ANLN and Lung Adenocarcinoma Prognosis and Immune Infiltration Research

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Abstract

Objective: This study aims to explore the role of aniline-actin binding protein (ANLN) in the prognosis of lung adenocarcinoma (LUAD) and its role in immune infiltration, and to provide new ideas for clinical diagnosis and treatment.

Methods: In this study, we applied bioinformatics methods to analyze the expression pattern and prognostic value of ANLN in LUAD ,and confirmed its independent prognostic value through Cox analysis. TIMER was used to evaluate the correlation between ANLN and tumor infiltrating immunity. LinkedOmics was used to study the co-expressed genes and functional networks related to ANLN.The TIMER database was used to analyze the correlation between ANLN co-expressed genes and tumor infiltrating immune cells.

Results: ANLN is highly expressed in most cancers. In the TCGA-LUAD cohort, ANLN was highly expressed in LUAD. ANLN was significantly related to the grade , T stage and N stage of patients with LUAD. It was verified in multiple independent queues. The high expression and highly mutation of ANLN predicts poor survival .Multivariate COX analysis showed that ANLN was an independent risk factor for survival . GSEA analysis shows that ANLN regulates cell cycle, and other pathways. The expression of ANLN was significantly correlated with the level of B cell infiltration.

Conclusions: ANLN is closely related to the prognosis of patients with LUAD and tumor immune cell infiltration suggesting that ANLN is a key factor regulating the recruitment of immune cells to LUAD and may play an important role in immune.

1. Introduction

Lung cancer is the main cause of cancer deaths (18.0% of total cancer deaths), followed by colorectal cancer (9.4%), liver cancer (8.3%), lung cancer is the most common malignant tumor, and it is also one of the cancel with highest morbidity and mortality rate of the world[(1)]. According to histological characteristics, lung cancer can be divided into two main subtypes: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), which account for 15% and 85% of all cases respectively [(2)] . Among them, lung adenocarcinoma (LUAD) is the main histological type of non-small cell lung cancer, accounting for about 40% of all lung cancers [(3)]. Although in the past few decades, great progress has been made in the research on the pathogenesis of the disease and new treatment methods, unfortunately, because there are no obvious symptoms in the early stage and it is difficult to be detected, LUAD is still the most aggressive and one of the most lethal tumor types [(4)], the overall survival rate of patients with lung adenocarcinoma is still very poor, and the incidence of lung adenocarcinoma has been on the rise in recent years. The fundamental reason is the lack of understanding of the mechanism of lung adenocarcinoma and the lack of more specific target molecules for early diagnosis and treatment.

The actin binding protein ANLN is a highly conserved multi-domain protein and is considered to be a key factor in cell division [5]. Recent studies have shown that ANLN is highly expressed in a variety of malignant tumors [6], and is related to disease progression and poor prognosis. Overexpression of ANLN leads to poor prognosis of breast cancer [7], non-small cell lung cancer [8], colorectal cancer [9], pancreatic ductal adenocarcinoma [10], and bladder cancer [11]. Therefore, ANLN has great potential as a biomarker of tumor progression [12].

In the past, Chenghan Luo [13]et studied differentially expressed genes in lung adenocarcinoma tissues and found that ANLN is a key factor in the pathogenesis of lung adenocarcinoma. However, so far, there are few studies on the role of ANLN in the occurrence and development of lung adenocarcinoma. The pathogenesis of lung adenocarcinoma has not yet been fully elucidated. Therefore, in this study, we evaluated the expression and prognostic value of ANLN and other cancers, and explored its , genetic alteration, signaling pathways and tumor immunity to provide new directions, biological targets and strategies for the diagnosis, treatment and prognosis evaluation of LUAD.

2. Results

2.1 Gene expression analysis in different tumors.

We applied the TIMER2 approach to analyze the expression status of ANLN across various cancer types of TCGA. As shown in Figure 1, the expression level of ANLN in the tumor tissues of BLCA、BRCA、CHOL、COAD、ESCA、HNSC、 KIRC、KIRP、LIHC、LUAD、LUSC、PRAD、READ、SKCM、STAD、etc is higher than the corresponding control tissues (P < .01).

2.2. The relationship between ANLN and clinicopathological characteristics in patients with lung adenocarcinoma

The clinical characteristics of 594 patients were obtained from the TCGA-LUAD cohort, including age, gender, tumor stage and TNM classification. According to the median of ANLN mRNA levels, patients were divided into 268 cases of high ANLN expression group and 267 cases of low ANLN expression group. As shown in Table 1, the expression of ANLN was significantly correlated with the grade (P<0.001), gender (P=0.017), age (P=0.001), T stage (P<0.001) and N stage (P<0.001) of lung adenocarcinoma Related. The expression of ANLN has nothing to do with clinicopathological factors such as M staging (P=0.122).

2.3 Expression analysis

We analyzed the expression level of ANLN in 535 cases of lung adenocarcinoma tissues and 59 cases of adjacent lung tissues, and found that ANLN was highly expressed in lung adenocarcinoma tissues

(P<0.001, Figure 2A). At the same time, we also analyzed the expression of ANLN in 57 cases of lung adenocarcinoma tissues and their paired adjacent tissues. The results showed that ANLN was highly expressed in lung adenocarcinoma tissues (P<0.001, Figure 2B). We detected the expression of ANLN protein in the UALCAN database and found that it was significantly highly expressed in tumor tissues (Figure 2C). Overall, these results indicate that the expression of ANLN in lung adenocarcinoma tissues is higher than that in normal tissues.

In addition, we also used the ROC curve to analyze the effectiveness of ANLN expression levels in distinguishing lung adenocarcinoma tissues from non-tumor tissues. The area under the curve (AUC value) of ANLN is 0.978, suggesting that ANLN can be used as an ideal biomarker to distinguish lung adenocarcinoma from non-tumor tissues (Figure 2D).

2.4 Prognostic analysis

In order to understand the correlation between ANLN expression and the prognosis of LUAD patients, we used Kaplan-Meier survival curve to analyze the prognostic value of ANLN expression in lung adenocarcinoma patients. According to the median of ANLN mRNA expression, the cohort was divided into low expression subgroup and high expression subgroup. The overall survival of patients with low ANLN expression (HR=1.90, 95%CI: 1.42–2.55, P□0.001) was significantly higher than that of the high expression group (Figure 3A). Similarly, the disease- specific survival of the low expression group (HR=1.98, 95%CI: 1.37–2.88, P□0.001) was also significantly higher than that of the high expression group (Figure 3B). These results indicate that the expression of ANLN is significantly associated with a poor prognosis.

A Cox univariate and multivariate proportional hazard model for the overall survival of LUAD patients was constructed. As shown in (Figure 4A)□ in the COX univariate regression model, T stage (P=0.002), N stage (P<0.001), and M stage (P=0.006) (P<0.001), pathological grade (P<0.001), ANLN (P<0.001) affect the prognosis of LUAD patients. Multivariate analysis further showed that T stage (P=0.046), N stage (P=0.003), pathological grade (P=0.022), ANLN (P=0.032) expression levels have a significant impact on the prognosis of patients with lung adenocarcinoma(Figure 4B). As mentioned, ANLN is a potential independent risk factor for LUAD.

