



Alkaline phosphatase (ALPL) as a diagnostic and prognostic biomarker linked to immune response in thyroid cancer

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Background: Thyroid cancer (THCA) is a rapidly increasing endocrine malignancy. This study aims to explore alkaline phosphatase (*ALPL*) as a potential prognostic biomarker and therapeutic target, focusing on its role in tumor progression and immune infiltration.

Methods: We utilized RNA sequencing (RNA-Seq) data from The Cancer Genome Atlas (TCGA) to investigate the role of *ALPL* in THCA. Comprehensive analyses included differential gene expression, survival outcomes, immune cell infiltration, and RNA methylation correlations. Functional assays such as cell counting kit-8, colony formation, Transwell migration/invasion, 5-ethynyl-2'-deoxyuridine (EdU) staining, and flow cytometry were conducted to evaluate the effects of *ALPL* knockdown in THCA cell lines. At the same time, we constructed a nomogram.

Results: Our study identified *ALPL* as a key biomarker in THCA through TCGA analysis. The expression of *ALPL* was significantly elevated in tumor tissues and correlated with worse overall survival (OS). Moreover, we verified the expression of *ALPL* in patients with THCA. Functional assays showed that *ALPL* knockdown inhibited proliferation by 40–50%, reduced migration and invasion by 35–45%, and diminished clonogenic potential. Flow cytometry revealed a 2-fold increase in apoptosis. *ALPL* was also linked to immune infiltration, RNA methylation, and vascular endothelial growth factor (VEGF)/calcium signaling pathways, highlighting its role in tumor progression and potential as a therapeutic target. The nomogram that we constructed showed high value in predicting prognosis.

Conclusions: *ALPL* is a key biomarker in THCA, driving tumor progression and poor outcomes through its roles in proliferation, invasion, and immune modulation. These findings support its potential as a diagnostic and prognostic marker.

Keywords: Alkaline phosphatase (ALPL); thyroid cancer (THCA); predict prognosis; immune infiltration; biomarker

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Introduction

Background

Thyroid cancer (THCA) is the most common endocrine malignancy globally, with its incidence rising rapidly in recent decades, posing a significant public health challenge (1,2). Between 1990 and 2020, the global prevalence of THCA increased dramatically (3). Despite its often-indolent nature, THCA can exhibit metastatic behavior in certain patients by the time of initial diagnosis. Notably, a nationwide autopsy study utilizing the Dutch pathology registry found that distant metastases were present in as many as 35.1% of THCA patients, and most of these individuals harbored multiple metastatic lesions, which correlates with reduced total survival rates (4). This underscores the urgent need for reliable biomarkers to predict tumor aggressiveness and clinical outcomes. Identifying such markers would enable personalized treatment strategies, potentially improving survival rates and quality of life for patients.

Rationale and knowledge gap

Alkaline phosphatase (*ALPL*) encodes an enzyme critical for biological processes such as bone mineralization, lipid metabolism, and cell differentiation (5). Aberrant *ALPL* expression has been linked to tumor initiation, progression,

and prognostic outcomes in cancer research. In breast and colorectal cancers, elevated *ALPL* expression correlates with increased tumor aggressiveness, metastasis, and unfavorable clinical outcomes (6). Evidence suggests that *ALPL* contributes to immune evasion and tumor growth by modulating immune cell infiltration and inflammatory signaling within the tumor microenvironment. Additionally, *ALPL* influences cancer cell differentiation, complicating therapeutic interventions in certain tumor subtypes (7). *ALPL* also impacts the tumor microenvironment by regulating immune cell functions, including T regulatory cells (Tregs) and macrophage polarization, thereby altering anti-tumor immune responses (8). We hypothesize that *ALPL* has the potential to serve as a prognostic biomarker for THCA and is a promising target for therapeutic exploration.

Objective

This study aims to explore *ALPL* as a potential prognostic biomarker and therapeutic target, focusing on its role in tumor progression and immune infiltration.

We utilized data from The Cancer Genome Atlas (TCGA) to explore the relationship between *ALPL* expression and THCA. RNA sequencing (RNA-seq) data from TCGA were analyzed to compare *ALPL* expression levels in thyroid tumors versus normal tissues. We further examined the association between *ALPL* expression, clinical characteristics, and patient prognosis. Moreover, functional assays such as Cell Counting Kit-8 (CCK-8), colony formation, Transwell migration/invasion, 5-ethynyl-2'-deoxyuridine (EdU) staining, and flow cytometry were conducted to evaluate the effects of *ALPL* knockdown in THCA cell lines. Additionally, we identified a potential mechanism through which *ALPL* contributes to THCA progression, focusing on its role in immune infiltration. Our findings indicate that *ALPL* holds potential as a diagnostic/prognostic biomarker and a key therapeutic target for managing THCA. We present this article in accordance with the MDAR and REMARK reporting checklists (available at <https://gs.amegroups.com/article/view/10.21037/gs-2025-202/rc>).

Methods

Data collection and analysis

The RNA-Seq expression data for *ALPL* across various

Highlight box

Key findings

- Alkaline phosphatase (*ALPL*) is overexpressed in thyroid cancer (THCA) and promotes tumor progression and it correlates with poor prognosis and immune cell infiltration.

What is known and what is new?

- ALPL* has been studied in other tumor types, but its role in THCA remains poorly defined.
- This study systematically reveals *ALPL*'s oncogenic function in THCA using multi-omics analysis and in vitro validation, establishing it as both a prognostic biomarker and a potential therapeutic target.

What is the implication, and what should change now?

- The identification of *ALPL* as a driver of tumor progression and immune modulation in THCA suggests that it may be integrated into clinical prognostic models.
- Further mechanistic studies and clinical validation are warranted to explore *ALPL*-targeted therapies and their role in shaping the immune microenvironment.

cancers were sourced from the database of TCGA (<https://portal.gdc.cancer.gov/>). We used the “DESeq2” R package to recognize differentially expressed genes (DEGs) by comparing THCA samples grouped according to *ALPL* expression levels. The median expression value of *ALPL* was used as the cutoff to divide samples into high and low expression groups, a widely adopted approach in similar transcriptomic studies. We screened out the genes related to the diagnosis and prognosis in THCA by screening the genes related to the prognosis of THCA and intersecting with the differential genes in THCA cancer and adjacent cancer. The analysis included the expression of *ALPL* levels and corresponding clinical details such as age, histological stage, and survival outcomes in THCA.

Survival analysis

Furthermore, the association between the expression of *ALPL* and disease-specific survival (DSS) or overall survival (OS) was analyzed across pan-cancer using TCGA data. Furthermore, correlations between *ALPL* expression and OS across various clinical characteristics in THCA patients were assessed using R packages.

Immune characteristic analysis

The relationship between *ALPL* expression and immune cell infiltration was examined using the “Cibersort” R package (9). Additionally, the database of TISIDB (<http://cis.hku.hk/TISIDB/>) (10), a comprehensive platform for investigating tumor immune system interactions, was utilized. To further explore the immune associations of *ALPL* in cancer, we examined and evaluated its correlation with the expression levels of immunoregulatory genes, including immunoinhibitors, immunostimulators, chemokines, and chemokine receptors.

RNA methylation analysis

A comprehensive pan-cancer dataset, containing *ALPL* gene expression data and 44 marker genes linked to m1A, m5C, and m6A RNA modification pathways across various samples, was sourced from the University of California, Santa Cruz (UCSC) database (<https://xenabrowser.net>). Pearson correlation analysis was conducted to explore the relation between *ALPL* expression and the expression levels

of these RNA modification marker genes.

