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ORIGINAL RESEARCH

Expression Characteristics and Significant Diagnostic and Prognostic Values of ANLN in Human Cancers

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Introduction: In light of the increased demand for reliable cancer-associated biomarkers and ANLN oncogenic potential, the present study aimed to investigate *ANLN's* role in 24 human cancers.

Methods: The UALCAN, Kaplan–Meier (KM) plotter, TNM Plot, GENT2, GEPIA, HPA, cBioPortal, STRING, Enrichr, TIMER, Cytoscape, DAVID, MuTarget, and CTD online databases and bioinformatic tools were used in this study.

Results: In three of the cancers analyzed, ANLN expression was downregulated in tumor tissue, while it was overexpressed in the 21 other types of tumor tissue relative to controls. In CESC, ESCA, HNSC, and KIRC patients, *ANLN* overexpression was correlated with shorter overall survival, relapse-free survival, and metastasis. This suggests that *ANLN* is significantly involved in the development and progression of these four cancers. Further expression analysis revealed upregulation of ANLN in CESC, ESCA, HNSC, and KIRC patients with different clinical characteristics, regardless of the heterogeneity barrier. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analysis revealed that ANLN-associated genes were coexpressed with *ANLN* and were included in diverse BP, MF, and KEGG terms. Moreover, some interesting correlations were also documented between *ANLN* expression and its promoter-methylation level, genetic alterations, other mutant genes, and $CD8^+$ T- and $CD4^+$ T-cell infiltration. Moreover, we also identified *ANLN*-associated transcription factors, miRNAs, and chemotherapeutic drugs.

Conclusion: This pan-cancer study revealed the novel diagnostic and prognostic role of *ANLN* across four cancers, regardless of heterogeneity.

Keywords: cancer, ANLN, diagnostic, prognostic, biomarker

Introduction

Cancer is a multifactorial disease with various potential causes.[1] In 2020, around 8.7 million cancer deaths were reported worldwide and the disease declared the second-leading cause of death after cardiovascular disorders.[2] Although these massive figures show that the battle against cancer has not been won yet, recent advances in personalized medicine and

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treatment strategies like immunotherapy have significantly improved the survival of cancer patients.[3] However, cancer is still rising significantly and results in tremendous social and economic burdens around the world. As such, exploring the underlying biological mechanisms of carcinogenesis and investigating the possible potential diagnostic and prognostic biomarkers of cancer is urgent.

Anillin (*ANLN*) is an actin-binding protein that was originally discovered in *Drosophila* and is primarily involved in cytokinesis.[4] The ***ANLN*** gene is located at the 7p14.2 position in the human genome and is involved in the recruitment of numerous key cell division–associated genes, such as F-actin, myosin II, and septins, and is thus regarded as the major organizer of the cytokinetic machinery.[5] It has been reported that mutations and expression variations in *ANLN* contribute significantly to the development and progression of human cancers.[6] So far, *ANLN* dysregulation has been reported in different human cancers, ie, in breast, colorectal, liver, lung, endometrial, kidney, ovarian, brain, and pancreatic cancers.[7] A bioinformatics-based study revealed that the *ANLN* gene can be an ideal prognostic biomarker for evaluating the survival of cervical cancer patients.[8,9] In addition, *ANLN* was found to cause chemotherapy resistance in breast cancer cells by directly reducing the effectiveness of doxorubicin during a clinical trial.[10] As ANLN upregulates in tumor tissue and its expression increases as the disease progresses, this gene holds great promise as a potential biomarker of cancer development and progression.[7,11,12] Due to the increasing prevalence of cancer cases worldwide, elucidation of underlying molecular mechanisms and the discovery of more sensitive diagnostic and prognostic biomarkers of cancer is critical to managing the disease.

In this study, we carried out a systematic expression analysis and evaluated the diagnostic and prognostic values of *ANLN*. Additionally, we also analyzed the correlation of *ANLN* expression with its promoter-methylation level, other mutant genes, and $CD4^+$ and $CD8^+$ T cells. Finally, using online databases, we identified transcription factors (TFs), miRNAs, and important chemotherapeutic drugs targeting ANLN.Our results revealed the unique role of *ANLN* in cervical squamous-cell carcinoma (CESC), esophageal carcinoma (ESCA), head-and-neck squamous-cell carcinoma (HNSC), and kidney renal clear-cell carcinoma (KIRC), as well as suggesting this gene as a reliable diagnostic and prognostic biomarker in these cancers.

Methods

Ethics

This study was reviewed and approved by the ethics committee of Islamia University of Bahawalpur, Bahawalpur, Pakistan.

UALCAN Analysis

The UALCAN web tool facilitates multiomic analysis with respect to both MET500 and TCGA cancer genomic data.[13] In this work, we performed pan-cancer differential expression and promoter-methylation analysis of *ANLN* across multiple human cancer types using UALCAN. mRNA-expression level were normalized as transcripts per million reads, while promoter-methylation level were normalized as β values. As with all statistical analyses herein, Student's *t*-test was employed and $P<0.05$ taken as significant.

KM Plotter–Based Analysis

Kaplan–Meier (KM) plotter (https://kmplot.com/analysis) is a user-friendly online tool designed for survival analysis of the gene of interest in 21 different cancer patients. This tool contains mRNA-expression data and survival information of cancer patients obtained from the Gene Expression Omnibus (GEO).[14] Briefly, *ANLN* was searched to obtain KM overall survival (OS) and relapse-free (RFS) plots of the available 21 cancer subtypes. *P*-values, 95% CIs, and HRs) were determined.

TNM Plot

This database (https://www.tnmplot.com) is designed to analyze the relative expression of any gene of interest in normal, cancerous, and metastatic tissue.[15] It contains data on 3,691 normal, 29,376 tumor, and 453 metastatic samples from the

GEO and 730 normal, 9,886 tumor, and 394 metastatic samples from TCGA. We used TNM Plot to analyze *ANLN* expression in normal and metastatic tissue from different cancers.

TIMER-, GENT2-, GEPIA-, and HPA-Based Expression-Validation Analysis

TIMER (http://timer.cistrome.org), GENT2 (http://gent2.appex.kr), GEPIA (http://gepia.cancer-pku.cn), and the Human Protein Atlas (HPA) database (https://www.proteinatlas.org) are cancer transcriptomic data-analysis websites.[16–19] In the current study, we used TIMER, GENT2, and GEPIA for mRNA- and protein-expression validation of *ANLN* using new independent cohorts of cancer patients. In TIMER, GENT2, and GEPIA, mRNA expression was normalized as transcripts per million reads, while in the HPA protein expression was graded as not detected, low, medium, or high based on the intensity of staining and fraction of the stained cells.

MExpress

MExpress (https://mexpress.be) helps to analyze correlations between promoter-methylation levels and expression.[20] In this study, correlations between *ANLN* expression and promoter-methylation levels in cancer patients were computed using this tool with the help of Pearson correlation analysis.

cBioPortal

cBioPortal is a web tool that facilitates the multiomic analysis using TCGA cancer-genome data.[21] This tool provides comprehensive detail regarding copy-number variation (CNV) and genetic mutations in the gene of interest. Additionally, this tool also evaluates the association between gene mutation and the prognosis of cancer patients. We used cBioPortal to evaluate and analyze the *ANLN* genetic alterations and CNVs in TCGA's data set of cancers.

PPI-Network Construction, Visualization, Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway-Enrichment Analysis

The STRING database (http://string-db.org) is an online source for constructing PPI networks of genes of interest.[22] We utilized this user-friendly tool to construct a PPI network of ANLN-enriched genes. This was further visualized with Cytoscape software.[23] Then, GO and KEGG analysis of the *ANLN*-enriched genes were performed using DAVID (http://david.ncifcrf.gov/summary.jsp).[24]

Correlation of ANLN with Related Genes

GEPIA (http://gepia.cancer-pku.cn) was utilized to compute correlations between *ANLN* and related genes with the help of the "Correlation Analysis" module.

ANLN and $CD4^+$ and $CD8^+$ T Cells

TIMER (http://timer.cistrome.org) helps in the evaluation of immune-cell infiltration and clinical/therapeutic effects.[25] This resource helped us to compute Spearman correlations between *ANLN* expression and $CD4^+$ T- and $CD8^+$ T-cell infiltration in cancer patients.

Enrichr Database Analysis

Enrichr (https://maayanlab.cloud/Enrichr)[26] facilitates analysis of gene(s) of interest using enrichment terms, eg, functional, targeted miRNAs, and pathway-enrichment terms. In this study, we identified *ANLN*-targeted miRNAs and TFs using this web source. The top-ten significantly enriched terms are given in the Results section.