2.5 Genetic alteration analysis

We observed the genetic alteration status of ANLN in different tumor samples of the TCGA cohorts. As shown in (Figure 5A). Only "mutation" is the type were Uterine Carcinosarcom□Diffuse Large B-Cell Lymphoma□Kidney Renal Clear Cell Carcinoma□Kidney Renal Papillary Cell Carcinoma . The main types of mutations are Uterine Corpus Endometrial Carcinoma □Bladder Urothelial Carcinoma □Skin Cutaneous Melanoma □Lung Adenocarcinoma□Adrenocortical Carcinoma □Stomach Adenocarcinoma □Head and Neck Squamous Cell Carcinoma□Lung Squamous Cell Carcinoma□Cervical Squamous Cell Carcinoma□

Colorectal Adenocarcinoma. Only the "amplified" type is Pheochromocytoma and Paraganglioma, The main type of amplification are sophageal Adenocarcinoma、Glioblastoma Multiforme 、Ovarian Serous Cystadenocarcinoma、Prostate Adenocarcinoma、Sarcoma 、Brain Lower Grade Glioma、 Pheochromocytoma and Paraganglioma. In LUAD, "Mutation" accounted for 2.12%,“Amplification”accounted for 0.71%;"Deep Deletion" 0.35%;“Multiple Alterations”accounted for 0.18%.The types and sites of the ANLN genetic alteration are further presented in(Figure 5B), and case number of the ANLN genetic alteration are further presented in table3. We found that the missense mutation (R153Q/L) of ANLN was the major type of genetic change, and mutation sites could be observed in the 3D structure of ANLN protein (Figure 5C). In addition, we investigated the potential association between ANLN gene changes and clinical survival outcomes in patients with LUAD. Compared with LUAD patients without ANLN changes, LUAD patients with ANLN changes had poorer overall outcomes (p-value: 0.0238) (Figure 5D) and disease-specific survival (P-value: 0.309)(Figure 5E).

2.6 Correlation analysis between the expression of ANLN and six main infiltrating immune cells

The TIMER database was used to analyze the correlation between the expression of ANLN in LUAD and the six main infiltrating immune cells (B cells, CD4 T cells, CD8+T cells, neutrophils, macrophages and dendritic cells). The analysis showed that the expression level of ANLN was significantly correlated with the levels of B cells (r=−0.227, p value=4.33e-07) and neutrophils (r=0.16, p value=4.00e−04) (Figure 6A) .

In addition, we evaluated the prognostic value of six immune cells through Kaplan-Meier analysis and found that B cells (P<0.001) and ANLN expression (P<0.001) were significantly correlated with the prognosis of LUAD (Figure 6B). It is suggested that ANLN has a strong regulatory effect on the immune cell infiltration of lung adenocarcinoma, especially the regulation of B cell infiltration.

2.7 Co-expression analysis

In order to better understand the expression mechanism of ANLN in LUAD, use the "LinkFinder" module in LinkedOmics to check the co-expression mode of ANLN. The related genes co-expressed by ANLN were obtained by Pearson test, and the results are shown in a volcano graph (Figure 7A). (Figures 7B and 7C) show the heat maps of the top 50 genes that are positively and negatively correlated with ANLN, respectively.

GSEA is used to identify signal pathways related to ANLN. The GO annotation of GSEA shows (Figure 8A) that ANLN co-expressed genes mainly involve chromosome segregation, mitotic cell cycle phase transition, DNA replication, DNA recombination, and RNA localization. On the contrary, the drug catabolism process is inhibited, and the protein activates the cascade process. KEGG enrichment analysis shows (Figure 8B) that genes are mainly enriched in the cell cycle, RNA transport, pyrimidine metabolism, spliceosome, ubiquitin-mediated proteolysis, and Epstein-Barr virus infection.

In addition, the top 5 significant genes related to ANLN, including KIF4A, KIF23, CKAP2L, TPX2, KIF2C, were selected for further analysis, and their correlation with ANLN was verified in the TIMER database. The results show that ANLN and KIF4A (COR=0.888, p=3.4e−175), KIF23 (COR=0.889, p=1.09e−175), CKAP2L (COR=0.883, p=2.74e−170), TPX2(COR =0.876, p=9.24e−165), KIF2C (COR=0.87, p=8.31e−160) were significantly correlated (Figure 9A-E).

3. Discussion

Lung adenocarcinoma has become one of the tumors with high clinical mortality due to its difficult early detection and poor prognosis [14]. The most common type of LC is adenocarcinoma (ADC), which accounts for about 40% of the total number of LCs. Lung ADC develops from small airway epithelium, type II alveolar cells, which secrete mucus and other substances [15.16]. Unfortunately, lung ADC is still one of the most aggressive and fatal tumor types, with an overall survival time of less than 5 years. The discovery of oncogenic driver gene mutations and their role in predicting response to targeted therapy has changed the way clinicians diagnose and treat ADCs in the lungs. Although targeted therapy has shown promising results, almost all patients will eventually develop disease progression due to acquired drug resistance [17].

The (ANLN) gene is located on chromosome 7p14.2 and encodes 4 domains composed of 1124 amino acids, including myosin and actin binding domains, RhoA binding domain and C-terminal Pleckstrin homology domain [18.19]. ANLN protein is located in the nucleus, cytoplasm, cytoskeleton, cleavage groove and cell cortex, and is expressed in adult placenta, testis and spinal cord, and many fetal organs [20]. ANLN encodes an actin-binding protein that plays a role in cell growth, migration and cytokinesis [21]. ANLN is involved in the PI3K/PTEN signal transduction pathway [8.22]. Nowadays, with the development of bioinformatics technology, it is found that the disorder of ANLN is closely related to the occurrence and development of many tumors [7-11]. In several types of cancer patients, ANLN has undergone genetic changes, including amplification, deletion, and SNP mutation; the mutation frequency is 0.2% in clear cell renal cancer and 19.6% in prostate cancer [23.24]. 27 mutations were found in lung adenocarcinoma [25], including 12 amplifications, 2 deletions and 13 SNPs. ANLN has been developed as a clinically applicable prognostic biomarker for hepatocellular carcinoma based on immunohistochemistry [26]. Although the role of ANLN in the occurrence and prognosis of certain cancers has been clarified, there is no report on the different expression and role of ANLN in LUAD.