Search Tool for the Retrieval of Interacting Genes (STRING) and Gene Multiple Association Network Integration Algorithm (GeneMANIA) database analysis

The database of STRING (www.string-db.org; doi: 10.1093/nar/gky1131) was utilized to analyze protein-protein interactions (PPIs) related to *ALPL*. In addition, GeneMANIA (www.genemania.org) was employed for functional gene analysis (11). Based on our study, we constructed a gene-gene interaction (GGI) network for *ALPL* by identifying the 20 most significant associated genes.

Functional analysis

We expanded our analysis to identify the 100 genes most strongly correlated, both positively and negatively, with *ALPL* expression. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were conducted to assess gene enrichment, and the results were visualized via the “ggplot2” R package. Additionally, the “GSVA” R package was applied, utilizing the single-sample gene set enrichment analysis (ssGSEA) method (12) to further enrich and enhance the analysis.

Nomogram construction for survival prediction

In this study, independent clinicopathological prognostic factors were identified using Cox regression analysis, and a nomogram was constructed to evaluate 1-, 5-, and 10-year OS probabilities for THCA patients. To evaluate the accuracy of these predictions, calibration curves were generated to compare the predicted OS probabilities with the actual observed outcomes, presented as line charts for visual comparison.

Drug sensitivity prediction

The platform Gene Set Cancer Analysis (GSCA) (<http://bioinfo.life.hust.edu.cn/GSCA/#/>) offers access to data on over 750 small-molecule drugs derived from the Genomics of Cancer Therapeutics Response Portal (CTRP) (13). Leveraging this extensive resource, we analyzed the sensitivity of *ALPL* to small-molecule drugs using the GSCA platform.

Cell culture and transfection

The BHT101 and Cal62 THCA cell lines were bought from the Cell Bank of the Chinese Academy of Sciences (Shanghai). These cells were grown in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS) and 100 U/mL penicillin-streptomycin (Pen/Strep), maintained at 37 °C in a humidified atmosphere with 5% CO₂. *ALPL* small interfering RNA (siRNA) (forward 5'-GCGCAAGAGACACUGAAAUTT-3' and reverse 5'-AUUUCAGUGUCUCUUGCGCTT-3') and a negative control (NC) were obtained from GenePharma (Shanghai, China). When the BHT101 and Cal62 cells reached 80% confluence, they were seeded into six-well plates and transfected with Lipofectamine 3000 (Thermo Fisher, USA) containing the corresponding siRNA. The cells were incubated in a transfection medium with 2% FBS and 10 mg/mL polybrene for 12 hours. Then, cells were harvested 8 hours post-transfection.

Real-time polymerase chain reaction (RT-PCR)

Samples of tumor and adjacent normal tissues were collected from 18 THCA patients for verification studies. The study was conducted in accordance with the Declaration of Helsinki and its subsequent amendments. The study was approved by the Ethics Committee of Sir Run Run Hospital Affiliated to Nanjing Medical University (No. 2024-SR-055). All patients signed informed consent.

SiALPL was purchased to assess knockdown efficiency in cell lines. Total RNA was converted into cDNA using the High-Capacity cDNA Reverse Transcription Kit (Life Technologies, Carlsbad, CA, USA) for the quantitative RT-PCR (qRT-PCR) analysis. qRT-PCR was subsequently performed on the synthesized cDNA using Power SYBR Green PCR Master Mix (Life Technologies) according to the manufacturer's protocol. The experiments were conducted on either the Agilent Mx3005P or Applied Biosystems AB7500 Fast Real-Time PCR Systems. The primer sequences employed for qRT-PCR were as follows: *ALPL* (forward: 5'-ACTGGTACTCAGACAACGAGAT-3' and reverse: 5'-ACGTCAATGTCCCTGATGTTATG-3') and *GAPDH* (forward: 5'-GGAGCGAGATCCCTCCAAAAT-3' and reverse: 5'-GGCTGTTGTCATACTTCTCATGG-3').

CCK-8 assay

The role of *ALPL* in regulating THCA cell proliferation

was assessed. Following cell counting, 2,000 cells were seeded into each well of a 96-well plate, with 3–6 replicate wells per experimental group. At designated time points, CCK-8 solution, at a volume equal to 1/10th of the culture medium, was added to each well. Then the cells were incubated in a cell culture incubator for 2 hours, after which the absorbance at 450 nm was measured using a microplate reader. To assess cell viability over time, absorbance readings were taken at 0, 24, 48, 72, and 96 hours post-*ALPL* transfection.

Colony formation assay

After transfection, THCA cells in the exponential growth phase were counted and seeded into 6-well plates at a density of 500 cells per well. The plates were then incubated at 37 °C in a 5% CO₂ atmosphere for 2–3 weeks to facilitate colony formation. Following incubation, the cells were rinsed twice with PBS and left to air-dry. Methanol was used to fix the cells for 15 minutes, followed by another air-drying step. The fixed cells were treated with crystal violet solution for 15 minutes, then gently rinsed with tap water to remove excess stain. Once dried, colonies were photographed using a digital camera.

Transwell migration and invasion assay

For the invasion assay, the upper surface of the Transwell inserts was pre-coated with Matrigel (BD, Franklin Lakes, NJ, USA), while this step was omitted for the migration assay. THCA cells were transfected and cultured to the exponential growth phase. The cells were enumerated and seeded into the upper compartments of the Transwell inserts at a concentration of 2,000 cells per insert, suspended in 500 µL of serum-free medium. The lower compartments of the 24-well plates were filled with 600 µL of medium enriched with 10% FBS, acting as a chemoattractant. The plates were maintained at 37 °C in a humidified atmosphere containing 5% CO₂ for 24 hours. Following incubation, the inserts were treated with methanol for 30 minutes to fix the cells, stained with 0.1% crystal violet solution for 15 minutes, and carefully rinsed to eliminate any residual stain. Migrated or invaded cells were visualized as well as counted in six fields using by microscope.

EdU staining

Seed THCA cells into a 6-well plate and treat them with

a pre-warmed 2× EdU working solution for 2 hours. After fixing the cells, apply the Click reaction mixture to each well and incubate in the dark for 30 minutes. Finally, stain the nuclei with Hoechst and visualize the cells using a fluorescence microscope to capture images.

Flow cytometry

THCA cells were cultured in 6-well plates, and 24 hours post-transfection with ALPL siRNA, they were collected and stained using the Annexin V/7-AAD staining kit (BD) containing PI and Annexin V/7-AAD dyes. The staining process lasted for 10 minutes. Subsequently, apoptotic cells were identified and analyzed through flow cytometry.

Statistical analyses

All statistical analyses were performed using R software (version 4.2.1). Gene expression and clinical data were visualized using the “ggplot2” package. Comparisons between two groups were conducted using the unpaired Student’s *t*-test (for normally distributed data) or the Wilcoxon rank-sum test (for non-normally distributed data), as appropriate. For comparisons involving more than two groups, one-way analysis of variance (ANOVA) or Kruskal-Wallis test was applied.

For high-throughput analyses, such as differential gene expression and gene set enrichment analysis (GSEA), multiple hypothesis testing correction was performed using the Benjamini-Hochberg false discovery rate (FDR) method. An FDR-adjusted P value (q-value) <0.05 was considered statistically significant.