MuTarget Analysis

The MuTarget (https://www.mutarget.com/result) platform is principally designed to associate gene expression with mutational status in cancer samples.[27] We identified mutant genes responsible for expression alterations in *ANLN* using

this tool. Based on Mann–Whitney *P*-values and mean FC, significant genes were selected for testing, where we used default thresholds of $P \leq 0.05$ and $0.714 > FC > 1.4$.

ANLN Gene–Drug Interaction-Network Analysis

The Comparative Toxicogenomics Database (CTD; http://ctdbase.org) is used to retrieve information on chemotherapeutic drugs that can change the expression of genes of interest.[28] *ANLN* was queued in CTD and a gene–drug interaction network visualized with Cytoscape to recognize ANLN-interacting drugs that may help in the treatment of distinct human cancers by regulating ANLN expression.

Results

Expression-Level Analysis of ANLN in Distinct Types of Human Cancer

To find differences in *ANLN* expression in tumors and normal tissue, TCGA expression profiles across 24 types of tumor samples and paired normal tissue were utilized through the UALCAN platform. Results demonstrated that *ANLN* was down-regulated in glioblastoma, skin cutaneous melanoma, and thymoma, while it was overexpressed in all the remaining 21 types of cancer samples compared to normal controls, including CESC, ESCA, HNSC, and KIRC (Figure 1).

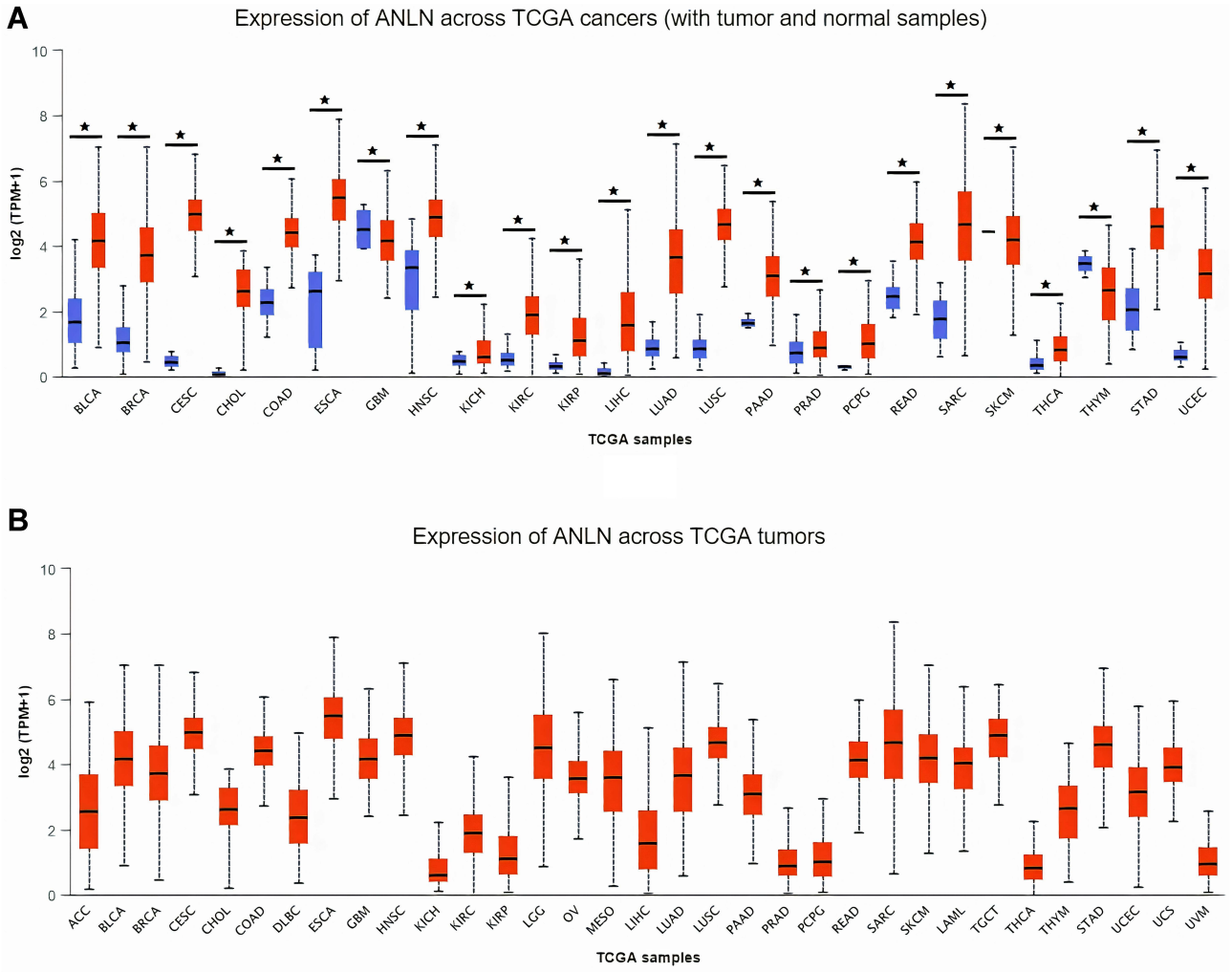


Figure 1 *ANLN* expression across cancers and normal tissue visualized by UALCAN. (**A**) *ANLN* expression in cancer subtypes paired with normal controls; (**B**) ANLN expression in cancer subtypes only. *$P<0.05$.

ANLN Prognostic Potential in Different Types of Cancer

KM Plotter was used to investigate the prognostic potential of *ANLN* in different types of cancer. Overexpression of *ANLN* was significantly associated with decreased OS and RFS duration of CESC (HR 1.77, 95% CI 1.1–2.83, *P*=0.016; HR 2.14, 95% CI 0.98–4.68, *P*=0.05), ESCA (HR 2.64, 95% CI 1.36–5.15, *P*=0.003, HR 271493743.64, 95% $CI_{0–Inf}$; *P*=0.028), HNSC (HR 1.36, 95% CI 1.2–1.81, *P*=0.034; HR 2.08, 95% CI 0.88–4.09, *P*=0.0087), and KIRC (HR 2.25, 95% CI 1.68–3.04, $P=8.2^{-8}$, HR 1.83, 95% CI 0.65–5.16, *P*=0.024) patients (Figure 2A and B). *ANLN* overexpression was also positively correlated with metastasis of CESC, ESCA, HNSC, and KIRC (Figure 2C). Taken together, these data suggest that ANLN might contribute significantly to the development and progression of CESC, ESCA, HNSC, and KIRC, and thus the next part of our study mainly focused on the unique role of *ANLN* in these four cancer types.

Transcription and Translational Expression–Level Validation of ANLN Using New Independent Cohorts

To further validate *ANLN* expression at transcription and translational levels, we reanalyzed its expression using new independent cohorts of CESC, ESCA, HNSC, and KIRC via TIMER, GENT2, GEPIA, and the HPA. Reanalysis also revealed significant overexpression of ANLN at both transcription and translational level in the new cohorts of patients relative to normal controls (Figure 3).

ANLN Overexpressed in CESC, ESCA, HNSC, and KIRC Patients with Different Clinicopathological Features

The distinct cancer types (CESC, ESCA, HNSC, and KIRC) in which *ANLN* showed significant negative correlations with OS and RFS duration were reanalyzed to verify the significance of *ANLN* expression in normal and cancerous samples with clinicopathological parameters: cancer stage, patient age, patient ethnicity, and nodal metastasis. *ANLN* was significantly overexpressed in all CESC, ESCA, HNSC, and KIRC patients with different clinicopathological parameters compared to the normal controls (Figure 4). Taken together, this implies that *ANLN* is overexpressed in CESC, ESCA, HNSC, and KIRC patients, regardless of the heterogeneity barrier.