This study first found that the expression of ANLN the expression of ANLN in LUAD, LUSC and other tumors was higher than that in the normal control group, suggesting that ANLN plays a role in a variety of tumors, in lung adenocarcinoma tissues was significantly higher than that in normal tissues through the bioinformatics analysis of TCGA database data. The ROC curve showed that ANLN can be used as a potential clinical indicator for the diagnosis of lung adenocarcinoma. We divided the lung adenocarcinoma patient specimens from the TCGA database into high gene expression/low expression groups. Kaplan-Meier survival analysis showed that the expression of ANLN affects the overall survival of

lung adenocarcinoma. The higher the ANLN expression level, the overall lung adenocarcinoma patients. The shorter the survival time. We further applied the clinicopathological data of lung adenocarcinoma patients in the TCGA database and found that the expression level of ANLN has an impact on tumor size, lymph node metastasis and pathological staging, and can be used as a potential indicator for the diagnosis of lung adenocarcinoma malignancy. Multivariate Cox regression analysis found that the risk of death in patients with lung adenocarcinoma with high expression of ANLN mRNA was about 1.4 times that of the low expression group, suggesting that ANLN can be used as an independent predictor of the clinical prognosis of lung adenocarcinoma. This study found that ANLN is mainly a missense mutation occurring at R153Q/L, which is related to the occurrence and development of tumors. In addition we studied the co-expression and regulatory network of ANLN. Through the analysis of GO and KEGG pathways, it is found that ANLN is mainly involved in cell division and cell cycle regulation, and these pathways are of great significance. It is related to the occurrence and development of LUAD. These results provide a basis for the follow-up study of the mechanism of ANLN in tumors and are worthy of further clinical verification.

Immune cell infiltration has been shown to affect the occurrence and recurrence of tumors, and plays an important role in tumor immunotherapy and clinical effects [(27)]. Another important result of this study is the discovery that the expression level of ANLN gene is correlated with the level of infiltration of a variety of immune cells. It is found that the infiltration of ANLN in B cells may be one of the factors affecting its prognosis. It shows that the expression difference of ANLN has an impact on the immune microenvironment of LUAD, and participates in the regulation of LUAD tumor immunity, and can be used as a prognostic indicator of LUAD patients, and can reflect the immune status of patients. Provide new insights and ideas for the immune mechanism of tumorigenesis and cancer treatment. However, the analysis at the transcriptome level cannot fully explain the impact of immune composition and immune status on tumor cells, but only reflects the overall level of cells, so more experiments are needed to verify this result.

This article is based on the TCGA database to determine that ANLN can be used as a potential clinical indicator for diagnosing early lung adenocarcinoma and predicting the prognosis of patients with lung adenocarcinoma. At the same time, it also provides new ideas for screening potential benefit populations of immunotherapy, and early diagnosis and prognosis of lung adenocarcinoma. Judgment and immunotherapy have important practical significance. However, more experimental studies are needed to confirm our results, so as to promote the clinical application of ANLN as a prognostic indicator of LUAD or as a target for immunotherapy.

4. Material And Methods

4.1 Gene expression analysis in cancers

We input ANLN in the “Gene_DE” module of TIMER2 (Tumor immune estimation resource, version 2) web (http://timer.cistrome.org/) and observed the expression difference of ANLN between tumor and adjacent

normal tissues for the different tumors or specific tumor subtypes of the TCGA project.[28].

4.2 Data Sources

First, obtain the gene expression data and clinical information of lung adenocarcinoma patients from the TCGA database (https://portal.gdc.cancer.gov/), including (age, gender, tumor grade, TNM staging, survival status, etc.). Filter all data to remove lost and duplicate results, and save it in TPM format.

4.3 ANLN gene expression analysis in LUAD

In the TCGA-LUAD cohort, Wilcoxon test (including unpaired and matched test) was used to analyze the difference in mRNA expression of ANLN in tumor tissues and normal tissues adjacent to cancer. The analysis was visualized by the R software "ggplot2" package, and $p<0.001$ was considered a significant difference.

The UALCAN database (http://ualcan.path.uab.edu/) is an online tool for analyzing cancer transcriptome data based on TCGA data [29]. In this study, we explored the effects of ANLN in LUAD and UALCAN normal lungs. The expression level of ANLN protein in the tissue.

We also used the ROC curve to analyze the effectiveness of ANLN expression in distinguishing lung adenocarcinoma tissues from non-tumor tissues.

4.4 Survival prognosis analysis

According to the median expression value of ANLN, TCGA-LUAD patients were divided into ANLN high expression group and low expression group. We analyzed the relationship between ANLN expression and patient OS (overall survival) and DSS (disease-specific survival) through Kaplan-Meier survival curve. Period) [30]. The analysis is visualized by the R software "Survminer" package.

The relationship between the clinicopathological characteristics and prognosis of patients with lung adenocarcinoma was analyzed by univariate Cox and multivariate Cox, and the independent prognostic value of ANLN in LUAD was determined. The analysis was visualized by the R software "survival" package.

4.5 Genetic alteration analysis

After logging into the cBioPortal web (https://www.cbioportal.org/) [31.32], we chose the “TCGA Pan Cancer Atlas Studies” in the “Quick select” section and entered “ANLN” for queries of the genetic alteration characteristics of ANLN. The results of the alteration frequency, mutation type and CNA (Copy

number alteration) across all TCGA tumors were observed in the “Cancer Types Summary” module. The mutated site information of ANLN. can be displayed in the schematic diagram of the protein structure or the 3D (Three-dimensional) structure via the “Mutations” module. We also used the “Comparison” module to obtain the data on the overall, disease-specific survival differences for the TCGA cancer cases with or without ANLN genetic alteration. Kaplan-Meier plots with log-rank P-value were generated as well.

4.6 Immune Infiltration Analysis

The TIMER database (https://cistrome.shinyapps.io/timer/) is used to systematically analyze the immune infiltration of different cancer types [28.33]. We use the "Gene" module to explore through the partial Spearman correlation analysis of tumor purity correction The relationship between ANLN expression and immune cell infiltration in lung adenocarcinoma tissue [28]. Calculate the immune scores of 6 immune cell types including B cells, CD4 + T cells, CD8 + T cells, neutrophils, macrophages and dendritic cells. In the "survival" module, the Kaplan-Meier method was used to analyze and evaluate the prognostic value of ANLN and six immune cells. The data used Spearman correlation test, $P<0.05$.

4.7 Co-expression analysis

The LinkedOmics (http://www.linkedomics.org) database includes multi-omics data from all 32 TCGA cancer types [34]. In the "LinkFinder" module of LinkedOmics, we use Pearson's test to perform statistical analysis on ANLN co-expression, and the results are displayed in the form of volcano maps and heat maps. And select the top 5 significant genes related to ANLN for further analysis, and verify their correlation with ANLN in the TIMER database.

The "LinkInterpreter" module of LinkedoMics is applied to GO (BP) and KEGG pathways for analysis through gene set enrichment analysis (GSEA). The grading standard is false discovery rate (FDR)<0.05, and the number of gene set permutations is set to 500.

Abbreviations

aniline-actin binding protein (ANLN)

lung adenocarcinoma (LUAD)

Declarations

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request https://portal.gdc.cancer.gov/

Contributions:

Mei-Hua Ru provided the experimental design, Mei-Hua Ru and Ying-Bo Liu provided technical support, and all of authors wrote and read the paper.

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All participants in this article. Competing interests. The authors declare that they have no competing interests, and all authors should confirm its accuracy.

Conflicts of interest

There is No conflict of interest exits in the submission of this manuscript.

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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.

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Tables

Table 1

Clinical characteristics of 522 patients obtained from the TCGA-LUAD cohort, including age, gender, tumor stage, and TNM classification.

