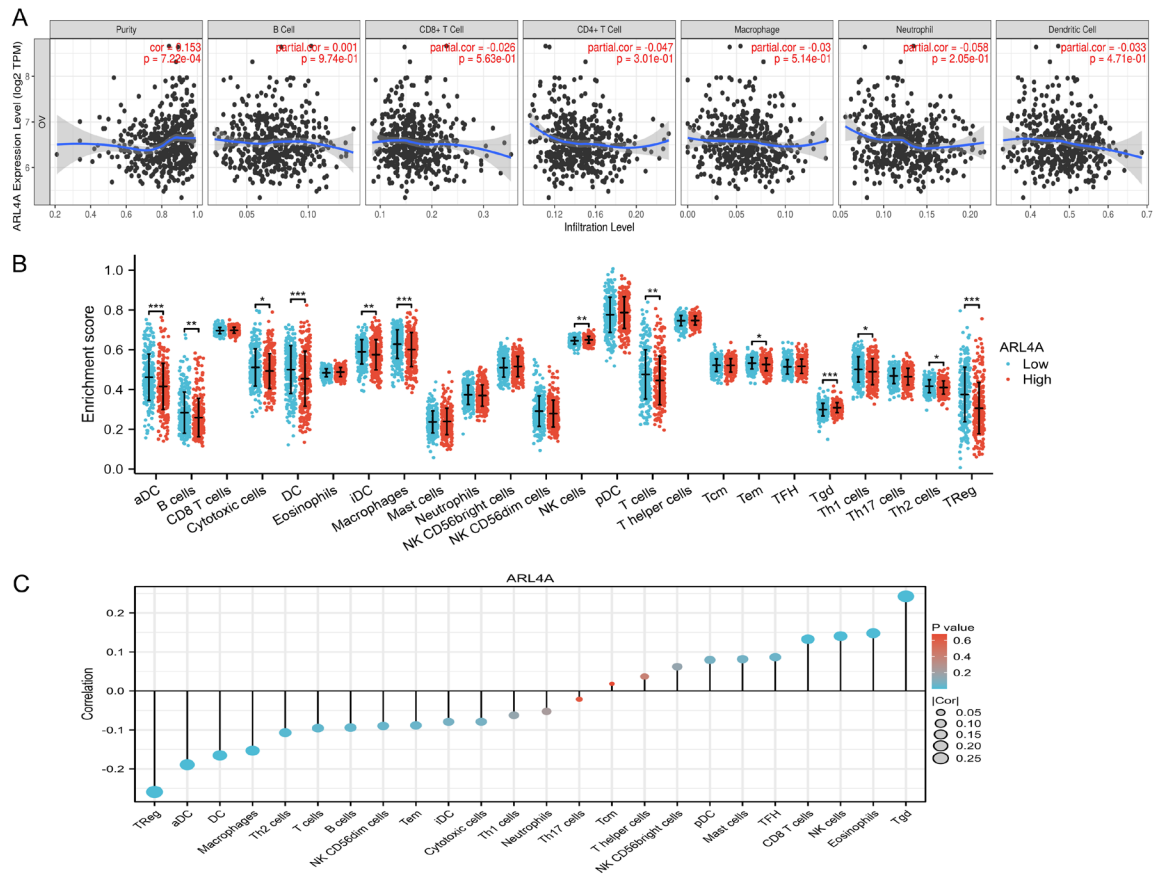 component; MF, molecular function. (E, F) Heatmaps showing the top 50 genes with positive (E) and negative (F) correlations with ARL4A in THCA.