Promoter-Methylation Analysis of ANLN

It is known that inactivation of tumor-suppressor genes due to hypermethylation of the promoter region leads to cancer.[29] To assess *ANLN* promoter methylation in defined cancer types, we utilized the UALCAN database. The results suggested

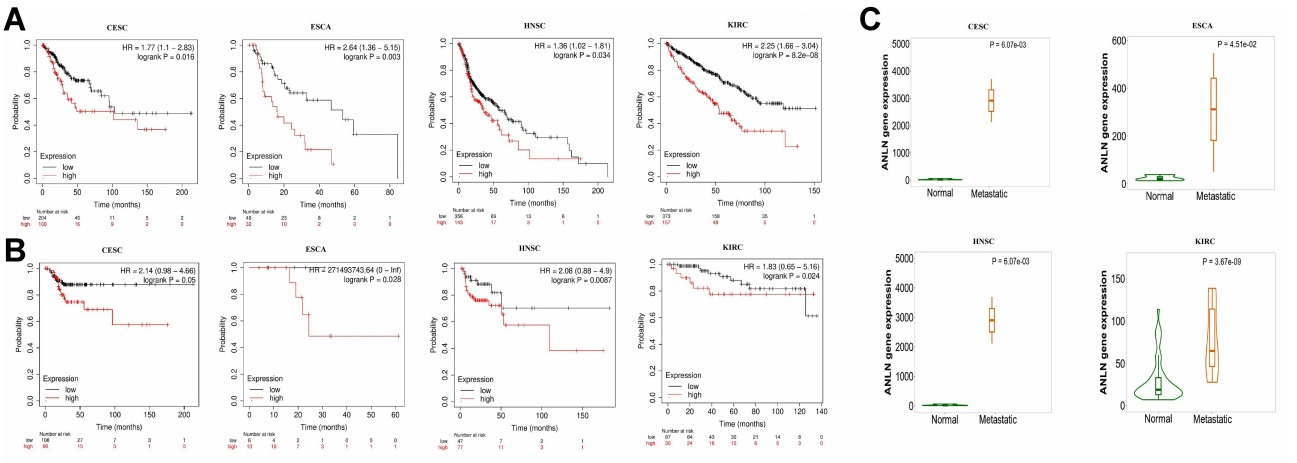


Figure 2 Prognostic values and correlation analysis of ANLN with tumor metastasis in cancers. (**A**) OS in CESC, ESCA, HNSC, and KIRC; (**B**) RFS in CESC, ESCA, HNSC, and KIRC; (**C**) correlation analysis of ANLN with metastasis in CESC, ESCA, HNSC, and KIRC tissue.

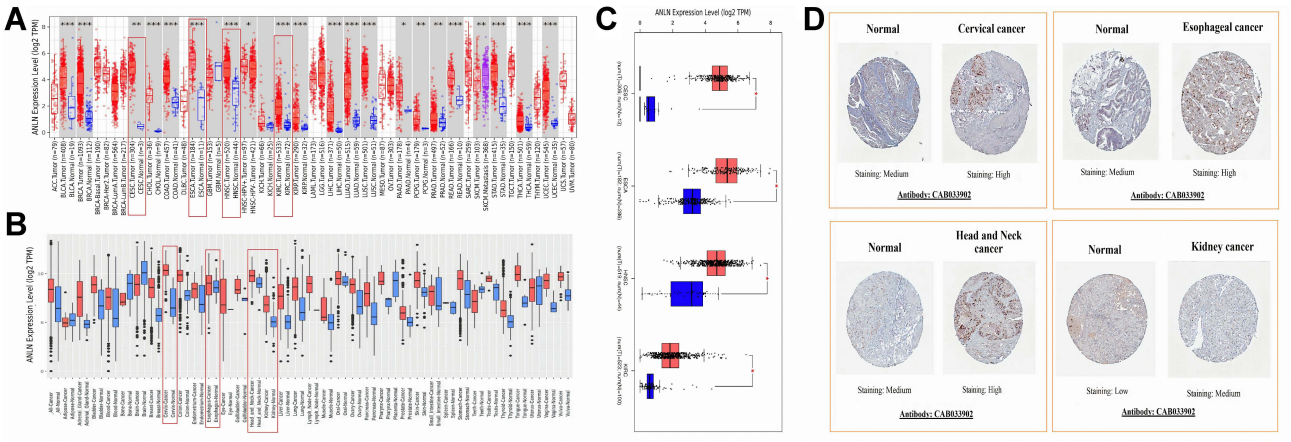


Figure 3 Transcription and translational expression–level validation of *ANLN* in new independent cohorts of CESC, ESCA, HNSC, and KIRC via TIMER, GENT2, GEPIA, and HPA databases. (**A**) Transcription level–expression validation of *ANLN* via TIMER; (**B**) transcription level–expression validation of *ANLN* via GENT2; (**C**) transcription level–expression validation of *ANLN* via GEPIA; (**D**) translational level expression validation of *ANLN* via HPA. **P*<0.05, ***P*<0.001, ****P*<0.0001.

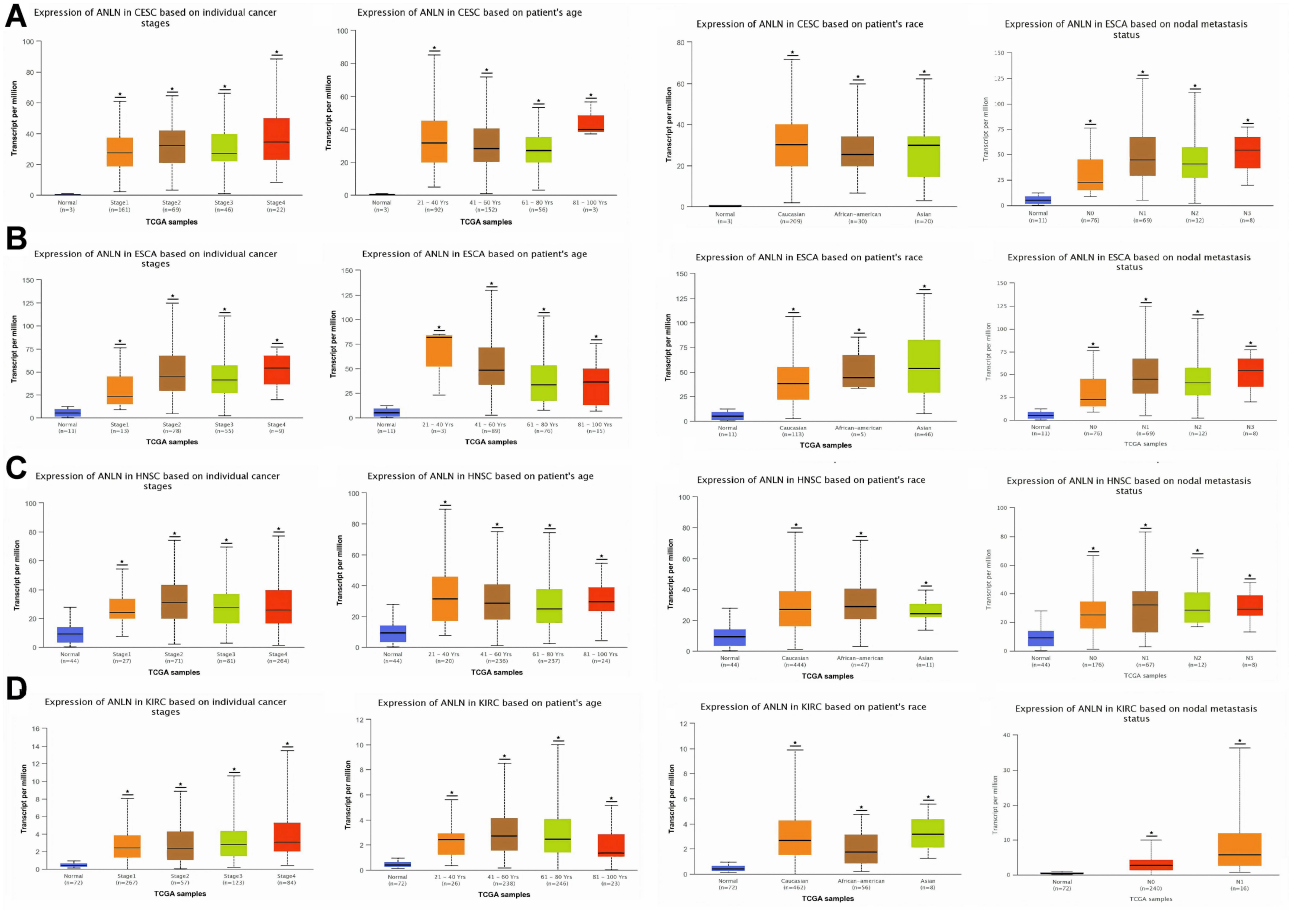


Figure 4 Clinicopathological feature–specific expression profiling of *ANLN* in CESC, ESCA, HNSC, and KIRC via UALCAN. (**A**) Individual cancer stage–specific expression profiling of *ANLN*; (**B**) patient age–specific expression profiling of *ANLN*; (**C**) patient ethnicity–specific expression profiling of *ANLN*; and (**D**) nodal metastasis–specific expression profiling of *ANLN*. **P*<0.05.

that the promoter level of *ANLN* was significantly lower in CESC, ESCA HNSC, and KIRC samples (Figure 5). Therefore, the overall data suggested a negative correlation between *ANLN* expression and promoter methylation in CESC, ESCA, HNSC, and KIRC samples compared to the normal controls.