Characteristic	Low expression of ANLN	High expression of ANLN	p
n	267	268	
T stage, n (%)			< 0.001
T1	113 (21.2%)	62 (11.7%)	
T2	122 (22.9%)	167 (31.4%)	
T3	23 (4.3%)	26 (4.9%)	
T4	8 (1.5%)	11 (2.1%)	
N stage, n (%)			< 0.001
N0	192 (37%)	156 (30.1%)	
N1	37 (7.1%)	58 (11.2%)	
N2	25 (4.8%)	49 (9.4%)	
N3	0 (0%)	2 (0.4%)	
M stage, n (%)			0.122
M0	181 (46.9%)	180 (46.6%)	
M1	8 (2.1%)	17 (4.4%)	
Pathologic stage, n (%)			< 0.001
Stage I	169 (32.1%)	125 (23.7%)	
Stage II	52 (9.9%)	71 (13.5%)	
Stage III	31 (5.9%)	53 (10.1%)	
Stage IV	9 (1.7%)	17 (3.2%)	
Gender, n (%)			0.017
Female	157 (29.3%)	129 (24.1%)	
Male	110 (20.6%)	139 (26%)	
Age, n (%)			0.001
<=65	109 (21.1%)	146 (28.3%)	

Table 2

Case number of ANLN mutation

Sample ID	Cancer Type Detailed	Protein Change	Mutation Type	Copy	Allele Freq (T)
TCGA-A5-A0G2-01	Uterine Serous Carcinoma/Uterine Papillary Serous Carcinoma	***R153Q***	**Missense**	ERROR	0.23
TCGA-75-6214-01	Lung Adenocarcinoma	***R153L***	**Missense**	ERROR	0.37
TCGA-D3-A5GU-06	Cutaneous Melanoma	***R153Q***	**Missense**	ERROR	0.42
TCGA-G4-6302-01	Mucinous Adenocarcinoma of the Colon and Rectum	***R153Q***	**Missense**	ERROR	0.09
TCGA-BA-A6DA-01	Head and Neck Squamous Cell Carcinoma	***R153L***	**Missense**	ERROR	0.26

Figures

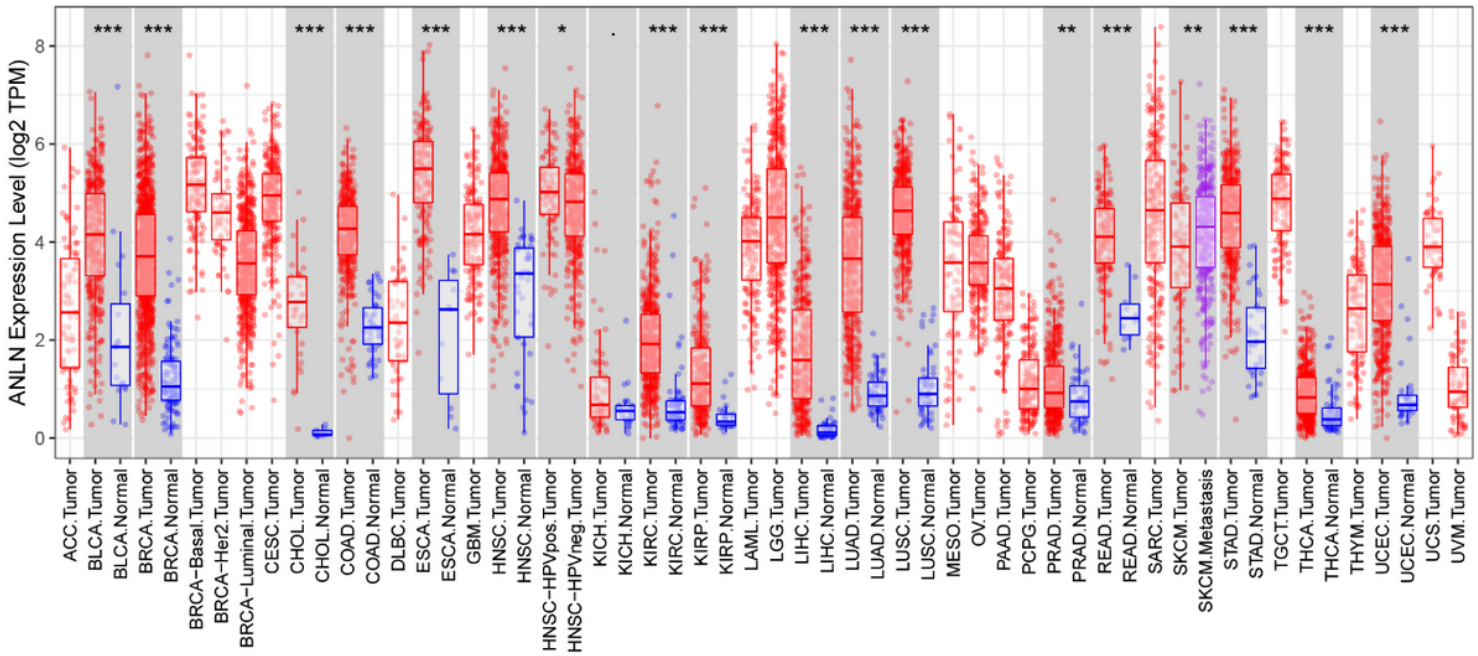

Figure 1

Expression levels of ANLN gene in different tumors. The expression of ANLN gene in different tumors or specific tumor subtypes was analyzed by TIMER2. . * P < .05; ** P < .01; *** P <.001.