Figure 6. Analysis of ARL4A on immune cells in THCA. A. TIMER database was used to understand the correlation between ARL4A expression in THCA and 6 immune cells. B. Comparison of 24 immune cell subtypes in high and low ARL4A expression groups in tumor tissue. C. Correlation analysis between ARL4A and 24 immune cells. DC, dendritic cell; aDC, activated DC; pDC, plasmacytoid DC; iDC, immature DC; Th, T helper cells; Th1, type 1 Th cell; Th2, type 2 Th cells; Th17, type 17 Th cell; Treg, regulatory T cell; Tgd, T gamma delta; Tcm, T central memory; Tem, T effector memory; Tfh, T follicular helper; NK, natural killer.

diagnosed as differentiated thyroid cancer, which is less malignant. However, patients with undifferentiated thyroid cancer often experience metastasis and recurrence, attributed to the high heterogeneity of thyroid cells, loss of thyroid cell characteristics, and a medullary pattern [18]. In addition, the insidious and slow progression of thyroid cancer [19] means that some patients may have tumor metastasis at the initial diagnosis, significantly impacting their health.

ARL4A, an ARF small GTPase, is involved in cell morphology, cell migration, and actin cytoskeleton remodeling [6]. Cell migration, as a pheno-

type of cancer cells, plays a crucial role in cancer cell development [20]. Pan-cancer analysis revealed varied expressions of ARL4A across different cancers, with high expression in GBM leading to tumor proliferation and migration [9], and low expression in THCA potentially inhibiting these processes. Thus, ARL4A exhibits dual functional roles in various human cancers. However, the functional role of ARL4A in cancer, particularly in THCA, has not yet been reported, necessitating further investigation into its function in THCA. In our study, we found that ARL4A was underexpressed in THCA, both in tissue specimens and cell lines, while a lower clinical stage corresponded with higher

ARL4A in thyroid cancer

A

Subgroud	Number	HR(95%CI)		pvalue
Basophils				
enriched	210	5.35(0.68-41.96)		0.074
decreased	291	355256549.2(0-Inf)		0.11
B-cells				
enriched	237	454215177.61(0-Inf)		0.086
decreased	264	5.85(0.72-43.35)		0.064
CD4+ memory T-cells				
enriched	203	19.66(2.05-189.08)		0.00052
decreased	298	293348682.43(0-Inf)		0.049
CD8+ T-cells				
enriched	401	395229395.63(0-Inf)		0.028
decreased	100	4.61(1.1-1933)		0.022
Eosinophils				
enriched	145	502456420.18(0-Inf)		0.041
decreased	356	320516906.97(0-Inf)		0.031
Macrophages				
enriched	285	39449096.99(0-Inf)		0.028
decreased	216	3.59(0.44-29.21)		0.2
Mesenchymal stem cells				
enriched	77	673830730.28(0-Inf)		0.066
decreased	424	334665275.63(0-Inf)		0.015
Natural killer T-cells				
enriched	162	448168511.76(0-Inf)		0.037
decreased	339	296203053.57(0-Inf)		0.061
Regulatory T-cells				
enriched	390	351952371.02(0-Inf)		0.0062
decreased	111	0(0-Inf)		0.27
Type 1 T-helper cells				
enriched	75	2025845257.05(0-Inf)		0.27
decreased	426	326022440.5(0-Inf)		0.011

0.0e+00 5.0e+08 1.0e+09 1.5e+09 2.0e+09

B

Kaplan-Meier survival curves (ARL4A expression) for immune cell subgroups: Enrichened B cell, Decreased B cell, Enrichened Basophils, Decreased asophils, Enrichened CD4⁺ T cell, Decreased CD4⁺ T cell, Enrichened CD8⁺ T cell, Decreased CD8⁺ T cell, Enrichened Eosinophils, Decreased Eosinophils, Enrichened Macrophages, Decreased Macrophages, Enrichened Mesenchymal stem cells, Decreased Mesenchymal stem cells, Enrichened Natural killer T-cells, Decreased Natural killer T-cells, Enrichened Regulatory T-cells, Decreased Regulatory T-cells, Enrichened Type 1 T-helper cells, Decreased Type 1 T-helper cells.