Receiver operating characteristic (ROC) curves were constructed using the “pROC” package to evaluate the diagnostic performance of *ALPL* and determine optimal cutoff values. Correlation analyses between *ALPL* expression and immune-related or methylation-related markers were assessed using Spearman’s rank correlation test. There is no missing data.

Survival analyses were carried out using the “survival” and “survminer” packages. Kaplan-Meier curves with log-rank tests were used to evaluate differences in OS between high and low *ALPL* expression groups. Univariate and multivariate Cox proportional hazards regression models were used to assess the prognostic value of *ALPL* while adjusting for relevant clinical covariates.

Statistical significance is indicated by P values below 0.05.

Results

Identification of DEGs and OS related genes in THCA

The mRNA expression profiles in THCA were analyzed for differential expression. A total of 1,747 DEGs were identified from RNA-seq-HTSeq-Counts, meeting the criteria of $\log_2$ fold change ($\log_2FC$) >1.5 and $P < 0.05$ (Figure 1A). From TCGA data, 712 genes associated with OS in THCA were identified. A Venn diagram revealed 125 overlapping genes between THCA-related DEGs and OS-associated genes (Figure 1B).

Clinical features analysis in THCA

As demonstrated in Figure 1C, the level of *ALPL* expression was markedly higher in THCA tissues compared to adjacent tissues ($P < 0.001$). The association between *ALPL* expression and the clinical characteristics of THCA patients is summarized in Table S1. Notably, *ALPL* mRNA expression was significantly elevated in patients over 45 years of age compared to those aged 45 or younger ($P_{adj} = 0.02$) (Figure 1D). Additionally, *ALPL* expression was reduced in individuals with the follicular histological subtype than in those with the tall cell subtype ($P_{adj} = 0.03$) (Figure 1E). Moreover, *ALPL* mRNA levels were significantly elevated in patients who experienced OS events (dead) compared to those who did not (alive) ($P_{adj} = 0.03$) (Figure 1F).

Expression analysis of data in pan-cancer

In our comprehensive analysis of *ALPL* expression across a pan-cancer cohort from TCGA, as summarized in Table S2, *ALPL* expression was significantly higher in tumor tissues compared to adjacent non-tumor tissues in several cancers, including uterine corpus endometrial carcinoma (UCEC), head and neck squamous cell carcinoma (HNSC), stomach adenocarcinoma (STAD), and THCA. Conversely, reduced *ALPL* expression was detected in several cancer types, such as breast invasive carcinoma (BRCA), kidney renal papillary cell carcinoma (KIRP), colon adenocarcinoma (COAD), lung squamous cell carcinoma (LUSC), cholangiocarcinoma (CHOL), kidney chromophobe (KICH), lung adenocarcinoma (LUAD), liver hepatocellular carcinoma

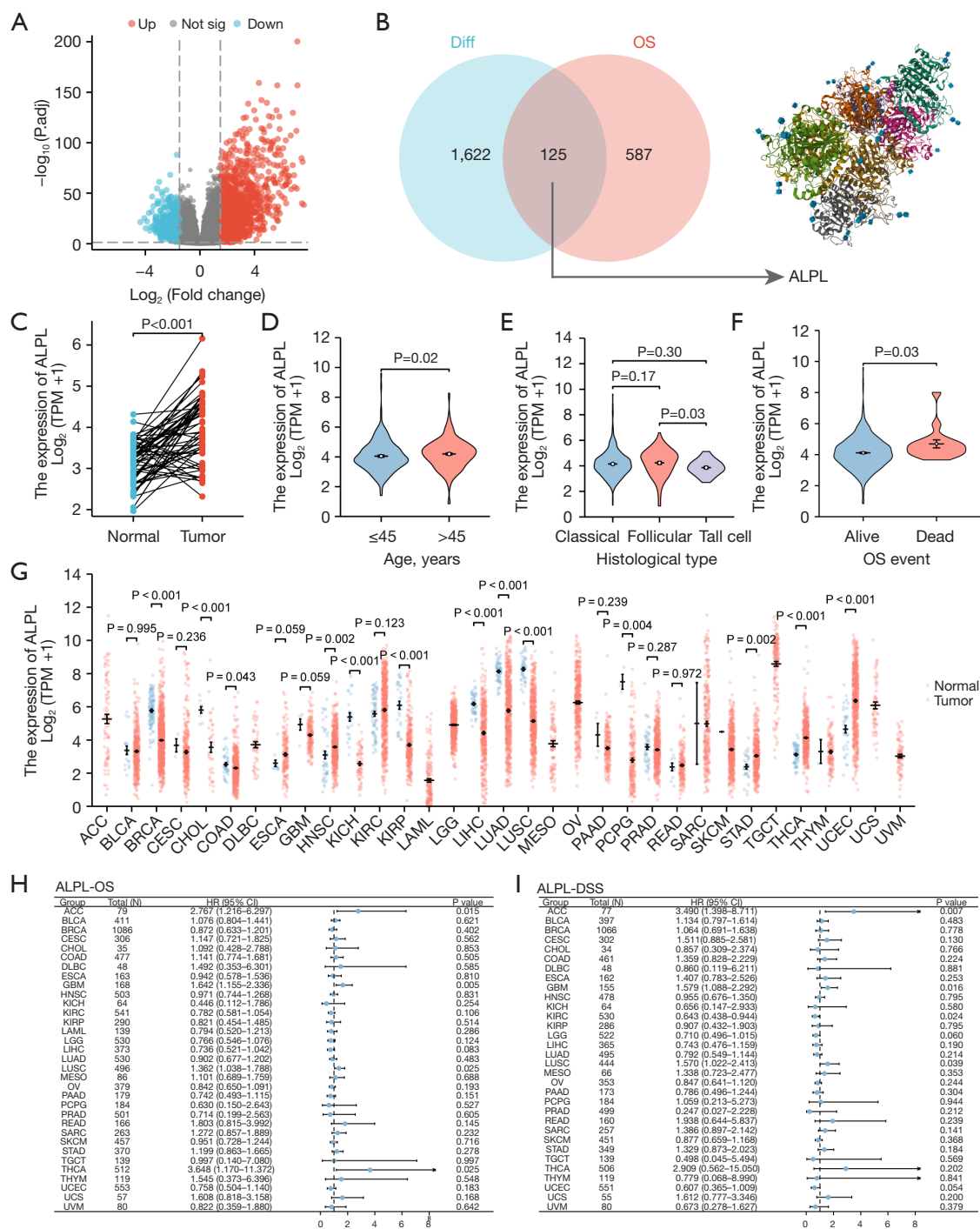


Figure 1 The expression and prognosis of *ALPL* in pan-cancer and the relationship between *ALPL* expression and clinical features of thyroid cancer. (A) Volcano plot of differentially expressed genes in THCA. (B) Venn diagram between expressed differential gene and OS gene in THCA. (C) The *ALPL* mRNA expression in THCA. (D-F) Expression level of *ALPL* in tumor tissues from patients with different clinical characteristics in TCGA database (age, histological type, OS event). (G) The *ALPL* mRNA expression in pan-cancer. Kaplan-Meier estimates of the (H) OS and (I) DSS probability of patients in pan-cancer. *ALPL*, alkaline phosphatase; CI, confidence interval; DSS, disease-specific survival; HR, hazard ratio; OS, overall survival; TCGA, The Cancer Genome Atlas; TPM, transcript per million.

(LIHC), and pheochromocytoma and paraganglioma (PCPG) (Figure 1G).