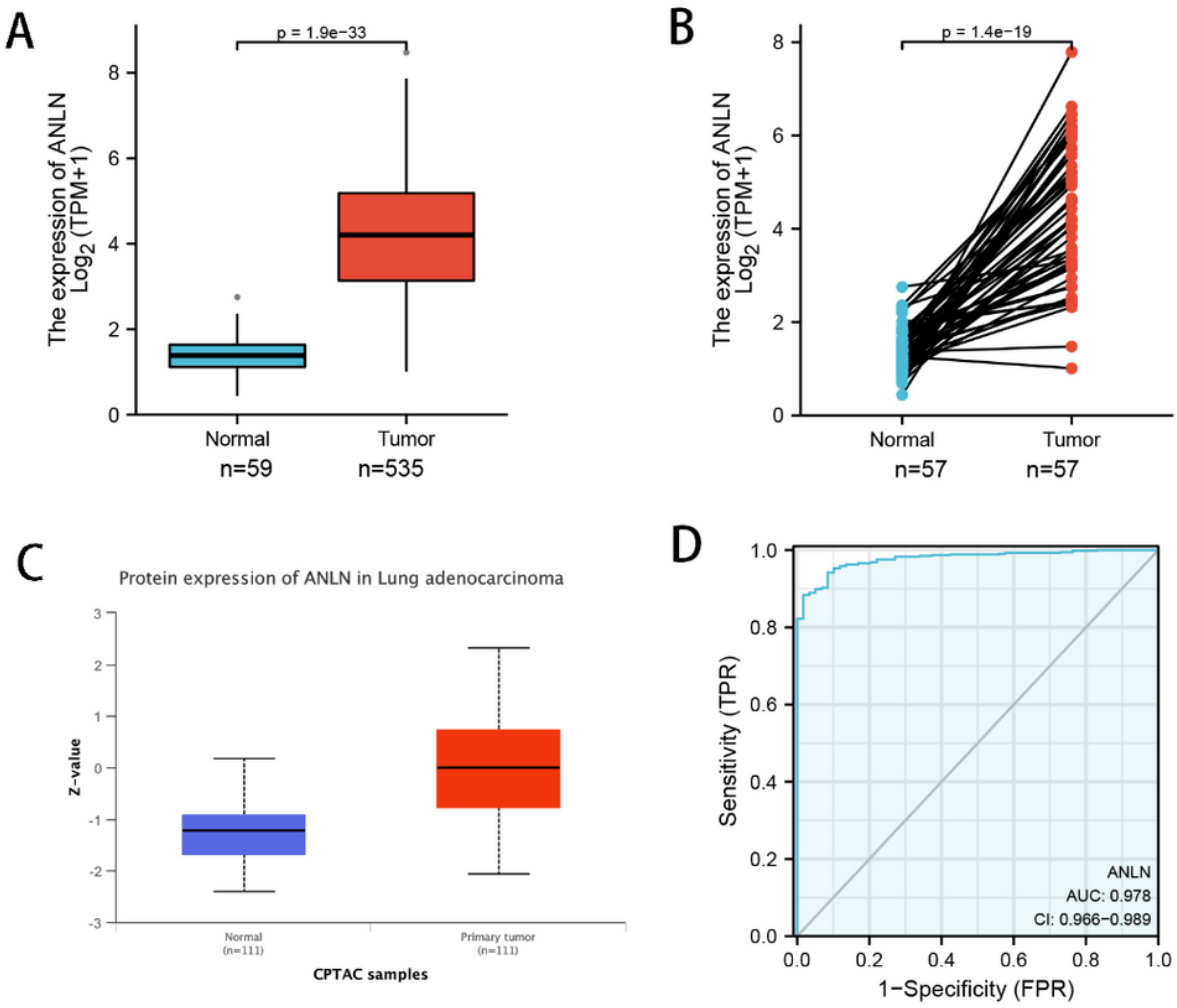

Figure 2

ANLN is highly expressed in LUAD. (A) The unpaired Wilcoxon test was used to analyze the comparison of ANLN mRNA expression in normal tissues and tumor tissues in the TCGA-LUAD cohort. (B) A paired Wilcoxon test was used to analyze the comparison of ANLN mRNA expression in normal tissues and adjacent tissues in the TCGA-LUAD cohort. (C) Comparison of ANLN protein expression in normal tissues and tumor tissues obtained from the UALCAN network tool (Wilcoxon test). (D) ROC curve shows that the expression level of ANLN can effectively distinguish LUAD tissues from non-tumor tissues. The X axis represents the false positive rate, and the Y axis represents the true positive rate.

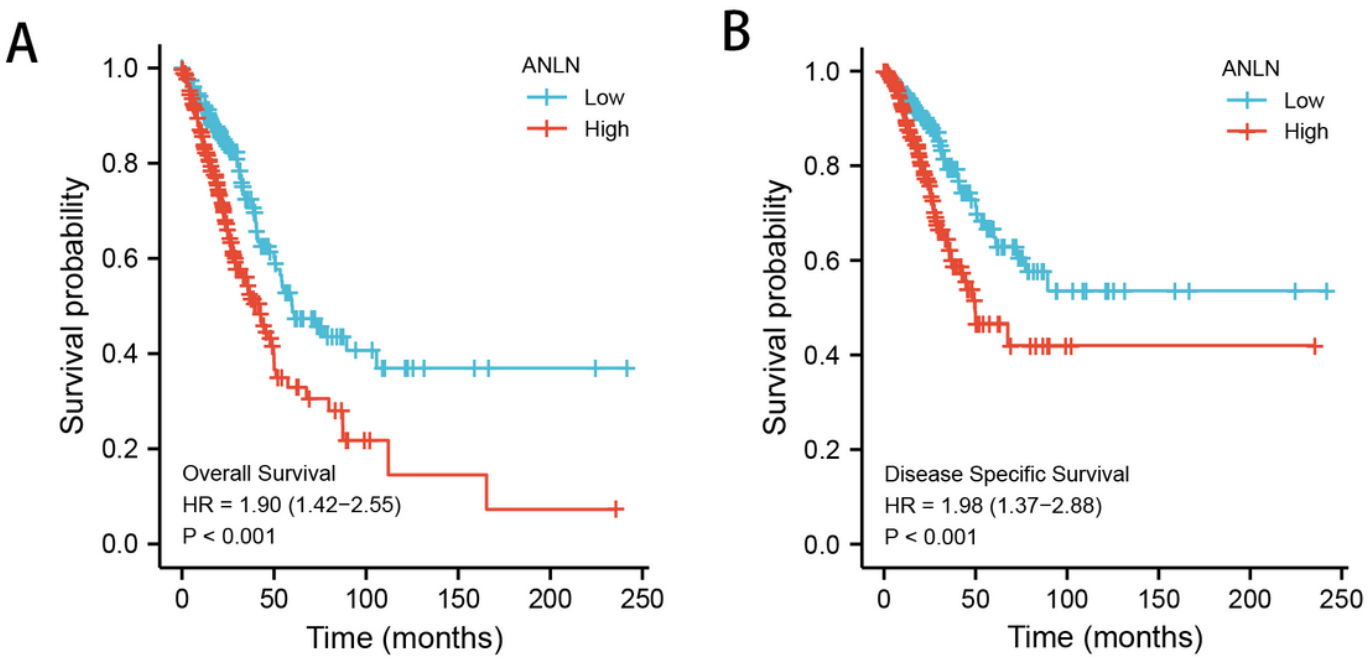

Figure 3

Kaplan-Meier survival curve of lung adenocarcinoma patients with high and low expression of ANLN. (A) Using TCGA data to analyze the OS (overall survival) of LUAD patients with high ANLN expression and low ANLN expression. (B) Using TCGA data to analyze DFS (disease-free survival) in patients with high ANLN expression and low ANLN expression LUAD

