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Research Article

ANLN as an Oncogenic Biomarker Associated with Immunosuppressive Microenvironment and Unfavorable Prognosis in Cervical Cancer

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Abstract:

Background: Cervical cancer remains a significant global health burden with a poor prognosis for patients with advanced disease. Identifying novel prognostic biomarkers is crucial for improving patient outcomes. Anillin (ANLN), an actin-binding protein involved in cytokinesis, has been implicated as an oncogene in various solid tumors, but its role in cervical cancer is not well defined.

Methods: This study integrated bioinformatic analysis of public datasets (TCGA, GEO) with experimental validation using clinical samples and qRT-PCR/Western blot. The prognostic value of ANLN was assessed via Kaplan-Meier and Cox regression analyses. A predictive nomogram was constructed. Functional mechanisms were explored through gene enrichment analysis and a pan-cancer analysis of ANLN's expression and prognostic significance.

Results: ANLN was significantly overexpressed at both the mRNA and protein levels in cervical cancer tissues. High ANLN expression was an independent predictor of poor overall survival (HR(High) = 1.85, 95%CI: 1.15 - 2.90, p = 0.01) and was incorporated into an accurate prognostic nomogram (C-index: 0.70, p=1.85e-07). Bioinformatic analysis revealed that ANLN co-expressed genes were predominantly enriched in cell cycle-related pathways. Pan-cancer analysis confirmed ANLN's widespread overexpression and association with poor prognosis in multiple cancers.

Conclusion: ANLN is a potential oncogene and a powerful independent prognostic biomarker in cervical cancer, possibly through promoting cell cycle progression. It represents a promising target for prognostic stratification and the development of novel therapeutic strategies.

Keywords: Anillin, Cervical cancer, Prognosis, Pan-cancer analysis

1. Introduction

Cervical cancer remains the fourth most common malignancy among women worldwide, posing a significant threat to female health [1]. Although the widespread adoption of human papillomavirus (HPV) prophylactic vaccines and early screening techniques has led to a decline in its incidence and mortality in some regions, the prognosis for cervical cancer patients, particularly those with advanced, recurrent, or metastatic disease, remains poor [2-4]. The International Federation of Gynecology and Obstetrics (FIGO) staging system is still the primary clinical tool for prognostic assessment [5-6]; however, it fails to fully capture the intrinsic molecular heterogeneity of tumors, resulting in significant variations in treatment response and survival outcomes among patients within the same stage [7-8]. Therefore, identifying novel molecular biomarkers capable of

accurately predicting prognosis and elucidating their underlying mechanisms in driving tumor progression is crucial for developing individualized treatment strategies and improving patient survival [9-10].

In recent years, the rapid development of high-throughput sequencing and bioinformatics technologies has ushered cancer research into the omics era, enabling the systematic screening and identification of key oncogenic genes [11-12]. Anillin (ANLN), an actin-binding protein involved in regulating cytokinesis during the final stages of cell division, plays a vital role in maintaining cell polarity and morphology [13]. Notably, a growing body of evidence indicates that ANLN is frequently overexpressed in various solid tumors (such as, breast cancer, lung cancer, and hepatocellular carcinoma) and contributes to tumorigenesis and progression by promoting cell proliferation, invasion, and metastasis [14-15]. Its overexpression is strongly associated with poor patient survival, suggesting its potential role as an oncogene [16]. However, the expression pattern, clinical prognostic value, and underlying biological functions of ANLN in cervical cancer remain largely unexplored, and its role in modulating the tumor immune microenvironment and chemotherapy resistance is entirely unknown [17].

To address this gap, this study aims to comprehensively investigate the oncogenic role of ANLN in cervical cancer by integrating bioinformatic analyses with experimental validation. We first examined the expression of ANLN at both the mRNA and protein levels in cervical cancer tissues using public databases (TCGA, GEO) and clinical samples from our institution. We subsequently evaluated the association between its expression levels and clinicopathological features and patient prognosis to determine its potential as an independent prognostic factor. A nomogram was constructed to translate the predictive value of ANLN into a quantifiable clinical tool.