Figure 7. Prognostic impact of ARL4A expression in THCA patients. (A) Summary and (B) detailed distribution of various cellular immune cell subsets on the prognosis of THCA patients with different ARL4A expression levels.

expression of ARL4A in the pathological stage. Paradoxically, high expression of ARL4A was associated with poor prognosis, suggesting that although ARL4A may act as an oncogene, its pathway could be generally suppressed or regulated, leading to low expression yet still impacting tumor growth [16].

In this study, we found that FRMD4A and JDP2 were closely associated with ARL4A. The FRMD4A gene is important in cancer biology, particularly in tumor development and metastasis. As part of the FERM family of proteins, FRMD4A is involved in cell polarity, migration, and signaling, crucial for cancer development [21]. It plays a key role in cytoskeleton remodeling and cell attachment sites, essential for the migration and invasion of cancer cells, such as in rectal [22] and pancreatic cancers [23]. By interacting with other proteins, FRMD4A influences various cell signaling pathways critical for tumor growth and survival, making it a potential therapeutic target [24]. JDP2, a basic region-zipper-type (bZIP) transcription factor, is significant in cancer development. It affects cell proliferation, differentiation, and apoptosis primarily through gene expression regulation. JDP2 acts as a tumor suppressor in hepatocellular carcinoma [25] and as an oncogenic factor in T-cell acute lymphoblastic leukemia, associated with disease progression and therapy resistance [26]. Its role in regulating activating transcription factor 4 (ATF4) and modulating tumor necrosis factor-associated apoptosis-inducing ligand (TRAIL) sensitivity through the ATF4-DR5 axis [27] and its impact on key signaling pathways, such as Mc2r during cellular stress response and apoptosis [28], are noteworthy. Given the roles of FRMD4A and JDP2 in cancer development and their influence on cell signaling, the positive correlation between ARL4A, FRMD4A, and JDP2 implies that ARL4A may regulate similar pathways or interactions in cancer development. This relationship is particularly critical in thyroid cancer, where cancer development may involve complex signaling and cell migration processes, warranting further research.

To better understand the functional role of ARL4A in THCA, we performed GO and KEGG

analyses. ARL4A was primarily associated with GTP-binding protein function and cell migration and adhesion. Immune function, which involves the body's resistance to disease through the interaction of immune cells, their products, and the immune recognition process [29], was also a focus.

In conditions like cancer, specific gene expression patterns not only affect immune cell function-such as maturation, activation, and pathogen response-but also determine the extent of their infiltration into the tumor microenvironment. Gene expression can promote the migration and infiltration of specific immune cells, such as T cells or macrophages, into tumor tissue, often closely linked with patient prognosis. In osteosarcoma, analysis of immune-related gene expression identified key genes associated with disease progression and prognosis [30]. Similarly, in prostate cancer, gene expression significantly correlated with immune cell infiltration levels, particularly T-cells γδ, with important implications for understanding disease progression and personalized treatment strategies [31]. These studies highlight the critical interaction between gene expression and immune cell infiltration in understanding the immune microenvironment and improving patient prognosis. However, the correlation between ARL4A expression in THCA and immune cells remains relatively unexplored. Our study showed that ARL4A expression negatively correlated with CD4+ T cells, macrophages, neutrophils, CD8+ T cells, and dendritic cells. The enrichment of high immune cells (macrophages, natural killer T-cells, and regulatory T-cells) and low immune cells (mesenchymal and type 1 T-helper cells) both contributed to poor prognosis in THCA patients through ARL4A expression. In contrast, CD4+ memory T-cells, CD8+ T-cells, and eosinophils, regardless of levels, were poorly correlated with prognosis.

These findings indicate that immune cell infiltration, combined with ARL4A expression, influences the prognosis of THCA patients, suggesting that ARL4A could be combined with immunotherapy for THCA treatment, though this requires further research.