Kaplan-Meier survival curve analysis in pan-cancer

In this study, we utilized pan-cancer data from TCGA to evaluate the impact of *ALPL* expression on survival outcomes by categorizing patients into high- and low-expression groups. Kaplan-Meier survival analysis revealed a significant association between elevated *ALPL* expression and decreased OS in several cancer types, including adrenocortical carcinoma (ACC) [hazard ratio (HR) =2.767, $P=0.02$], glioblastoma multiforme (GBM) (HR =1.642, $P=0.005$), LUSC (HR =1.362, $P=0.03$), and THCA (HR =3.684, $P=0.03$). For DSS, high *ALPL* expression correlated with worse outcomes in cancers such as ACC (HR =3.490, $P=0.007$), GBM (HR =1.579, $P=0.02$), and LUSC (HR =1.570, $P=0.04$). Conversely, lower *ALPL* expression was correlated with worse DSS in Kidney renal clear cell carcinoma (KIRC) (HR =0.643, $P=0.02$) (Figure 1H,1I).

Prognostic significance in THCA

In this study, THCA patients were classified into low- and high-*ALPL* expression groups to assess its effect on survival outcomes (Figure 2A). Kaplan-Meier analysis showed a significant correlation between elevated *ALPL* expression and poorer OS ($P=0.001$). Further subgroup analyses showed that high *ALPL* expression was consistently linked to poor outcomes across diverse demographic and clinical categories, including age >45 years ($P=0.004$, Figure 2B), White race ($P=0.002$, Figure 2C), male ($P=0.03$, Figure 2D) and female ($P=0.02$, Figure 2E) gender, pathological T3 ($P=0.02$, Figure 2F) and T4 ($P=0.03$, Figure 2G) stages, pathological N1 ($P=0.004$, Figure 2H), pathological M0 ($P=0.003$, Figure 2I), stage III ($P=0.02$, Figure 2J), R0 ($P=0.04$, Figure 2K) and R1 ($P=0.005$, Figure 2L), residual tumor status, presence ($P=0.002$, Figure 2M) or absence ($P=0.02$, Figure 2N) of extrathyroidal extension, and the classical histological subtype ($P<0.001$, Figure 2O).

Relationship between ALPL and immunity

We created heatmaps to examine the relationship between *ALPL* and 22 immune cell types, aiming to better understand immune infiltration patterns across different cancers. In most cancer types, *ALPL* expression showed

a positive correlation with M2 macrophage infiltration. However, in ACC, BRCA, acute myeloid leukemia (LAML), skin cutaneous melanoma (SKCM), and testicular germ cell tumors (TGCT), *ALPL* expression was negatively associated with M2 macrophages (Figure 3A). Among THCA patients, those with high *ALPL* expression was associated with significantly reduced infiltration of several immune cell types, including naive B cells, Tregs, resting dendritic cells (DCs), and activated DCs. In contrast, patients with low *ALPL* expression displayed higher infiltration levels of CD8⁺ T cells and M2 macrophages (Figure 3B-3H).

Then, we analyzed the relationship between *ALPL* expression and chemokine/chemokine receptor levels in THCA using TISIDB. The heatmap results revealed significant associations between the expression of *ALPL* showed a positive correlation with several chemokines and their respective receptors (Figure 4A,4B). Specifically, *ALPL* expression was positively correlated with CCL3 ($r=0.163$, $P<0.001$; Figure 4C), CXCL12 ($r=0.343$, $P<0.001$; Figure 4D), CCL14 ($r=0.321$, $P<0.001$; Figure 4E), and CCR10 ($r=0.191$, $P<0.001$; Figure 4F).

We further investigated the connection between *ALPL* expression and immunoinhibitory/immunostimulatory molecules in various cancers using the TISIDB database (Figure 4A,4B,4G,4H). In THCA, *ALPL* expression was positively related to kinase insert domain receptor (KDR) ($r=0.329$, $P<0.001$; Figure 4I), transforming growth factor beta 1 (TGFB1) ($r=0.21$, $P<0.001$; Figure 4J), and tumor necrosis factor superfamily member 15 (TNFSF15) ($r=-0.220$, $P<0.001$; Figure 4K). Conversely, *ALPL* expression showed a negative correlation with TNFSF18 ($r=-0.220$, $P<0.001$; Figure 4L). These results indicate that *ALPL* is a key factor in tumor immunity and the migration of immune cells.

Relationship between ALPL expression and methylation

The role of *ALPL* in influencing cancer prognosis may be partially mediated through its impact on RNA methylation. In our analysis, *ALPL* expression demonstrated significant positive correlations with m6A, m5C, and m1A RNA methylation regulatory genes across multiple cancer types. Specifically, in THCA, *ALPL* showed strong associations with the majority of m6A, m5C, and m1A regulatory genes (Figure S1). This suggests that *ALPL* may contribute to tumor progression by modulating the expression of RNA methylation regulators.