Finally, through functional enrichment analysis, we explored the potential molecular mechanisms by which ANLN contributes to cervical cancer progression, particularly its possible involvement in cell cycle regulation. This study aims to provide a novel molecular biomarker for prognostic assessment in cervical cancer and to offer a theoretical foundation for developing new therapeutic strategies targeting ANLN.

2. Materials and Methods

2.1. Data Acquisition and Processing

Transcriptomic data and corresponding clinical information for the TCGA-CESC cohort were downloaded from the UCSC Xena portal (https://xenabrowser.net/). The gene expression dataset GSE67522 for cervical cancer was obtained from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). Gene expression levels were log2-transformed for further analysis. For the pan-cancer analysis, RNA-seq data and clinical data for 33 cancer types were retrieved from the TCGA database via the UCSC Xena platform.

2.2. Patient Tissue Samples

Four pairs of fresh cervical cancer tissues and matched adjacent non-tumor tissues were collected during surgical resection at Zhuzhou Central Hospital. The use of human tissues was approved by the Institutional Ethics Committee of Zhuzhou Central Hospital (No. 20231046), and written informed consent was obtained from all patients. All tissues were immediately frozen in liquid nitrogen and stored at -80°C until RNA and protein extraction.

2.3. RNA Extraction and Quantitative Real-Time PCR (qRT-PCR)

Total RNA was extracted from tissues using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. cDNA was synthesized using a PrimeScript RT reagent kit (Takara). qRT-PCR was performed on an Applied Biosystems QuantStudio system using SYBR Green Premix Pro Taq HS (Accurate Biology). GAPDH was used as an internal control for normalization. The relative expression of ANLN was calculated using the 2^(-ΔΔCt) method.

2.4. Western Blot Analysis

Tissues were lysed in RIPA buffer containing protease inhibitors. Protein concentrations were determined using a BCA assay kit. Equal amounts of protein were separated by SDS-PAGE and transferred onto PVDF membranes. After blocking, the membranes were incubated overnight at 4°C with primary antibodies against ANLN (abcam, ab99352) and GAPDH (CST,

5174T), followed by incubation with HRP-conjugated secondary antibodies. Protein bands were visualized using an enhanced chemiluminescence (ECL) detection system.

2.5. Survival and Statistical Analysis

Patients in the TCGA-CESC cohort were stratified into high and low ANLN expression groups based on the median expression value. Overall survival (OS) was analyzed using the Kaplan-Meier method, and differences between groups were compared with the log-rank test. Univariate and multivariate Cox proportional hazards regression models were employed to assess the independent prognostic value of ANLN. A p-value < 0.05 was considered statistically significant. All statistical analyses were performed using R software (v4.0.3, foundation for statistical computing, 2020).

2.6. Construction of the Nomogram

Independent prognostic factors identified by multivariate Cox analysis were integrated to construct a predictive nomogram using the rms package in R. The performance of the nomogram was evaluated by the concordance index (C-index) and calibration curves plotted for 1-, 3-, and 5-year overall survival.

2.7. Bioinformatic Analysis

Co-expressed genes with ANLN in the TCGA-CESC cohort were identified using Pearson correlation analysis. Genes with |Pearson R| > 0.4 and an adjusted p-value < 0.001 were considered significantly correlated. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses for the top correlated genes were performed using the clusterProfiler R package.

2.8. Pan-Cancer Analysis

ANLN expression levels across different TCGA cancer types and corresponding normal tissues were compared. The prognostic value of ANLN in various cancers was evaluated using Kaplan-Meier survival analysis and univariate Cox regression. The association between ANLN expression and pathological stage was analyzed using Kruskal-Wallis tests.