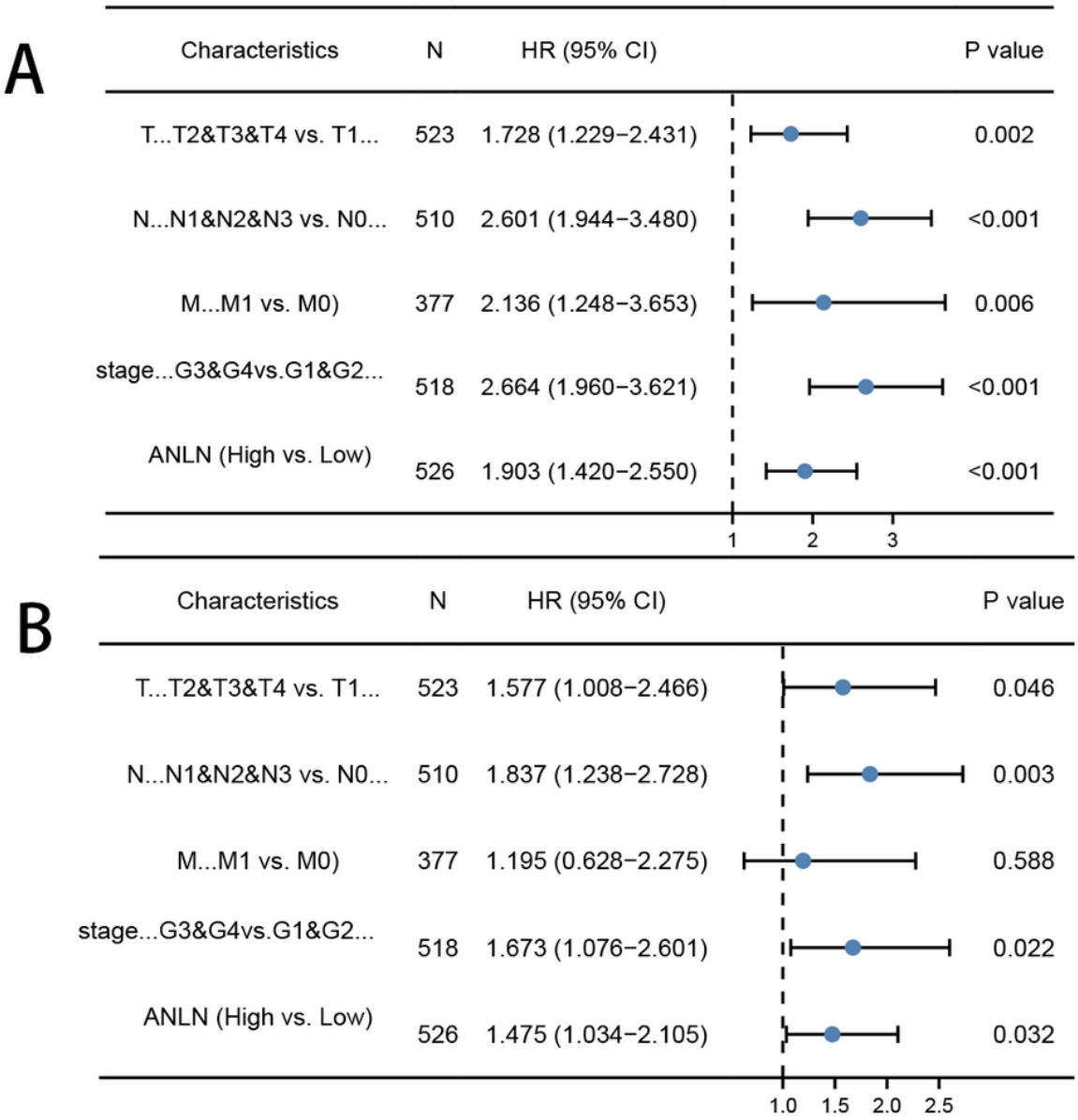

Figure 4

The relationship between Cox regression analysis and progression-free survival and clinicopathological features in patients with TCGA

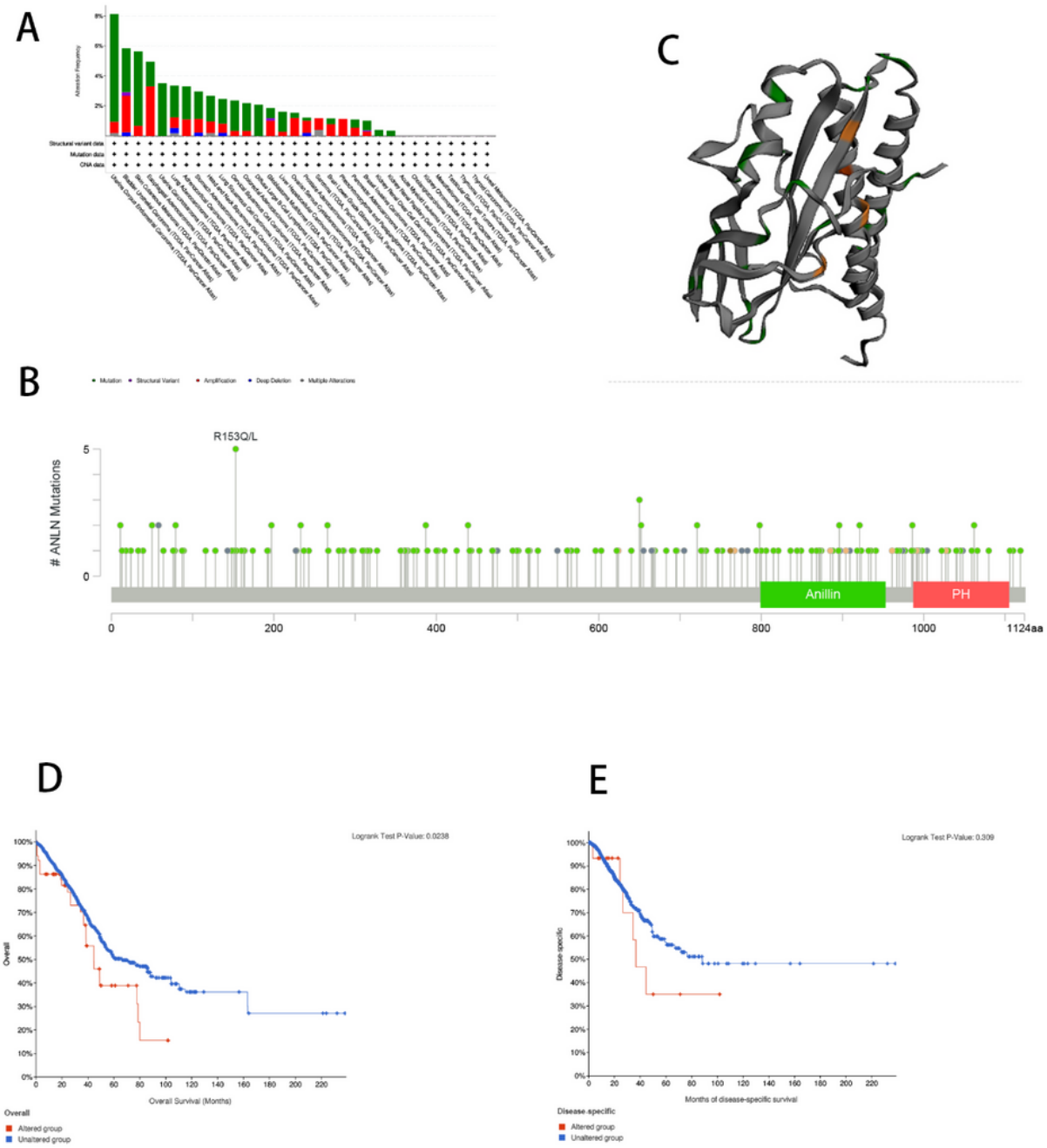

Figure 5

Mutation feature of ANLN in different tumors of TCGA. We analyzed the mutation features of ANLN for the TCGA tumors using the cBioPortal tool(A). The alteration frequency with mutation type are displayed(B). We can see the mutation site in the 3D structure of ANLN(C). We also analyzed the potential correlation between mutation status and overall, disease-specific survival(Figure 4E).