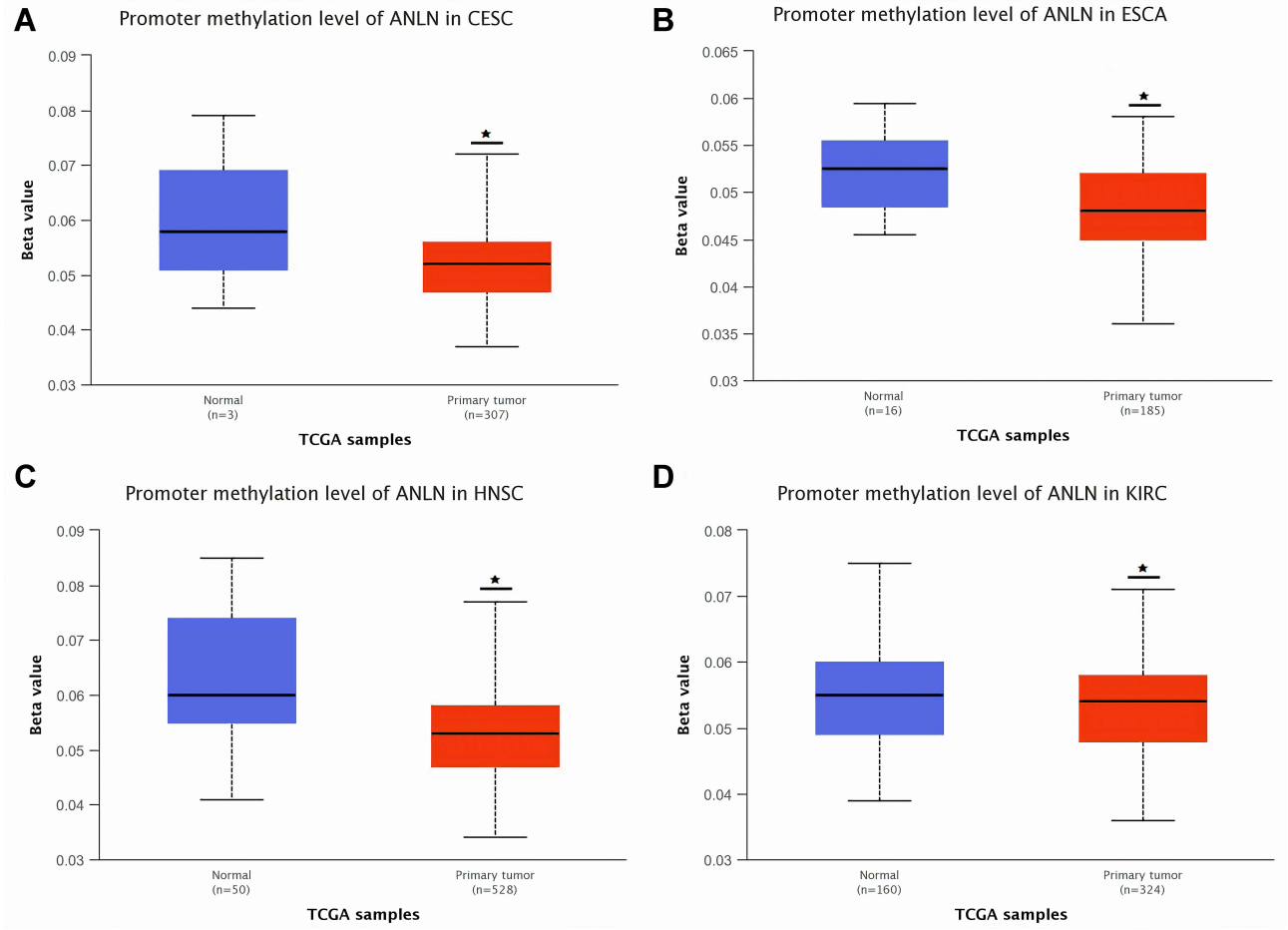


Figure 5 Promoter-methylation analysis of *ANLN* in CESC, ESCA, HNSC, and KIRC via UALCAN. (**A**) CESC; (**B**) ESCA; (**C**) HNSC; (**D**) KIRC. **P*<0.05.

Promoter Methylation–Level Validation of ANLN Using New Independent Cohorts

To validate *ANLN* promoter methylation in the new independent cohorts of CESC, ESCA, HNSC, and KIRC, we used MExpress. It was observed that promoter methylation values obtained from the different methylation probes in CESC, ESCA, HNSC, and KIRC were also significantly negatively correlated with ANLN expression levels (Figure 6).

Copy-Number Variations and Mutational Analysis of ANLN

Information related to *ANLN* genetic alterations — deep amplification, deep deletion, genetic mutation, and fusion — in CESC, ESCA, HNSC, and KIRC was obtained from TCGA CESC, ESCA, HNSC, and KIRC (TCGA, PanCancer Atlas) data sets consisting of 297, 182, 523, and 512 cancerous samples, respectively. Results revealed that ANLN harbored genetic alterations in only 0.8% cases of CESC, 5% cases of ESCA, 2.8% cases of HNSC, and 0.6% cases of KIRC, with maximum missense mutations in CESC, HNSC, and KIRC and maximum deep amplification in ESCA (Figure 7).

PPI Network of ANLN, GO, and KEGG Analysis

We constructed a PPI of *ANLN*-associated genes via STRING and visualized it through. In total, ten ANLN interacting genes were identified through this network. We then performed GO and KEGG enrichment analysis of *ANLN*-associated genes. *ANLN*-associated genes were significantly involved in mitotic cytokinesis, positive regulation of cytokinesis, mitotic nuclear division, microtubule-based movement, and regulation of small GTPase–mediated signal-ransduction biological process (BP) terms, protein-kinase binding, microtubule binding, ATP binding, and microtubule motor-activity

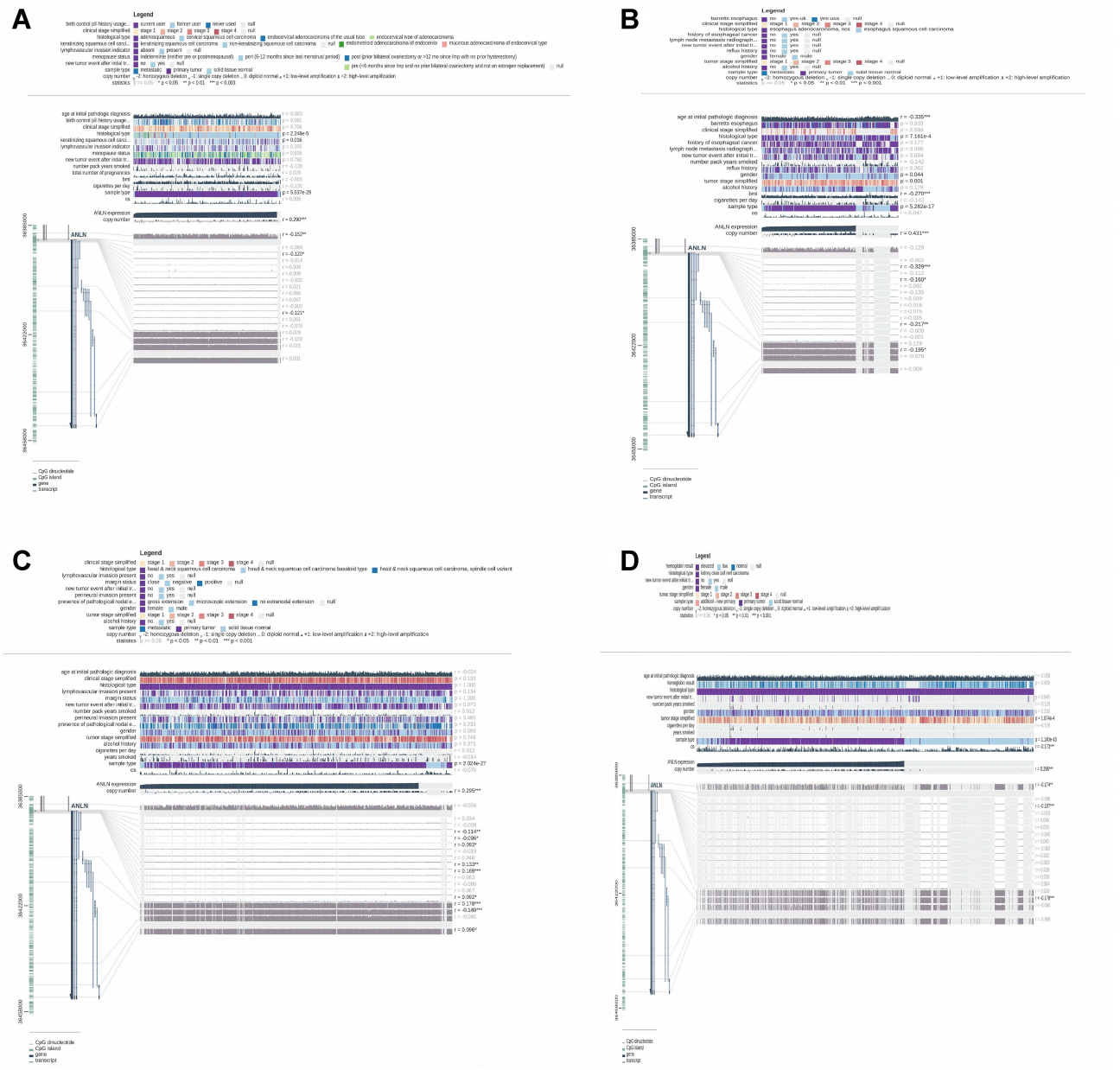


Figure 6 Promoter methylation–level validation of *ANLN* in CESC, ESCA, HNSC, and KIRC via MEXPRESS. (**A**) CESC; (**B**) ESCA; (**C**) HNSC; (**D**) KIRC. **P*<0.05, ***P*<0.001, ****P*<0.0001.