2.9. Statistical Analysis

All statistical analyses were performed using R software (v4.0.3, foundation for statistical computing, 2020). A p-value < 0.05 was considered statistically significant. Differences in ANLN expression between groups were assessed using the Mann-Whitney U test or Student's t-test/Wilcoxon test, as appropriate. Survival analysis was conducted with the Kaplan-Meier method and compared by the log-rank test. Univariate and multivariate Cox proportional hazards regression models were used to identify independent prognostic factors, with results reported as hazard ratios (HRs) and 95% confidence intervals (CIs). Gene expression correlations were evaluated using Pearson correlation. The predictive accuracy of the nomogram was quantified by the concordance index (C-index) and visually assessed with calibration curves.

3. Results

3.1. Overexpression of ANLN in Cervical Cancer

To investigate ANLN expression in cervical cancer (CC), transcriptomic data from public databases were analyzed. ANLN mRNA was found to be significantly upregulated in cervical cancer tissues compared to normal adjacent tissues in both the TCGA-CESC and GSE67522 datasets (all p < 0.0001, Figure 1A—B). Immunohistochemical staining results for ANLN in cervical tissues are publicly available in the Human Protein Atlas database (Figure 1C).

To validate these findings, four pairs of clinical cervical cancer and matched non-tumor tissues were examined. Western blot analysis confirmed abundant ANLN protein levels in cancerous tissues, with minimal expression in normal samples (p < 0.01, Figure 2D-E), and qRT-PCR results further showed significantly elevated ANLN mRNA expression in tumor samples (p < 0.01, Figure 1F). These results demonstrate that ANLN is highly overexpressed in cervical cancer at both the protein and transcriptional levels. These results demonstrate that ANLN is highly overexpressed in cervical cancer.

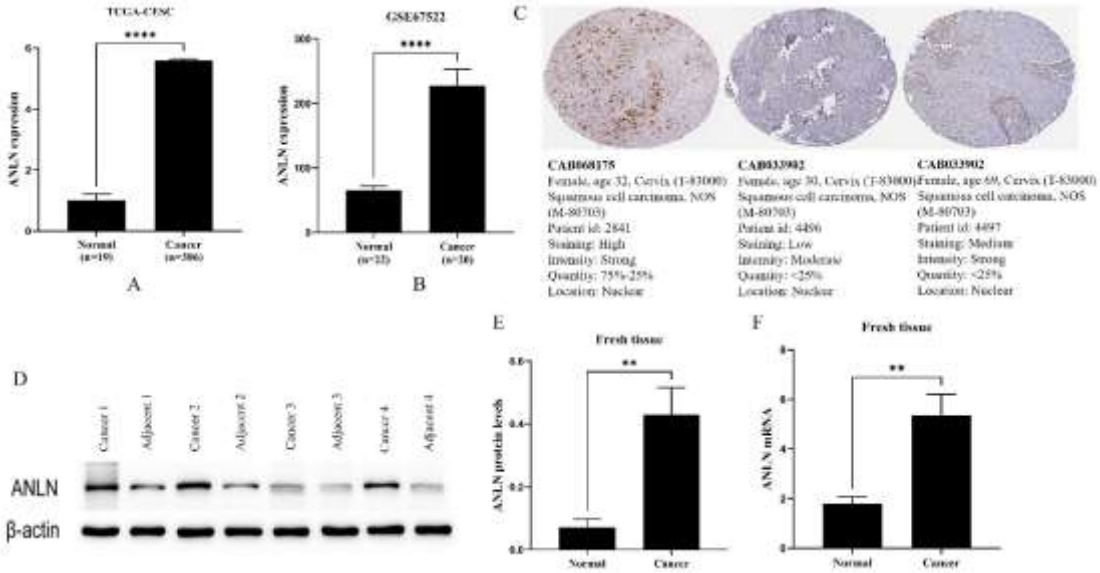

Figure 1, ANLN is overexpressed in cervical cancer

(A-B) ANLN mRNA expression levels in cervical cancer tissues (Tumor) and normal adjacent tissues (Normal) from the TCGA-CESC and GSE67522 datasets. (C) qRT-PCR analysis of ANLN mRNA expression in four paired clinical samples (T: tumor; N: adjacent normal tissue). (D) Representative western blot images showing ANLN protein expression in two of the four paired samples. GAPDH was used as a loading control. **p < 0.01, ****p < 0.0001.