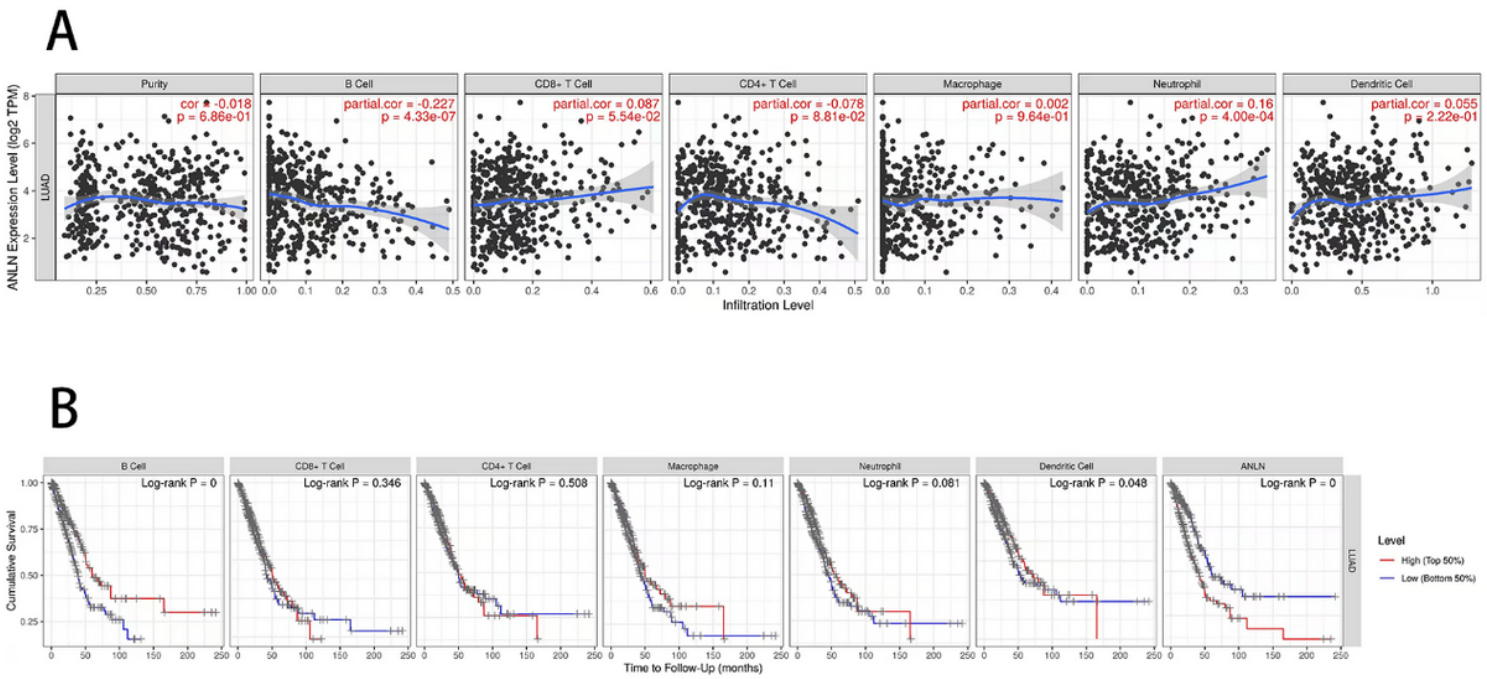

Figure 6

The relationship between the expression level of ANLN and immune cell infiltration. (A) The expression of ANLN in lung adenocarcinoma is related to the degree of immune invasion. (B) Kaplan-Meier plot of lung adenocarcinoma immune infiltration and ANLN expression level. (Spearman correlation test, P<0.05).

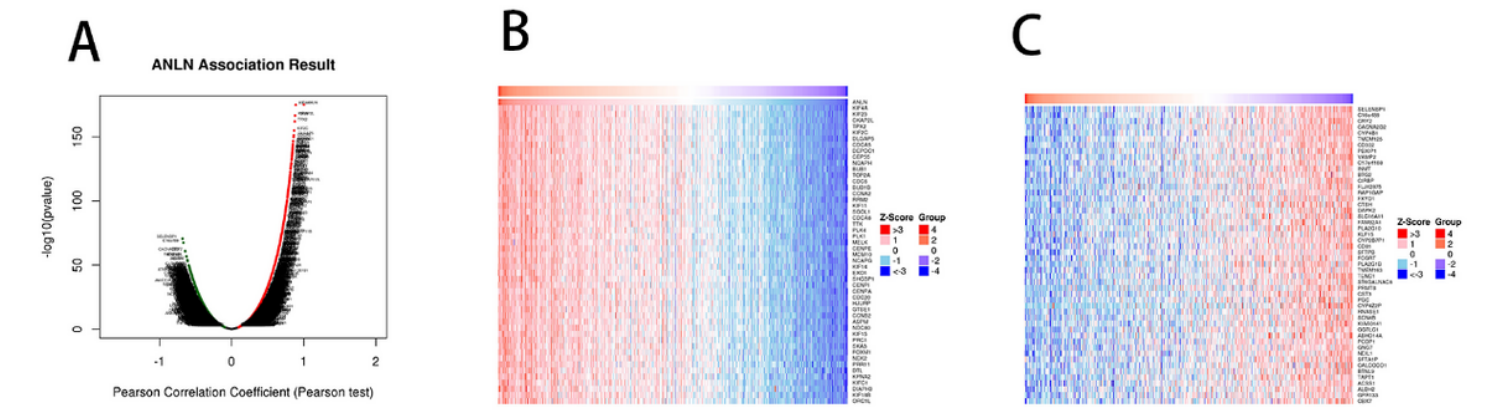

Figure 7

Study on ANLN co-expression gene in LUAD(LinkedOmics). (A) ANLN highly correlated genes identified by Pearson at LUAD. The red and green dots represent positively and negatively correlated genes with RRM2, respectively. (B) The heat map showed that the top 50 genes in LUAD were positively correlated with ANLN.(C) The heat map showed that the top 50 genes in LUAD were negatively correlated with ANLN.