molecular function (MF) terms, and KEGG terms: progesterone-mediated oocyte maturation, oocyte meiosis, and the cell cycle (Figure 8 and Table 1).

Correlation Analysis Between ANLN and Associated Gene Expression

We used GEPIA to analyze correlations between *ANLN* and associated gene (explored via STRING) expression in CESC, ESCA, HNSC, and KIRC. ANLN expression was strongly positively correlated with expression levels of PHOA, RACGAP1, KIF23, KIF20A, KIF11, ECT2, DLGAP5, CDK1, CDC5L, and BUB1 (Figure 9). Based on these results, we speculated that along with ANLN, dysregulation of its other associated genes may also play a tumor-promoting role in CESC, ESCA, HNSC, and KIRC.

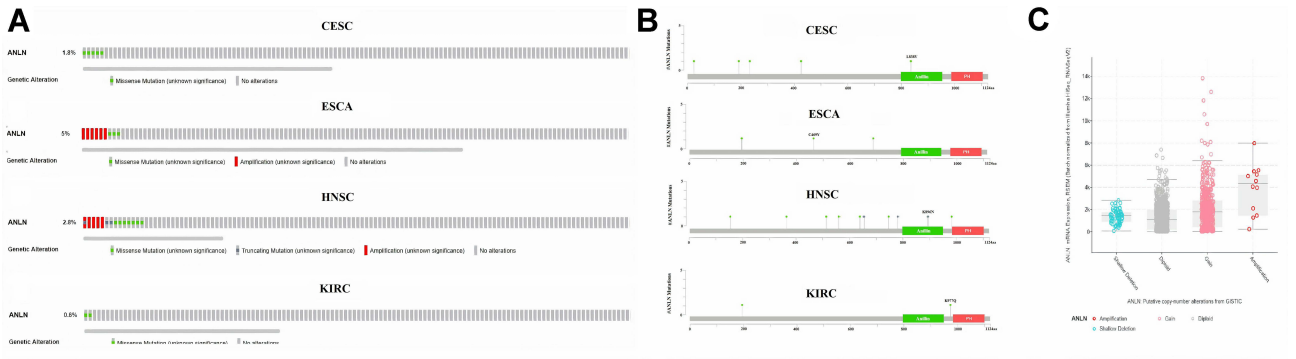


Figure 7 Copy-number variations (CNVs) and genetic alteration analysis of *ANLN* in CESC, ESCA, HNSC, and KIRC data sets. (**A**) Overview of CNVs and genetic alterations of *ANLN* in CESC, ESCA, HNSC, and KIRC samples; (**B**) types of genetic mutation observed in CESC, ESCA, HNSC, and KIRC samples, altering the amino acids of the encoded proteins; (**C**) types of CNV observed in CESC, ESCA, HNSC, and KIRC samples.

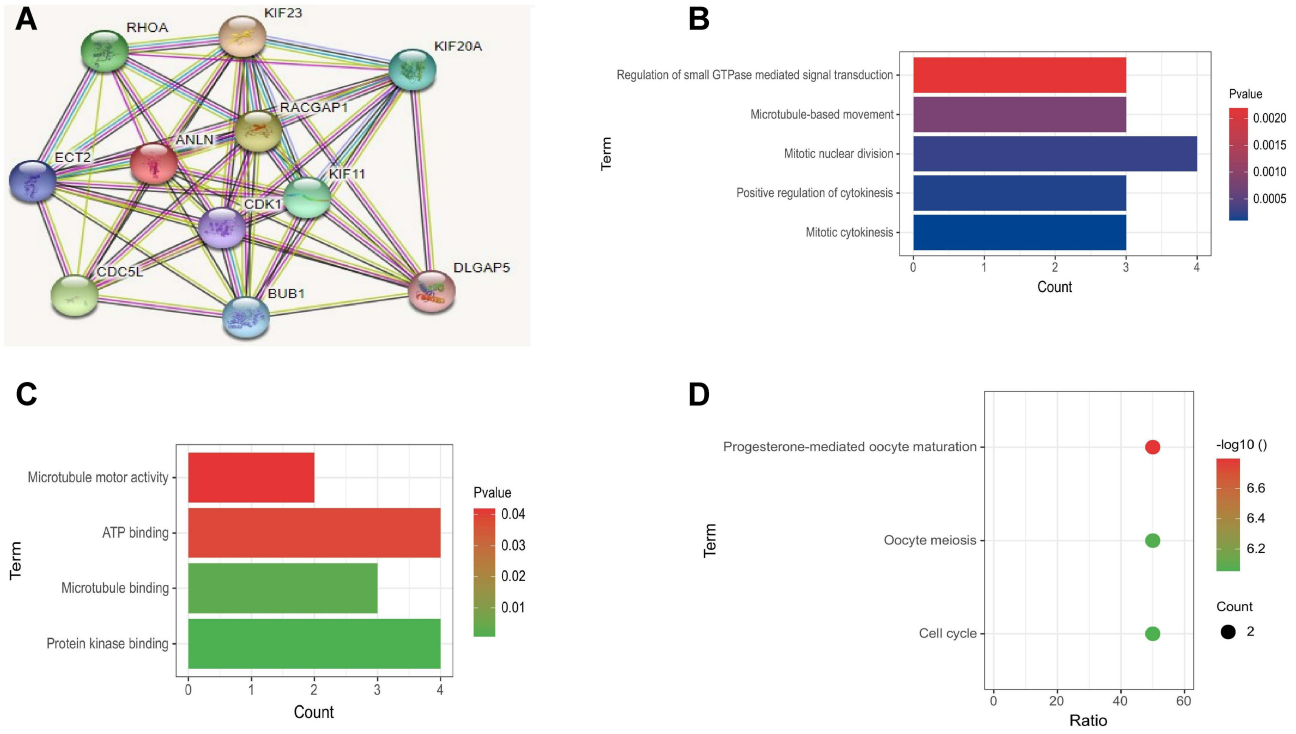


Figure 8 PPI network, GO, and KEGG enrichment analysis of *ANLN*-enriched genes via STRING and DAVID. (**A**) PPI network of *ANLN*-enriched genes; (**B**) BP GO terms of ANLN-enriched genes; (**C**) MF GO terms of *ANLN*-enriched genes; (**D**) KEGG terms of *ANLN*-enriched genes.

ANLN and Infiltrating Levels of $CD8^+$ and $CD4^+$ T Cells

Functions of and interactions between the innate and adaptive immune systems are vital for anticancer immunity. Cytotoxic T cells expressing cell-surface $CD8^+$ and $CD4^+$ T cells are the most powerful effectors in anticancer immune response and form the backbone of current successful cancer immunotherapies.[30] In the current study, Spearman correlations between expression of *ANLN* and $CD8^+$ and $CD4^+$ T cells was calculated using TIMER. Results revealed a significant egative correlation between the mRNA expression of *ANLN* and $CD8^+$ T–cell levels in CESC, ESCA, and HNSC and significant positive correlation in KIRC (Figure 10). On the other hand, a significant positive correlation between mRNA expression of *ANLN* and $CD4^+$ T–cell levels was also found in CESC, ESCA, HNSC, and KIRC (Figure 10).