3.2. ANLN as an Independent Prognostic Factor and Construction of a Predictive Nomogram

To assess the prognostic significance of ANLN, we stratified patients in the TCGA-CESC cohort into high- and low-expression groups based on the median expression level. Kaplan-Meier analysis revealed that high ANLN expression was associated with significantly worse overall survival (log-rank test, p=0.01; Figure 2A). This was further substantiated by univariate Cox regression analysis, which identified high ANLN expression, along with age, FIGO stage, and grade, as significant risk factors (all $p < 0.05$). Crucially, multivariate analysis confirmed that ANLN expression remains an independent prognostic predictor after adjusting for other clinical variables (HR(High) = 1.85, 95%CI: 1.15 −2.90, p = 0.01).

To translate these findings into a quantitative clinical tool, we integrated these independent prognostic factors to construct a nomogram (Figure 2B). This nomogram allows for the individualized prediction of 1-, 3-, and 5-year overall survival probabilities by summing the points assigned to each variable. The model demonstrated strong predictive accuracy, with a concordance index (C-index=0.70, p =1.85e-07), and its calibration curves showed excellent agreement between predicted and observed survival outcomes (Figure 2C).

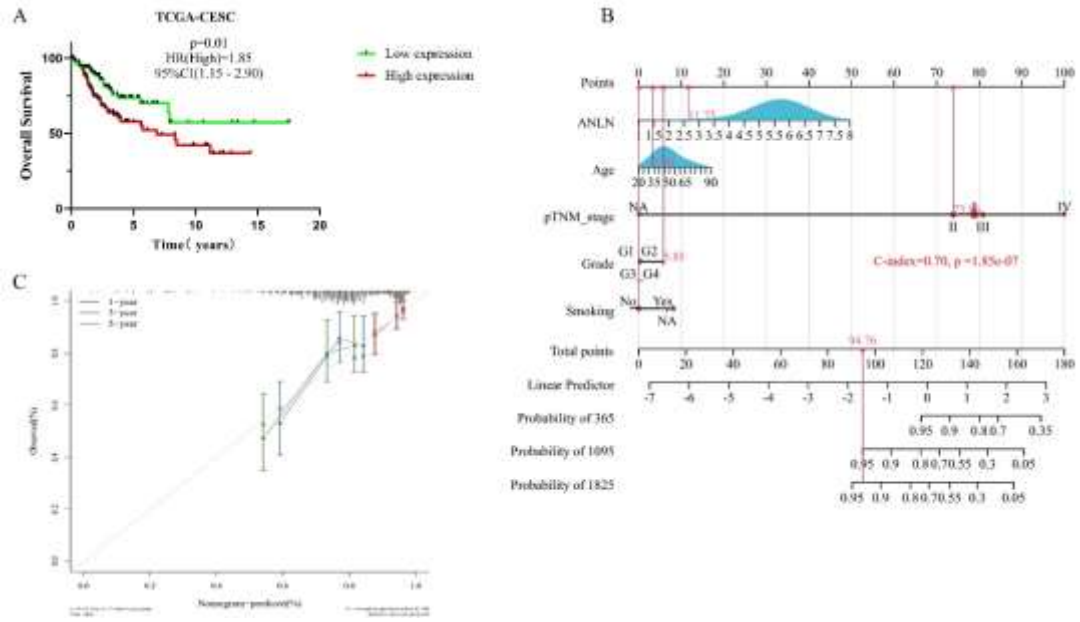

Figure 2. Prognostic value of ANLN in cervical cancer.
(A) Kaplan-Meier survival analysis based on ANLN expression (log-rank p=0.01). (B) Nomogram integrating ANLN expression and clinical variables for predicting 1-, 3-, and 5-year overall survival. (C) Calibration curves demonstrating agreement between predicted and observed survival outcomes.