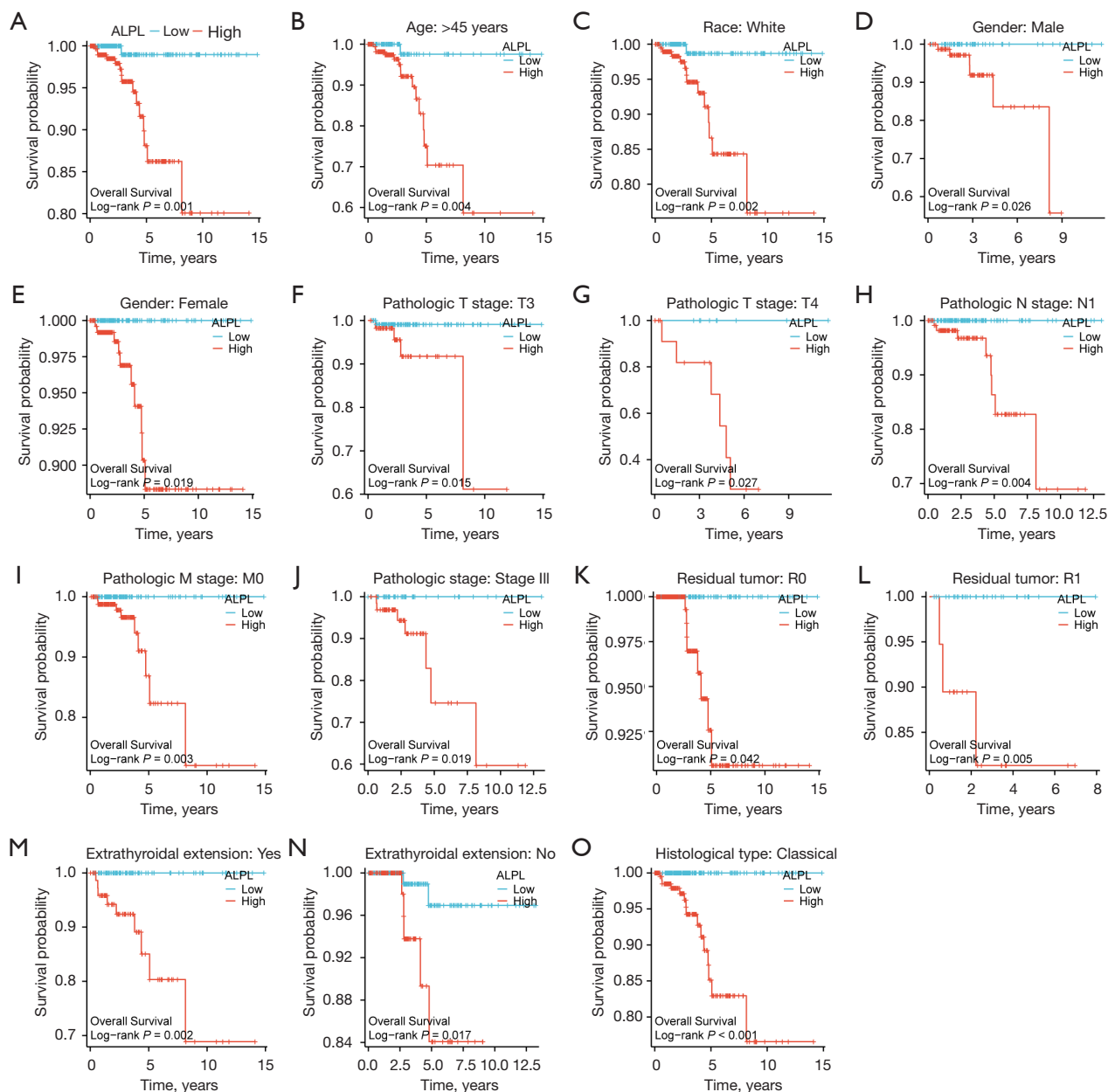


Figure 2 Kaplan-Meier survival curve analysis of the prognostic significance of a high and a low expression of *ALPL* in THCA. (A) Kaplan-Meier estimates of the OS probability of TCGA patients in all THCA patients; (B) age greater than 45 years; (C) race: White; (D) gender: male; (E) gender: female; (F) T3; (G) T4; (H) N1; (I) M0; (J) stage III; (K) residual tumor: R0; (L) residual tumor: R1; (M) extrathyroidal extension: yes; (N) extrathyroidal extension: no; (O) histological type: classical. *ALPL*, alkaline phosphatase; M, metastasis; N, node; OS, overall survival; T, tumor; THCA, thyroid cancer.

PPI, GGI, network, and functional enrichment analysis

A comprehensive analysis of PPI involving *ALPL* at various transcriptional levels was conducted using the STRING database. The resulting network, shown in *Figure 5A*,

highlights *ALPL* and its interactions with 20 closely related proteins. Additionally, a GGI network for *ALPL* was constructed, as illustrated in *Figure 5B*, where the central *ALPL* node is connected to 20 other nodes, representing

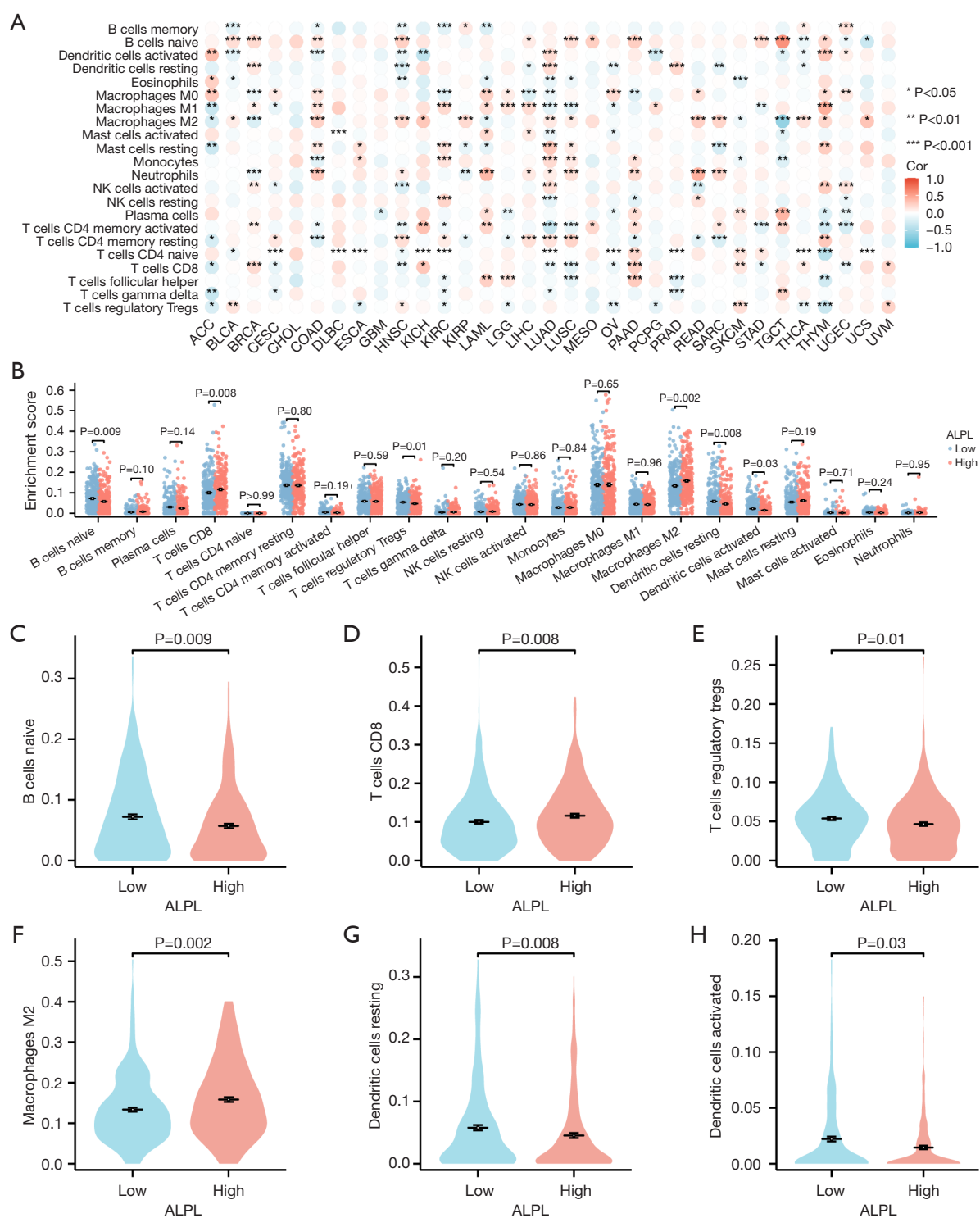
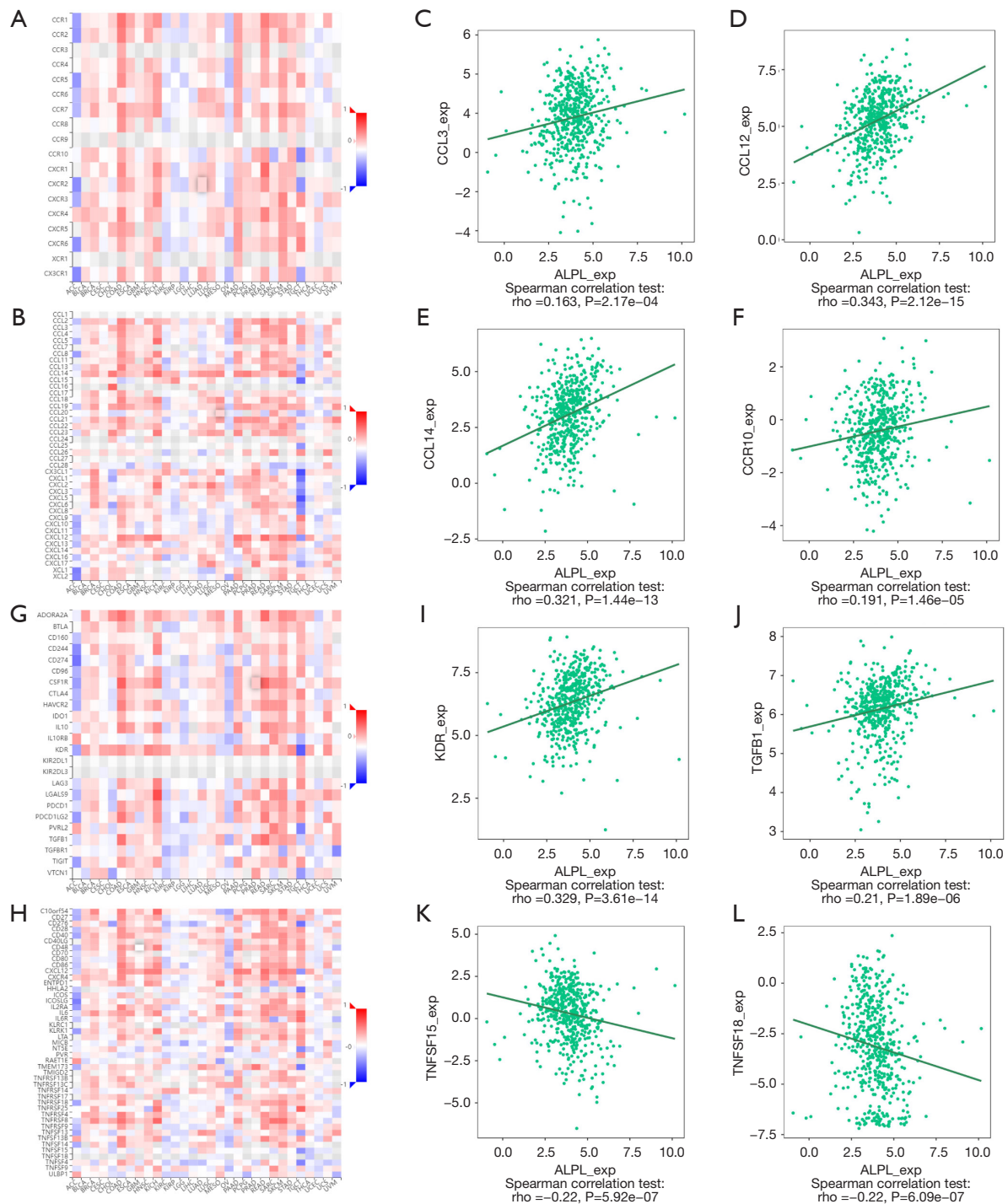