Table 1 GO and KEGG enrichment analysis of ANLN-enriched genes

	Name	Genes	*P*	Gene symbols
GO BP terms				
GO:0000281	Mitotic cytokinesis	3	1.0290098284689253^{-4}	*ANLN*, *RACGAP1*, *KIF20A*
GO:0032467	Positive regulation of cytokinesis	3	1.6842308606935264^{-4}	*RACGAP1*, *ECT2*, RHOA
GO:0007067	Mitotic nuclear division	4	2.5032649977440837^{-4}	*ANLN*, *CDK1*, *KIF11*, *BUB1*
GO:0007018	Microtubule-based movement	3	8.093894290048242^{-4}	*RACGAP1*, *KIF20A*, *KIF11*
GO:0051056	Regulation of small GTPase–mediated signal transduction	3	0.0021934816623356197	*RACGAP1*, *ECT2*, RHOA
GO MF terms				
GO:0019901	Protein-kinase binding	4	8.333346238382384^{-4}	*RACGAP1*, *CDC5L*, *KIF20A*, *KIF11*
GO:0008017	Microtubule binding	3	0.003138105842022134	*RACGAP1*, *KIF20A*, *KIF11*
GO:0005524	ATP binding	4	0.03878566879527679	*CDK1*, *KIF20A*, *KIF11*, *BUB1*
GO:0003777	Microtubule motor activity	2	0.04186160016871645	*KIF20A*, *KIF11*
KEGG terms				
hsa04914	Progesterone-mediated oocyte maturation	2	1.6012127104389768^{-7}	*CDK1*, *BUB1*
hsa04114	Oocyte meiosis	2	8.572436716581579^{-7}	*CDK1*, *BUB1*
hsa04110	Cell cycle	2	8.841944543185589^{-7}	*CDK1*, *BUB1*

Abbreviations: GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; BP, biological process; MF, molecular function.

Identification of miRNAs and TFs That Potentially Regulate ANLN

Enrichr was utilized to predict the *ANLN* targeted TFs, and miRNAs. In total, the ten most significant TFs (E2F1, E2F3, TP53, E2F4, YBX1, TRP53 [mouse], RBL2 [mouse], FOXM1, MYC, and ARID3A) and ten miRNAs (hsa-miR215-5p, hsa-miR192-5p, hsa-miR193b-3p, hsa-miR34a-5p, hsa-miR92a-3p, hsa-miR16-5p, hsa-miR18b-5p, hsa-miR6507-5p, hsa-miR221-3p, and hsa-miR24-1-5p) were identified that potentially regulate *ANLN* expression (Figure 11). Taken together, these clues highlight that *ANLN* expression can be regulated by a variety of factors.

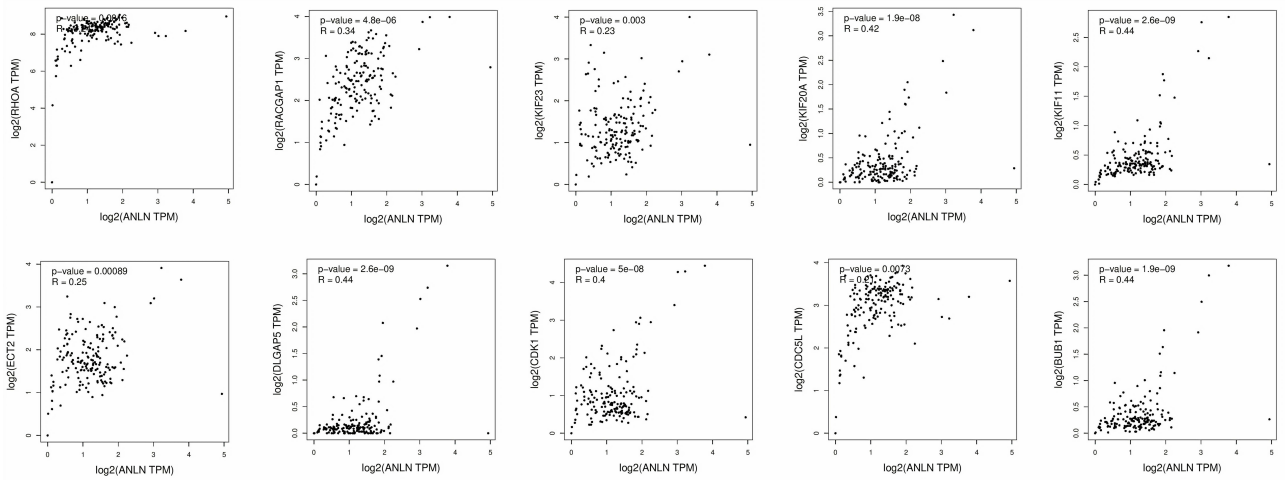


Figure 9 Correlation analysis of *ANLN* and associated gene expression in CESC, ESCA, HNSC, and KIRC samples.

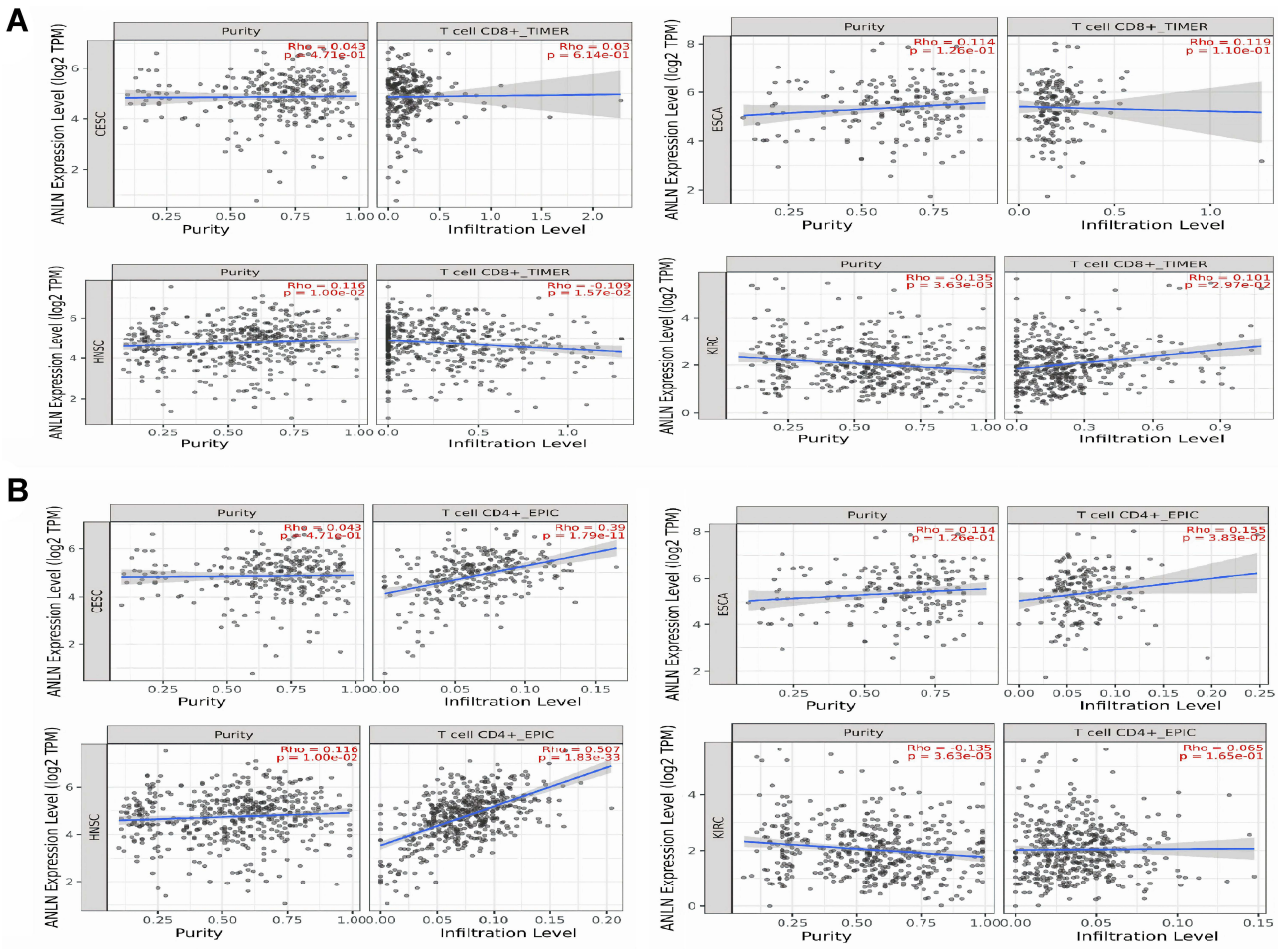
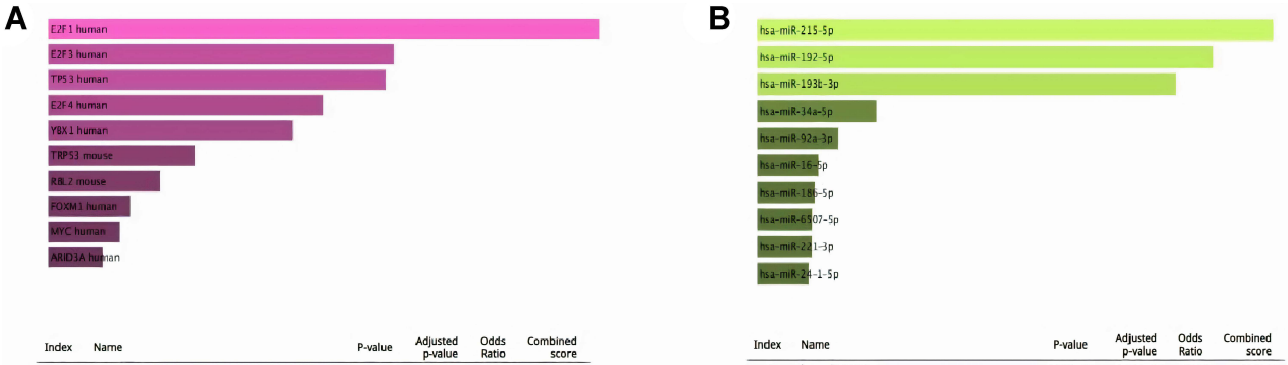