3.3 Comprehensive Bioinformatic Analysis of ANLN in CC

To further investigate the biological functions and mechanisms of ANLN in cervical carcinogenesis, a series of bioinformatic analyses were performed using data from the TCGA-CESC project.

Correlation analysis identified the top 10 positively and top 10 negatively correlated genes with ANLN expression (|Pearson R| > 0.4, $p < 0.001$) (Figure 3A, Supplementary Table S1 provides the complete list of 169 positively and 10 negatively correlated genes). Functional enrichment analysis of ANLN co-expressed genes revealed its pivotal role in cell cycle regulation. GO analysis identified significant associations with mitotic processes, including cell division, nuclear division, spindle organization, and microtubule cytoskeleton formation ($p < 0.05$; Figure 3B-D). KEGG pathway analysis further demonstrated enrichment in cell cycle, oocyte meiosis, cellular senescence, and viral carcinogenesis pathways ($p < 0.05$; Figure 3E). These results strongly suggest ANLN's central involvement in cell cycle progression and mitotic regulation in cervical cancer.

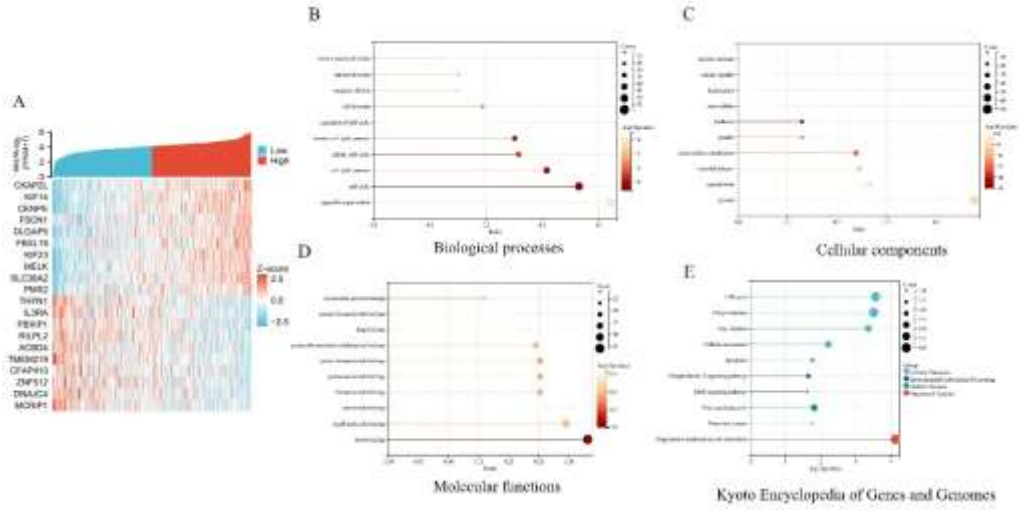

Figure 3, Functional enrichment analysis of ANLN co-expressed genes in cervical cancer.

(A) Heatmap of the top 10 positively and negatively correlated genes with ANLN expression. (B-D) Gene Ontology (GO) enrichment analysis showing significant terms in biological processes, cellular components, and molecular functions related to cell cycle regulation. (E) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis showing significantly enriched pathways.

3.4 Pan-cancer Analysis of ANLN Expression and Prognostic Significance

To investigate the potential oncogenic role of ANLN across human cancers, a comprehensive pan-cancer analysis was conducted using the TCGA database. ANLN expression was first evaluated across 33 cancer types and corresponding normal tissues. ANLN was significantly overexpressed in tumor versus normal tissues across most cancer types (Figure 4A). Elevated ANLN expression predicted poorer overall survival in multiple malignancies including KIPAN (HR=1.57, p=5.2e-18), KIRP (HR=1.99, p=1.5e-10), LUAD (HR=1.32, p=1.1e-7), and notably in CESC (HR=1.44, p=0.03), while showing protective effects in KICH and TGCT (Figure 4B).