Nevertheless, this study focused primarily on the differential expression of ARL4A in cell lines, supplemented by extensive online data analyses. However, it lacked comprehensive clinical data and experimental analyses on prognostic regulation and functional resolution, pointing to areas for future exploration.

Conclusion

In this study, we delved into the significance of ARL4A in THCA through a combination of online database analyses and experimental investigations. Our findings revealed that ARL4A holds considerable importance in the diagnosis, prognosis, and treatment of THCA. Furthermore, ARL4A was identified as playing a pivotal regulatory role in the immune microenvironment of THCA. However, these results warrant further experimental validations.

Disclosure of conflict of interest

None.

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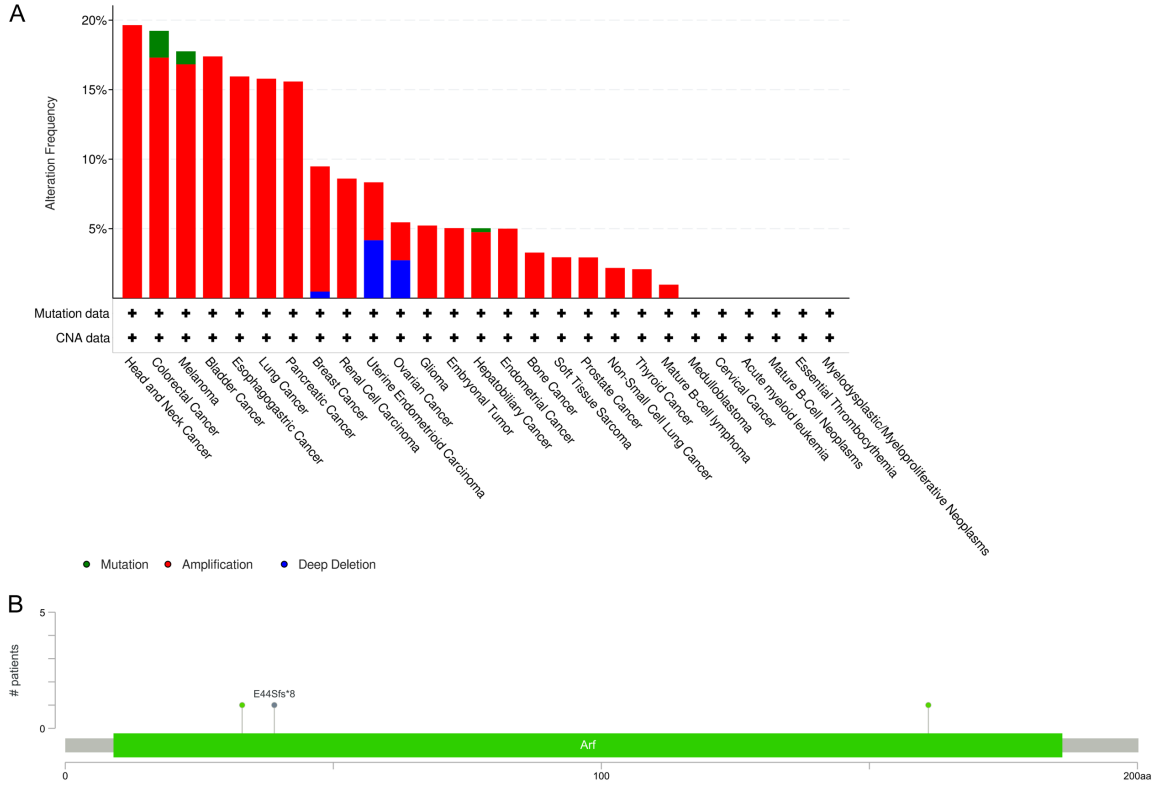
Figure S1. Analysis of ARL4A mutation features in various tumors of the TCGA cohort. Utilizing the cBioPortal tool, the mutation characteristics of ARL4A in different tumors of TCGA cohort were examined. This figure displays the alteration frequency along with the mutation type (A) and specific mutation sites (B).

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