Figure 10 TIMER-based Spearman correlation analyses of *ANLN* expression and CD4$^+$ T-, and CD8$^+$ T- cell levels in CESC, ESCA, HNSC, and KIRC. (**A**) ANLN expression and CD4$^+$ T cells; (**B**) *ANLN* expression and CD8$^+$ T cells.



Index	Name	P-value	Adjusted p-value	Odds Ratio	Combined score
1	E2F3 human	2.238e-11	1.018e-9	181.40	4448.31
2	E2F4 human	1.265e-9	2.877e-8	74.65	1529.56
3	E2F1 human	1.725e-16	1.569e-14	29.33	1064.72
4	YBX1 human	7.232e-9	1.316e-7	52.86	990.88
5	RBL2 mouse	0.00001419	0.0001844	87.89	981.15
6	ARID3A human	0.0003664	0.003335	101.51	803.12
7	CUX1 human	0.0006796	0.005622	67.67	493.57
8	TP53 human	3.469e-11	1.052e-9	17.72	426.70
9	FOXM1 human	0.00007838	0.0008916	43.93	415.32
10	TFDP1 human	0.001085	0.008228	50.74	346.39

Index	Name	P-value	Adjusted p-value	Odds Ratio	Combined score
1	hsa-miR-215-5p	1.237e-29	1.614e-26	17.13	1140.22
2	hsa-miR-192-5p	2.522e-26	1.646e-23	13.25	781.24
3	hsa-miR-193b-3p	3.263e-24	1.419e-21	13.16	711.43
4	mmu-miR-6951-3p	0.02963	0.3362	40.19	141.43
5	mmu-miR-7116-3p	0.02963	0.3362	40.19	141.43
6	hsa-miR-24-1-5p	0.0007682	0.1002	18.62	133.53
7	hsa-miR-4523	0.004434	0.1231	22.54	122.14
8	mmu-miR-193a-3p	0.03931	0.3780	28.71	92.90
9	hsa-miR-34a-5p	1.299e-7	0.00004237	5.47	86.77
10	hsa-miR-4520-5p	0.04412	0.3838	25.12	78.38

Figure 11 ANLN-targeted TFS and miRNAs in human cancers. (**A**) ANLN-targeted TFS; (**B**) ANLN-targeted miRNAs.

Correlations Between ANLN and Other Mutant Genes

To identify mutant genes responsible for *ANLN* overexpression, we used MuTarget and selected the top three mutant genes for CESC, ESCA, HNSC, and KIRC patient. As shown in Figure 12, the top three mutant genes positively correlated with the expression of *ANLN* were *LMO1*, *WHSC1L1*, and *SPRY1* in CESC, *FMNL2*, *DNM2*, and *CACNA11* in ESCA, *GYPA*, *SLCO1B1*, and *CRACR2A* in HNSC, and *XIRP2*, *ZGRF1*, and *FAM178A* in KIRC. Collectively, these results suggest that *ANLN* expression has strong correlations with different mutant genes in CESC, ESCA, HNSC, and KIRC.

Gene–Drug Interaction-Network Analysis of ANLN

In order to explore the relationship between *ANLN* and available cancer therapeutic drugs, a gene–drug interaction network was developed using CTD. *ANLN* expression can be influenced by a variety of drugs. For example, estradiol and ionomycin can elevate the expression level of *ANLN*, while sunitinib and cyclosporine can reduce *ANLN* expression (Figure 13).

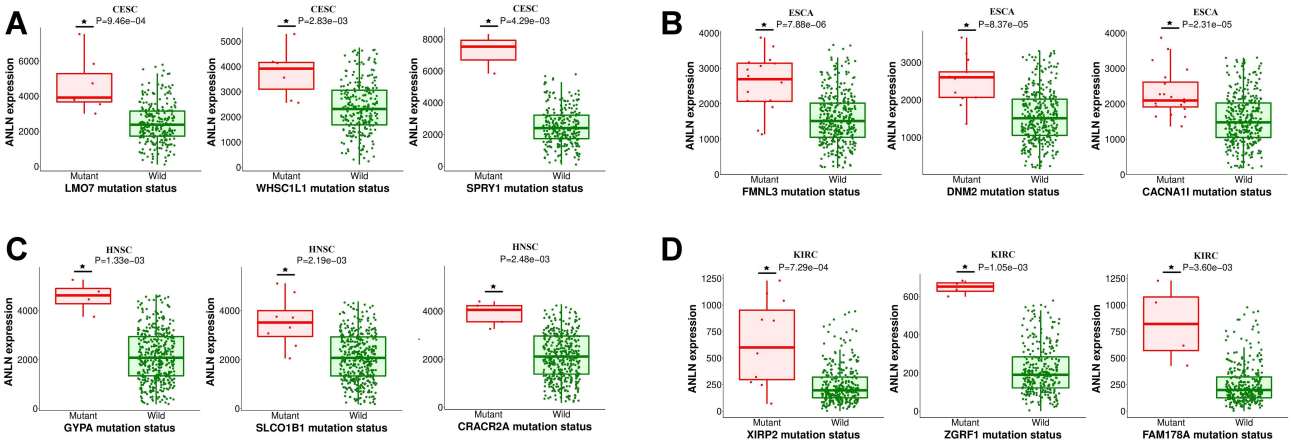


Figure 12 Mutant genes positively correlated with ANLN in CESC, ESCA, HNSC, and KIRC from MuTarget. (**A**) Top three genes correlated with *ANLN* in CESC; (**B**) top three genes correlated with *ANLN* in ESCA; (**C**) top three correlated genes with ANLN in HNSC; (**D**) top three genes correlated with *ANLN* in KIRC. *$P<0.05$.

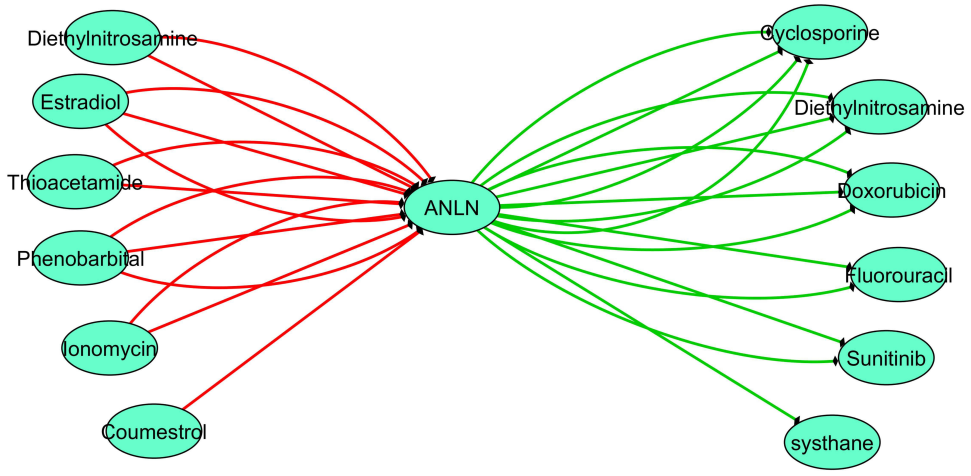


Figure 13 Gene–drug interaction network of *ANLN* and chemotherapeutic drugs. Red arrows, chemotherapeutic drugs that increase the expression of *ANLN*; green arrows: chemotherapeutic drugs that decrease the expression of *ANLN*; arrow counts, number of supported studies in the literature.