ANLN expression was significantly associated with advanced tumor stage (Stage III/IV) in KIRC, LIHC, KIRP and LUAD (Figure 4C), and with higher tumor grade (G3) in STAD, LIHC, PAAD and KIRC (Figure 4D). These results establish ANLN as both a consistently overexpressed gene and a powerful prognostic biomarker associated with tumor progression across human cancers.

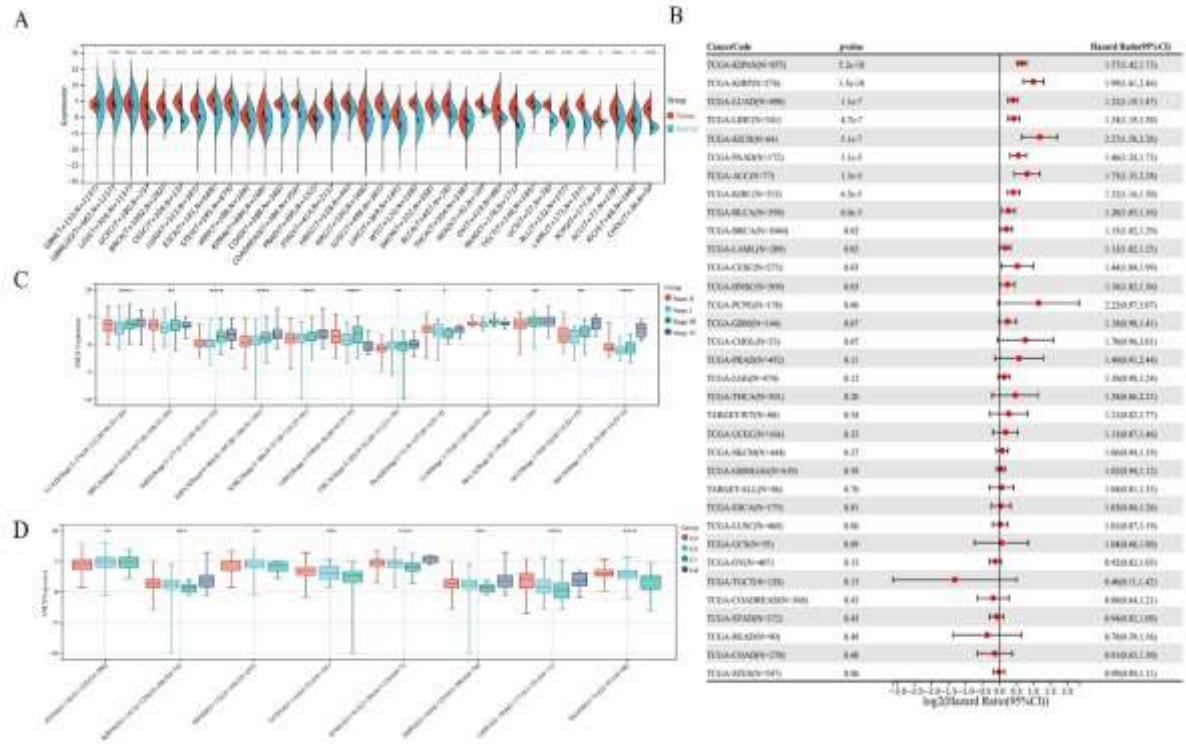

Figure 4. Pan-cancer analysis of ANLN

(A) ANLN expression across TCGA cancers. T, tumor; N, normal. (B) Forest plot of hazard ratios (HR) for overall survival. (C) ANLN expression across pathological stages. (D) Correlation heatmap between ANLN and immune checkpoint genes. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

4. Discussion

Despite advances in prevention and screening, cervical cancer remains a significant clinical challenge, particularly for patients with advanced or recurrent disease [18]. The identification of robust prognostic biomarkers is therefore critical for improving risk stratification and guiding personalized treatment strategies. In this study, we comprehensively demonstrate that ANLN serves as a novel oncogene and an independent prognostic biomarker in cervical cancer.