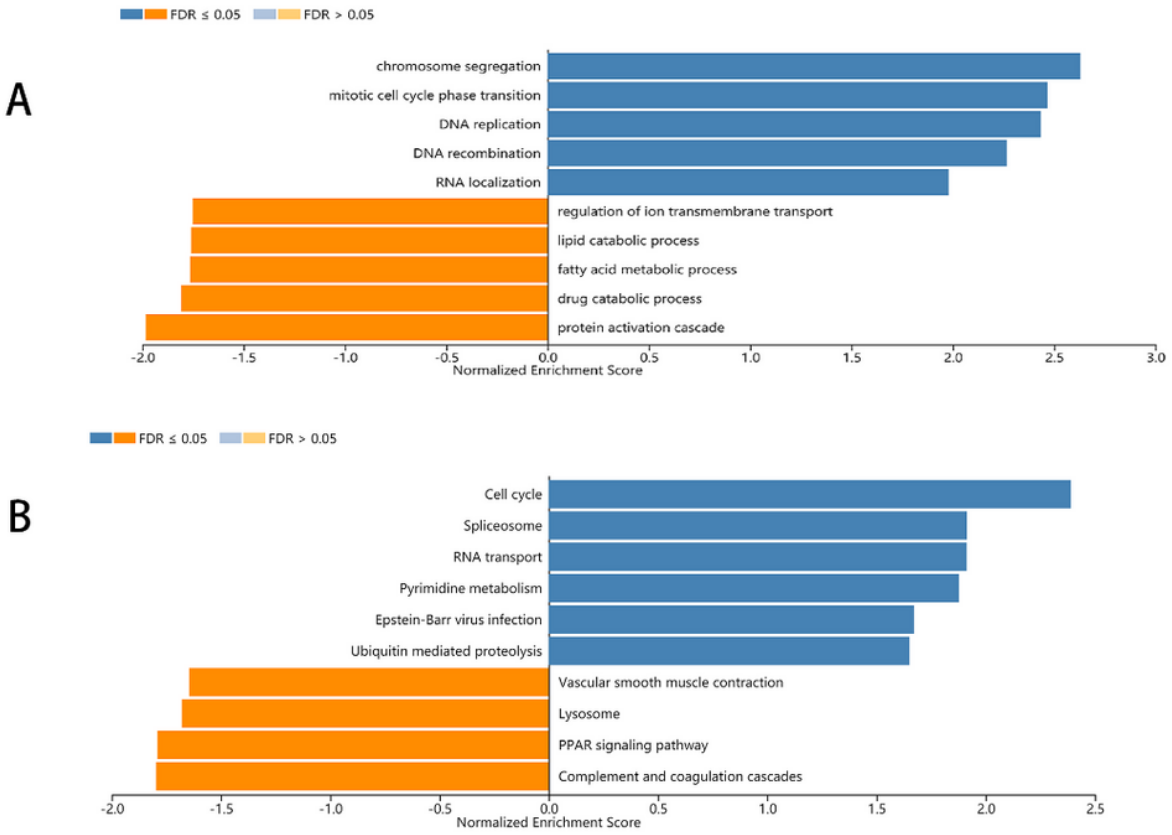

Figure 8

GSEA analysis of ANLN related pathways in LUAD. (A) Significant enrichment of GO-BP in ANLN in LUAD. (B). Significant enrichment of ANLN in LUAD KEGG

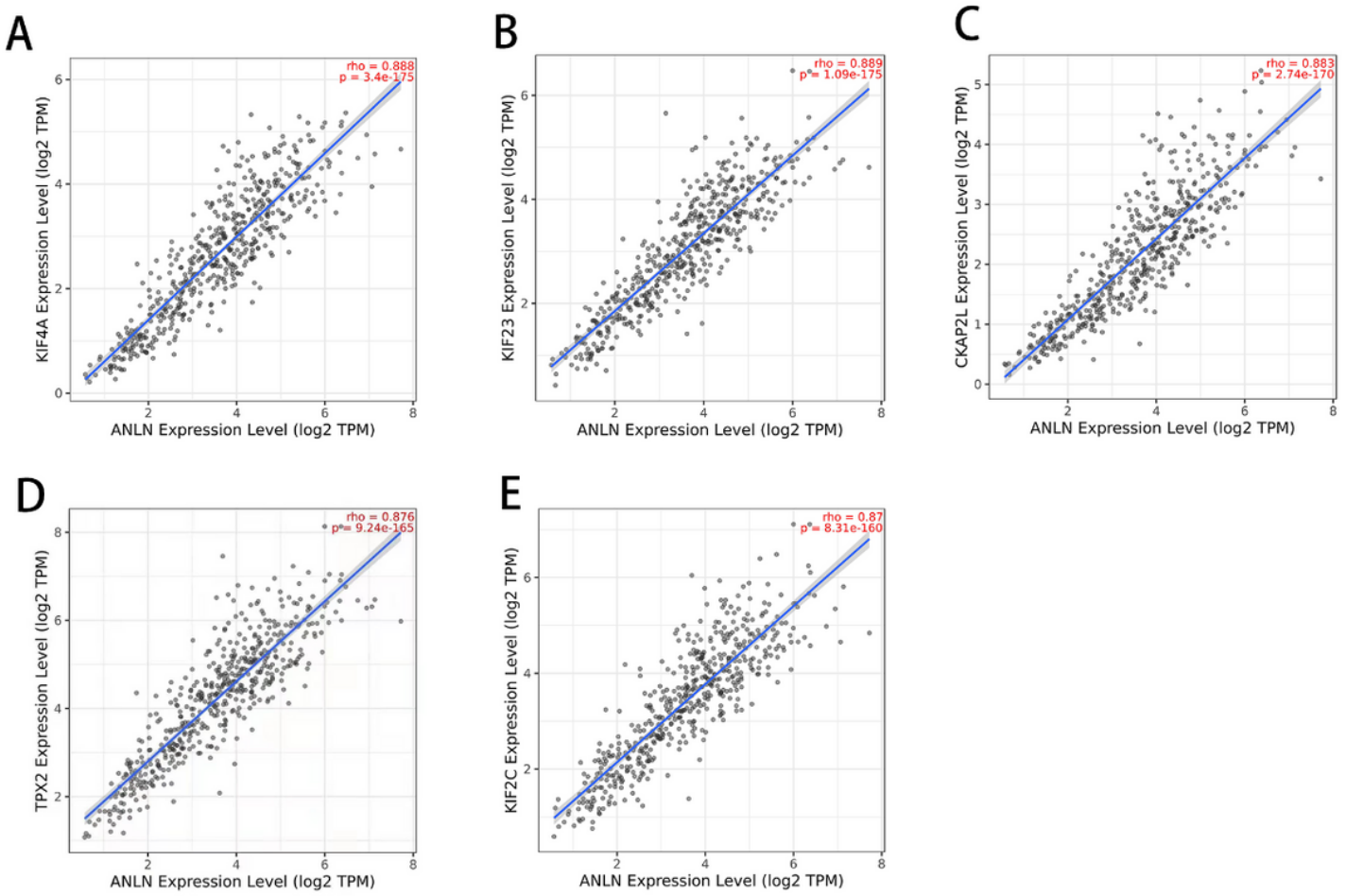

Figure 9

The TIMER database was used to evaluate genes co-expressed with ANLN in LUAD. (ae) ANLN and KIF4A (COR=0.888, p=3.4e−175), KIF23 (COR=0.889, p=1.09e−175), CKAP2L (COR=0.883, p=2.74e−170), TPX2(COR =0.876, p=9.24e−165), KIF2C (COR=0.87, p=8.31e−160) are significantly correlated