Discussion

Although *ANLN* has been explored in few cancers,[7,11,12] its role in other subtypes of human cancer has yet to be uncovered. In the current study, we identified significant associations between ANLN expression and CESC, ESCA, HNSC, and KIRC. We found that *ANLN* was downregulated in three types of cancer tissue and overexpressed in another 21 types relative to controls and its overexpression was significantly correlated with the decreased OS and RFS duration, and advanced metastasis of CESC, ESCA, HNSC, and KIRC. Altogether, these results support that ANLN may play a key role in the initiation and development of CESC, ESCA, HNSC, and KIRC. Therefore, in the present study, our main focus was these four cancers. Due to heterogeneity, CESC, ESCA, HNSC, and KIRC may remain a serious therapeutic issue for clinicians and doctors. Therefore, we further evaluated the clinicopathological feature–specific expression of ANLN in CESC, ESCA, HNSC, and KIRC patients. *ANLN* was also significantly overexpressed in CESC, ESCA, HNSC, and KIRC patients with clinicopathological features of different cancer stage, age, ethnicity, and nodal metastasis. Therefore, since *ANLN* expression was higher in CESC, ESCA, HNSC, and KIRC samples relative to controls, we believe that it is more important to reduce the expression of *ANLN* using different chemotherapeutics drugs to treat CESC, ESCA, HNSC, and KIRC patients.

Next, we tried to explore the possible causes of *ANLN* overexpression, so we carried out correlation analyses between *ANLN* overexpression and promoter-methylation level, CNV, and genetic mutations in CESC, ESCA, HNSC, and KIRC. ANLN harbored genetic alterations in very small proportions of the cancer patients (0.8% of CESC, 5% of ESCA, 2.8% of HNSC, and 0.6% of KIRC), with maximum missense mutations in CESC, HNSC, and KIRC and maximum deep amplification in ESCA. Therefore, we speculate that these genetic changes might participate in *ANLN* overexpression at a low level. Moreover, *ANLN* promoter-methylation analysis revealed that promoter-methylation level of *ANLN* was significantly (lower in CESC, ESCA, HNSC, and KIRC samples. Therefore, we speculate that *ANLN* overexpression can be the result of significant hypomethylation in CESC, ESCA, HNSC, and KIRC. To the best of our knowledge, this study is the first to correlate *ANLN* expression with genetic alterations and promoter-methylation levels in CESC, ESCA, HNSC, and KIRC.

Gynecological cancer, including CESC, is a leading cause of cancer-related deaths in women. Various studies have been conducted worldwide to identify CESC-specific diagnostic and prognostic biomarkers, eg, Berger et al[31] identified various mutated genes and CNV abnormalities in CESC by analyzing GEO datasets. Song et al[32] developed a long noncoding RNA–miRNA–mRNA network in CESC using GEO data sets and provided novel insights into CESC biology. However, none of the biomarkers has been generalized so far in CESC patients with various clinicopathological features for diagnosis and predicting the prognosis of CESC. We showed significant upregulation of *ANLN* expression in CESC patients with various clinicopathological features, ie, different cancer stage, age, ethnicity, and nodal metastasis compared to the normal controls.

Recently, various ESCA-related molecular biomarkers have been investigated, including *ASPM*, *AURKA*, *BUB1*, *CDC20*, *CENPF*, *DLGAP5*, *NEK2*, *TOP2A*, *TPX2*, and *UBE2C*.[33] However, none of these biomarker has been generalized in ESCA patients with various clinicopathological features for diagnosis and predicting the prognosis of ESCA. In the present study, we revealed significant upregulation of *ANLN* expression in ESCA patients with the aforementioned clinicopathological features.

At present, different molecular biomarkers, including *LAMC1*, *LAMC2*, *LAMC3*, and *HOX*, used for diagnosis and predicting the prognosis of HNSC, have certain limitations.[34] None of the biomarkers has been generalized in HNSC patients with various clinicopathological features for diagnosis and predicting the prognosis of HNSC. However, In the present study, we showed a significant upregulation of *ANLN* expression in HNSC patients with the aforementioned clinicopathological features.

For differentiating KIRC patients from normal individuals, expression profiling of *TIMP3* and *HMGCS1* genes and hsa-miR21-5p and hsa-miR365a-3p miRNAs were suggested to be reliable by Huang et al.[35] However, none of these or any other biomarker has been generalized so far in KIRC patients of various clinicopathological features for diagnosis and predicting the prognosis. We found significant upregulation of *ANLN* expression in KIRC patients with the aforementionedclinicopathological features compared to the normal controls. In sum, overexpression, promoter-

methylation levels, OS, and RFS suggest *ANLN* to be a novel diagnostic and prognostic potential biomarker of CESC, ESCA, HNSC, and KIRC patients with different clinical variables.

TFs and miRNAs are important regulators of gene expression, and abnormalities in their expression are known to cause cancer.[36,37] To discover possible roles of TFs and miRNAs in the dysregulation of *ANLN*, we predicted potential TFs and miRNAs of *ANLN* using Enrichr. Our results revealed the ten most significant TFs to be E2F1, E2F3, TP53, E2F4, YBX1, TRP53 [mouse], RBL2 [mouse], FOXM1, MYC, and ARID3A and the ten most significant miRNAs to hsa-miR215-5p, hsa-miR192-5p, hsa-miR193b-3p, hsa-miR34a-5p, hsa-miR92a-3p, hsa-miR16-5p, hsa-miR18b-5p, hsa-miR6507-5p, hsa-miR221-3p, and hsa-miR24-1-5p as potential regulator of *ANLN* expression. This important information might also help in understanding *ANLN's* oncogenic role in more detail.

Immunotherapy is one of the most reliable treatment options for cancer patients.[38] However, only a small proportion of cancer patients actually benefit from immunotherapy. $CD8^+$ and $CD4^+$ T cells are important components of the immunotherapy; therefore, based on these immune cells, the discovery of novel biomarkers is a clinical priority. In our study, we analyzed whether *ANLN* correlated with $CD8^+$ and $CD4^+$ T cells or not. We found interesting positive and negative correlations between *ANLN* expression and $CD8^+$ and $CD4^+$ T cells, which may open up new avenues for the treatment of CESC, ESCA, HNSC, and KIRC patients, especially those who are not getting benefits from the existing immunocheckpoint inhibitors/regulators. However, an *ANLN* and immune cell infiltration–based study is needed on a large scale for further confirmation of these results.

Despite the use of single-gene indicators, multigene diagnostic and prognostic approaches have recently gained traction.[39] CESC, ESCA, HNSC, and KIRC patients with different types of mutation still lack any sensitive, accurate therapy. Therefore, using MuTarget, we identified mutant genes responsible for altering *ANLN* expression. The top three mutant genes in each of CESC, ESCA, HNSC, and KIRC were *LMO1*, *WHSC1L1*, and *SPRY1* in CESC, *FMNL2*, *DNM2*, and *CACNA11* in ESCA, *GYPA*, *SLCO1B1*, and *CRACR2A* in HNSC, and *XIRP2*, *ZGRF1*, and *FAM178A* in KIRC. By linking these mutant genes with *ANLN* expression, it is easier to identify potential multigene-based therapies in CESC, ESCA, HNSC, and KIRC.

A PPI network of *ANLN*-associated genes via STRING revealed a set of ten *ANLN*-associated genes. GO and KEGG analysis of these highlighted their involvement in mitotic cytokinesis, positive regulation of cytokinesis, mitotic nuclear division, microtubule-based movement, and regulation of small GTPase–mediated signal-transduction BP terms, while protein-kinase binding, microtubule binding, ATP binding, and microtubule motor–activity MF terms, and KEGG terms for progesterone-mediated oocyte maturation, oocyte meiosis, and the cell cycle. Moreover, a correlation analysis between *ANLN* and *ANLN*-associated gene expression revealed a strong positive correlation further validating our findings regarding GO and KEGG analysis and paved the way for further exploration of *ANLN* molecular function. We also identified drugs that could be useful in the treatment of CESC, ESCA, HNSC, and KIRC by targeting *ANLN* for expression regulation.

Conclusion

ANLN is an actin-binding protein that participates in cytokinesis. Here, we systematically utilized several gene-expression databases and bioinformatic tools to verify the diagnostic and prognostic roles of *ANLN* in CESC, ESCA, HNSC, and KIRC patients with different clinical variables for the first time. Our findings suggested that *ANLN* is a diagnostic and prognostic biomarker of CESC, ESCA, HNSC, and KIRC patients. However, further studies are required to confirm these findings.

Disclosure

The authors report no conflicts of interest in this work.

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