Our findings consistently revealed a significant upregulation of ANLN at both the mRNA and protein levels in cervical cancer tissues compared to normal controls, a observation further corroborated by pan-cancer analysis which identified ANLN overexpression as a common event across multiple malignancies [19]. This conserved pattern of dysregulation strongly suggests a fundamental role for ANLN in tumorigenesis [14, 20]. Most importantly, elevated ANLN expression was powerfully associated with unfavorable overall survival in CC patients. Multivariate Cox regression analysis confirmed that ANLN is an independent prognostic factor, surpassing the predictive value of traditional clinicopathological parameters alone. To translate this finding into a clinically applicable tool, we developed a nomogram that integrates ANLN expression with other independent risk factors. This model demonstrated high predictive accuracy for 1-, 3-, and 5-year survival, offering a quantitative method for individualized prognosis assessment.

To elucidate the potential mechanisms driving ANLN's oncogenic role, we performed comprehensive bioinformatic analyses. The strong positive correlation between ANLN expression and genes enriched in cell cycle-related processes—such as nuclear division, chromosome segregation, and the mitotic spindle apparatus—directly implicates ANLN in the loss of cell cycle control, a hallmark of cancer [13,17,22]. This is further supported by KEGG pathway enrichment analysis, which highlighted significant involvement in the Cell cycle pathway. Essentially, our data suggest that ANLN is associated with cervical carcinogenesis and may contribute to it by promoting uncontrolled cellular proliferation [23]. However, it is important to note that the current study is primarily descriptive; the exact molecular mechanisms through which ANLN exerts its oncogenic effects require further functional validation in subsequent experiments.

Interestingly, our pan-cancer analysis revealed a context-dependent function of ANLN, wherein it acted as a favorable prognostic factor in KIRC. This intriguing paradox underscores the complexity of its biological roles and suggests that the functional impact of ANLN may be highly tissue-specific, influenced by the unique genetic and molecular background of different cancers [24-25].

Despite the compelling evidence presented, this study has certain limitations that should be acknowledged. Firstly, the number of clinical tissue samples used for experimental validation (qRT-PCR and Western blot) was relatively small (only four pairs). Although the results were highly consistent with the trends observed in large public databases, future studies with a larger, prospectively collected cohort are essential to further validate the clinical relevance and robustness of ANLN as a biomarker in cervical cancer. Secondly, while our bioinformatic analyses provide strong mechanistic insights, the exact molecular pathways through which ANLN influences the tumor immune microenvironment and confers chemoresistance require further functional validation through in vitro and in vivo experiments, such as gene knockdown or overexpression models.

In conclusion, our integrated multi-omics study establishes ANLN as a potential oncogene in cervical cancer. Our findings suggest that its overexpression is associated with tumor progression, potentially through directly regulating cell cycle proliferation. The robust prognostic performance of the ANLN-based nomogram highlights its potential clinical utility for improving patient stratification. Future studies should focus on validating these findings in larger, prospective cohorts and investigating the precise molecular mechanisms by which ANLN regulates chemoresistance. Ultimately, ANLN represents a promising prognostic biomarker. If its causative role in cervical cancer progression is further validated by functional studies, it could become a compelling candidate for targeted therapeutic intervention.

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Ethics approval: The protocol and methodology of the present study were approved by the Ethics Committee of Zhuzhou Central Hospital (No. 20231046), and each eligible participant provided written informed consent.

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Conflicts of interest: The authors have no known conflicts of interest to declare.

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