Figure 3 Correlation analysis of *ALPL* expression and immune infiltration. (A) The correlation between *ALPL* expression and immune cells in pan-cancer. (B) Correlation between the expression level of *ALPL* and immune infiltration in THCA: (C) B cells naive, (D) CD8⁺ T, (E) Tregs, (F) M2 macrophages, (G) DC resting, (H) DC activated. ALPL, alkaline phosphatase; DC, dendritic cell; THCA, thyroid cancer.



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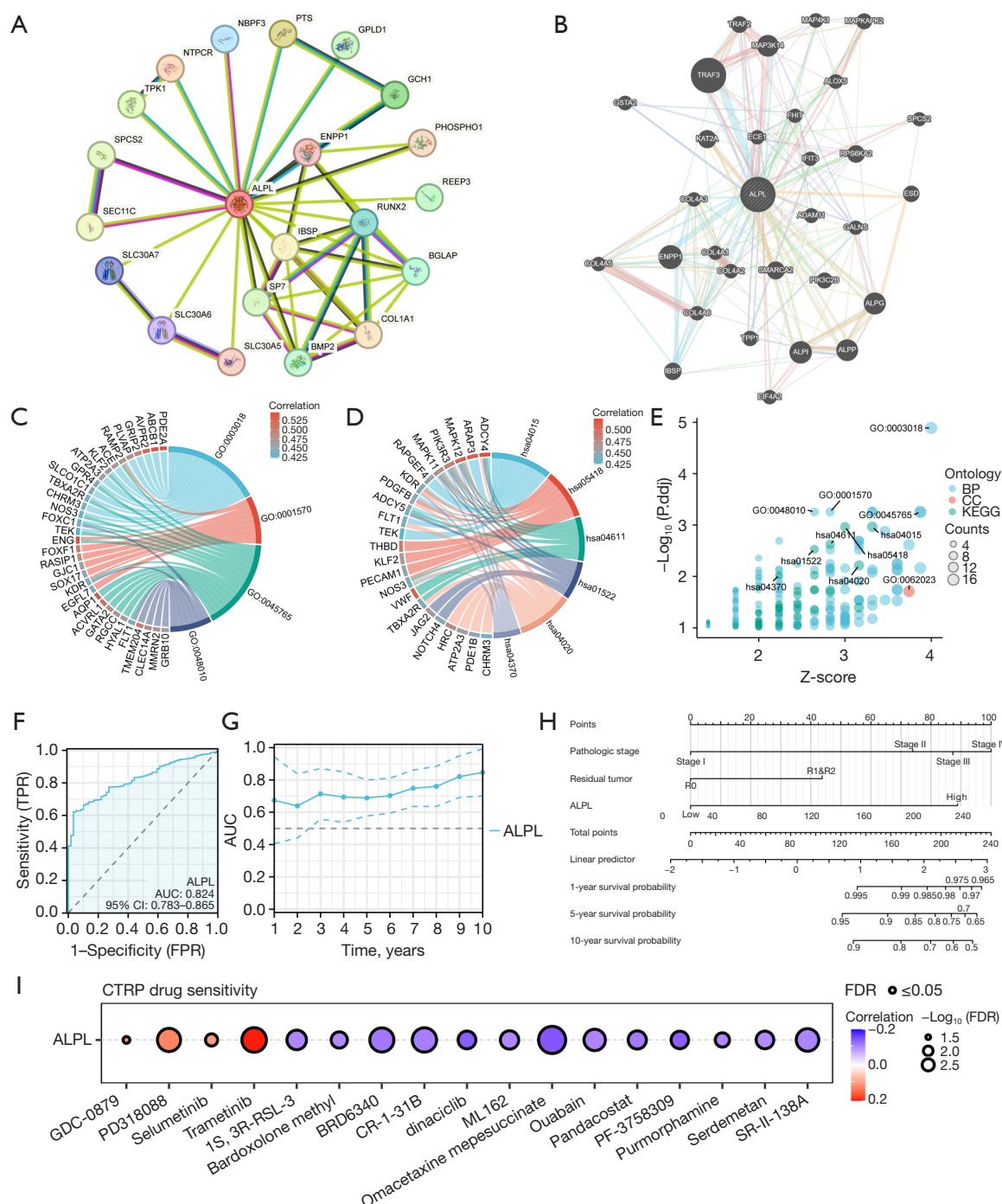


Figure 5 Interaction network, functions, diagnostic value, survival prediction, and drug sensitivity of ALPL. (A) PPI network of ALPL. (B) GGI network of ALPL. (C-E) GO term and KEGG pathway analyses of *ALPL*-related genes in THCA. (F) ROC curve and (G) time ROC curve analysis was performed to evaluate the diagnostic power of the *ALPL*. (H) Nomogram for predicting the 1-, 5-, and 10-year OS rates. (I) Correlation between *ALPL* expression and the sensitivity of CTRP drugs. ALPL, alkaline phosphatase; AUC, area under the curve; BP, biological process; CC, cellular component; CI, confidence interval; CTRP, Cancer Therapeutics Response Portal; FDR, false discovery rate; FPR, false positive rate; GGI, gene-gene interaction; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; OS, overall survival; PPI, protein-protein interaction; ROC, receiver operating characteristic; THCA, thyroid cancer; TPR, true positive rate.

genes strongly associated with *ALPL*.

To explore the biological roles of *ALPL*, we conducted GO and KEGG enrichment. The results are presented in *Figure 5C-5E* and detailed in *Table S3*. Among the pathways most positively correlated with *ALPL* expression were the Rap1 signaling pathway ($P=0.001$), Fluid shear stress and atherosclerosis ($P=0.001$), Platelet activation ($P=0.002$), Endocrine resistance ($P=0.003$), Calcium signaling pathway ($P=0.006$), and vascular endothelial growth factor (VEGF) signaling pathway ($P=0.009$). These findings underscore *ALPL*'s involvement in crucial signaling and regulatory pathways, supporting its potential role in tumor progression and related pathophysiological processes.

ROC and prognostic model of ALPL in THCA

To evaluate the potential of *ALPL* in differentiating THCA tissue from normal tissue, ROC curve analysis revealed an area under the curve (AUC) of 0.824 (95% confidence interval: 0.783–0.865) (*Figure 5F*), demonstrating the strong capability of *ALPL* expression to distinguish between THCA and normal tissue. Additionally, the diagnostic accuracy of *ALPL* improved as the disease advanced in THCA patients (*Figure 5G*).

To further refine prognosis prediction for THCA patients, a nomogram was constructed based on Cox regression analysis (*Figure 5H*). Analyses were performed using univariate and multivariate methods, incorporating four key prognostic factors into the model: pathologic stage, residual tumor status, and *ALPL* expression (*Table S4*).

Drug sensitivity prediction

The relationship between *ALPL* expression and the sensitivity of various cancer types to pharmacological agents was assessed using the CTRP database. *ALPL* expression was inversely correlated with the half maximal inhibitory concentration (IC_{50}) of drugs such as omacetaxine mepesuccinate, SR-II-138A, and CR-1-31B. Conversely, a positive correlation was observed for drugs like GDC-0879, PD318088, selumetinib, and trametinib, where higher *ALPL* expression corresponded to increased IC_{50} values (*Figure 5I*). These findings suggest a potential role of *ALPL* in influencing drug response across different cancer types.

ALPL expression validation

ALPL gene expression in THCA patients was further

validated using RT-PCR. Results demonstrated that the expression of *ALPL* was notably higher in THCA tissues compared to nearby tissues ($P<0.001$) (*Figure 6A*).

ALPL promotes cell growth

The correlation between elevated *ALPL* expression and poor clinical outcomes highlights its potential pro-carcinogenic role in cancer. To investigate the biological functions of *ALPL* in THCA, siRNA was employed to knock down *ALPL* in BHT101 and Cal62 cell lines, with successful knockdown confirmed by RT-PCR (*Figure 6B,6C*). The results showed that *ALPL* knockdown markedly inhibited the proliferation of both BHT101 (*Figure 6D,6F*) and Cal62 (*Figure 6E,6G*) cells.

Additionally, a transwell assay revealed that *ALPL* knockdown significantly decreased the invasion and migration capabilities of THCA cells (*Figure 6H,6I*). The clonogenic potential of these cells was also significantly diminished (*Figure 6J,6K*). Flow cytometry further demonstrated that *ALPL* depletion induced a notable increase in apoptosis in THCA cells (*Figure 6L,6M*). These findings show that *ALPL* is essential for promoting tumor growth and progression in THCA.

Discussion

Key findings

Our study shows that *ALPL* is significantly overexpressed in THCA, particularly in patients aged over 45 years, those with tall cell histology, and those with poor survival outcomes. Kaplan-Meier survival analysis demonstrated that elevated *ALPL* expression correlates with significantly worse survival rates across various subgroups, including age, sex, and pathological stage. High *ALPL* expression was consistently associated with worse outcomes in advanced tumor-node-metastasis (TNM) stages, residual tumor status, and the presence or absence of extrathyroidal extension. These findings underscore *ALPL*'s value as a prognostic biomarker for THCA and its potential role in stratifying risk.

Strengths and limitations

Although this study highlights the critical role of *ALPL* in THCA, there are several limitations to consider. First, our analysis relied primarily on publicly available

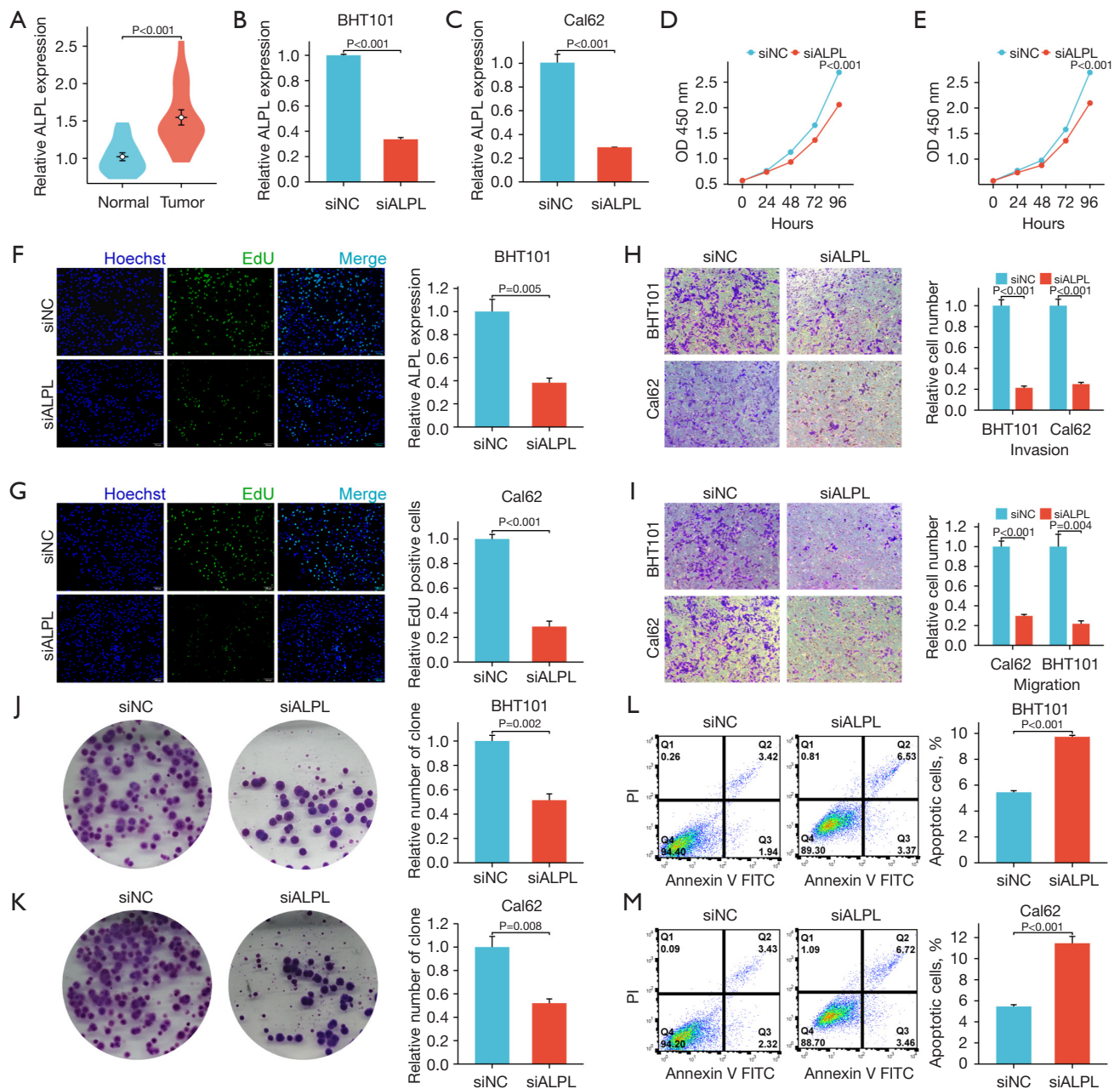


Figure 6 ALPL knockdown inhibits THCA cell growth *in vitro* and human tissues verification. (A) The expression of ALPL in thyroid cancer tissues; (B,C) chose siRNA for cell transfection and verified the expression of ALPL; (D,E) cell proliferation assays; (F,G) EdU staining assays with THCA cells upon ALPL knockdown(scale bar: 100 μ M); (H,I) transwell assays (stained with 0.1% crystal violet; Representative image at 20 $\times$ magnification bar); and (J,K) clone formation assays with THCA cells upon ALPL knockdown (stained with 0.1% crystal violet); (L,M) apoptosis flow cytometry analysis of THCA cells after ALPL knocking down. ALPL, alkaline phosphatase; EdU, 5-ethynyl-2'-deoxyuridine; FITC, fluorescein isothiocyanate; OD, optical density; PI, propidium iodide; siNC, small interfering negative control; THCA, thyroid cancer.

data from databases such as TCGA, and the quality and completeness of these data may impact the interpretation of the relationship between *ALPL* expression and clinical characteristics. Second, the study did not include laboratory validation or animal model experiments. While we observed a strong correlation between *ALPL* expression and immune cell infiltration, the specific molecular mechanisms underlying this relationship require further investigation. Third, although we identified associations between *ALPL* and the immune microenvironment, its exact role in immune evasion and tumor progression remains unclear and warrants deeper exploration. Finally, while our study proposes *ALPL* as a potential diagnostic and prognostic biomarker, its clinical application still needs to be confirmed through large-scale, multi-center clinical trials.

Comparison with similar research

THCA is the most common endocrine malignancy, and its incidence, especially of papillary thyroid carcinoma (PTC), has significantly increased in recent years (2). Its pathogenesis is complex, involving genetic, environmental, and immune factors (14). Although most patients have favorable outcomes, aggressive cases—especially anaplastic THCA—are characterized by high recurrence, metastasis, and poor survival rates. Current diagnostic methods, primarily based on imaging and cytology, have limitations in accurately assessing tumor invasiveness and predicting prognosis (15). This underscores the need for reliable biomarkers and targeted therapies to improve diagnostic precision, enable personalized treatment, and reduce the disease burden.

While there is limited research on *ALPL*'s role in tumor progression, existing studies suggest its involvement in regulating invasion in ovarian cancer (16) and inhibiting metastasis in lung adenocarcinoma (17). Additionally, *ALPL* downregulation has been associated with breast cancer progression to bone metastasis (6). However, its function in THCA has yet to be investigated.

Explanations of findings

Our analysis identified a notable association between *ALPL* expression and immune infiltration patterns. Elevated *ALPL* expression demonstrated a positive connection with CD8⁺ T cells and M2 macrophages while showing a negative correlation with naive B cells, DCs, and Tregs. M2 macrophages, known for their immunosuppressive

and tumor-promoting functions, secrete cytokines like interleukin-10 (IL-10) and TGF- β , which support tumor growth and metastasis (18–20). The positive correlation between *ALPL* and M2 macrophages suggests that *ALPL* may contribute to an immunosuppressive tumor microenvironment, aiding in immune evasion.

The negative correlation between *ALPL* expression and B cells implies that *ALPL* may suppress humoral immunity, potentially diminishing antibody-mediated anti-tumor responses (21). Additionally, DCs, as critical antigen-presenting cells responsible for initiating T cell-mediated immune responses (22,23), were negatively correlated with *ALPL* expression. This indicates that *ALPL* may impair antigen presentation and T cell activation, further promoting immune evasion.

Besides, our results indicated that *ALPL* may promote the recruitment and activation of M2 macrophages by upregulating *CCL3* expression (24). Similarly, the association with *CXCL12* implies that *ALPL* may play a role in promoting the migration of immunosuppressive cells within the tumor microenvironment (25,26). Additionally, *ALPL*-induced upregulation of TGF β 1 may enhance the immunosuppressive functions of M2 macrophages, contributing to tumor immune evasion (27).

Moreover, RNA methylation, as a critical epigenetic modification, controls multiple facets of RNA stability, splicing, translation, and decay, thereby affecting gene expression patterns and cellular functions (28,29). The observed correlations imply that *ALPL* might enhance tumorigenicity by altering the activity of key methylation regulators, potentially driving oncogenic pathways. Further investigation into the mechanistic link between *ALPL* and RNA methylation pathways could provide insights into its potential as a therapeutic target in THCA and other cancers.

In addition, our results highlight the significant diagnostic and prognostic value of *ALPL* in THCA, with ROC curve analysis showing its strong ability to differentiate THCA from normal tissues. Furthermore, a prognostic nomogram model incorporating *ALPL* expression, pathological stage, and residual tumor status was developed using Cox regression analysis, enhancing the prediction of patient outcomes. These findings establish *ALPL* as both a reliable diagnostic marker and an important prognostic indicator for THCA.

Additionally, drug sensitivity analysis further reveals the relationship between *ALPL* expression and responses to various anticancer drugs. Negative correlations were

observed with drugs like omacetaxine mepesuccinate, indicating increased drug sensitivity at lower *ALPL* expression levels, while positive correlations with drugs such as selumetinib and trametinib suggest reduced sensitivity with higher *ALPL* expression. These insights highlight the potential role of *ALPL* in guiding personalized treatment strategies and optimizing therapeutic efficacy in cancer management.

Implications and actions needed

The strong correlation between *ALPL* expression and immune cell infiltration suggests that *ALPL* may play a pivotal role in shaping the tumor immune microenvironment in THCA. This finding highlights the need for further mechanistic studies to elucidate *ALPL*'s functional role and its interaction with immune components. Additionally, integrating *ALPL* expression into diagnostic and prognostic frameworks could improve early detection and inform personalized therapeutic strategies for THCA patients.

Conclusions

Our study underscores the pivotal role of *ALPL* in THCA, highlighting its dual function as both a diagnostic biomarker and an immunoregulatory factor. The strong correlation between *ALPL* expression and immune cell infiltration emphasizes its potential significance in THCA therapy. A deeper understanding of *ALPL*'s molecular mechanisms could improve tumor detection, inform treatment strategies, and enhance patient prognosis.

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Footnote

Reporting Checklist: The authors have completed the MDAR and REMARK reporting checklists. Available at <https://gs.amegroups.com/article/view/10.21037/gs-2025-202/rc>

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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 and its subsequent amendments. The study was approved by the Ethics Committee of Sir Run Run Hospital Affiliated to Nanjing Medical University (No. 2024-SR-055). All patients signed informed consent.

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