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DECIPHERING THE ORCHESTRATED SYMPHONY: ANLN'S MULTIFACETED ROLE IN LIVER HEPATOCELLULAR CARCINOMA PATHOGENESIS AND PROGNOSIS

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Abstract

The role of Anillin Actin Binding protein (ANLN) in Liver Hepatocellular Carcinoma (LIHC) is investigated through comprehensive expression and methylation analyses, genetic mutation inquiry, and prognostic assessment. Utilizing the UALAN database, ANLN expression is found significantly elevated in LIHC samples compared to normal controls, suggesting its regulatory involvement in LIHC proliferation. Further examination based on different parameters such as cancer stages, patient demographics, and promoter methylation levels reveals consistent overexpression of ANLN across diverse conditions, emphasizing its pivotal role in LIHC pathogenesis. Interestingly, promoter hypomethylation of ANLN is observed in LIHC samples compared to normal controls, indicating a negative correlation between ANLN expression and promoter methylation, with therapeutic implications. Genetic mutation analysis via cBioPortal identifies a minimal proportion of ANLN mutations in LIHC, predominantly amplifications and missense mutations, indicating their basal significance in ANLN dysregulation within LIHC. Prognostic assessment utilizing KM plotter demonstrates a significant correlation between ANLN overexpression and decreased overall survival rates in LIHC patients, suggesting ANLN's potential as a prognostic biomarker for disease progression and patient outcome prediction. Collectively, these findings underscore the crucial involvement of ANLN in LIHC progression and development, providing insights into its potential as a therapeutic target and prognostic indicator in the clinical management of LIHC. Further

exploration of ANLN's molecular mechanisms and its interplay within the LIHC microenvironment may unveil novel avenues for targeted therapy and precision medicine approaches in combating this challenging disease.

Keyword: ANLN; LIHC; Biomarker; Prognosis

Introduction

Cancer remains a significant global challenge and stands as a primary cause of death in countries like China, the USA, and other developed nations (1-5). According to recent statistics, in 2021 alone, there were approximately 19.2 million cancer cases reported, resulting in 9.9 million deaths worldwide (6, 7). Over the years, China has seen a notable increase in the incidence, mortality rates, and overall death toll attributed to cancer since the year 2000 (8, 9). With over 200 different types of cancers, liver cancer stands out as a major burden globally, ranking as the second leading cause of tumor-related fatalities in China (10-12). Specifically, liver hepatocellular carcinoma (LIHC) emerges as the most prevalent form of primary liver cancer, accounting for roughly 90% of all liver cancer cases (13, 14). LIHC ranks fourth in terms of common cancers in China and fifth worldwide (15). Notably, major risk factors for LIHC include viral hepatitis, alcohol consumption, and non-alcoholic liver diseases, with approximately 85% of LIHC cases occurring in livers affected by cirrhosis (16-20).

The Anillin actin binding protein (ANLN) gene encodes a protein that binds to actin and plays essential roles in cell growth, migration, and cytokinesis. Previous studies have indicated that variations and mutations in ANLN contribute to the progression of various cancers, including LIHC, breast, kidney, brain, and ovarian cancer (21, 22). However, another study suggests that reducing ANLN levels suppresses cell proliferation, migration, and invasion in LIHC (23). Significantly, decreased ANLN expression is associated with inhibited cell proliferation, invasion, and migration in bladder urothelial carcinoma, underscoring its importance in regulating the cell cycle (24). ANLN's heightened expression in cancer cells has prompted discussions regarding its potential as an early predictor for cancer diagnosis (25-27). Moreover, ANLN has been found to counteract the effects of doxorubicin, thereby conferring resistance to chemotherapy in breast cancer (28). Given its pivotal role in the cell cycle, ANLN is considered a promising target for cancer treatment and diagnosis. Consequently, analyzing ANLN expression is crucial due to its significant impact on cell cycle regulation and cancer development. However, as of now, the role of ANLN in LIHC has not been explored through bioinformatics methods.

In the current investigation, our objective was to scrutinize ANLN mutations, expression levels, prognostic implications on survival, and functional aspects within the context of LIHC through bioinformatics analysis. Additionally, we explored the correlation between ANLN expression and promoter methylation levels. To achieve this, we employed various resources including the Cancer Genome Atlas (TCGA) database, UALCAN platforms, Kaplan-Meier database, and cBioPortal. The primary aim of this study was to assess ANLN expression patterns in LIHC and elucidate its potential significance in cancer treatment and diagnosis.

Materials and Methods

UALCAN Analysis

In our study, we leveraged UALCAN, a user-friendly and freely accessible web tool designed for the analysis of TCGA and CPTA cancer data (20, 29, 30). Specifically, we utilized the UALCAN database to examine ANLN expression levels and promoter methylation status in LIHC. Furthermore, we employed UALCAN to assess ANLN expression and promoter methylation levels across various demographic parameters, including patient race, age, gender, and ethnicity. This comprehensive analysis provided valuable insights into the association between ANLN expression patterns, promoter methylation, and demographic factors in LIHC patients.

Kaplan-Meier Plotter Analysis

The Kaplan-Meier Plotter (KM plotter) is a valuable online tool used for survival analysis and assessing gene expression patterns (31-33). This tool utilizes resources from TCGA and provides data on mRNA expression levels. Its user-friendly interface allows researchers to easily explore the prognostic significance of genes of interest. In our study, we employed the KM plotter database to investigate the impact of ANLN expression on overall survival (OS) in patients diagnosed with LIHC. This analysis provided important insights into the potential prognostic value of ANLN in LIHC patients.

cBioPortal

cBioPortal is a widely-used, open-access web tool for cancer genomic research, leveraging data from the TCGA database (34-36). It offers comprehensive information on copy number variations, genetic mutations, and other genomic alterations, drawing from a vast dataset of over 28,000 samples. In our study, we utilized cBioPortal to investigate ANLN mutations specifically within the context of LIHC, drawing upon the rich resources available in the TCGA database. This analysis allowed us to gain insights into the prevalence and characteristics of ANLN mutations in LIHC patients, contributing to our understanding of the molecular landscape of this cancer type.

Results

ANLN expression in LIHC

Initially, our investigation centered on comparing the expression levels of ANLN between cancerous and normal control samples. To conduct this analysis specifically in the context of LIHC, we employed the UALCAN database. Our findings revealed a significant overexpression of ANLN in LIHC samples compared to normal control samples (see Figure 1). This observation suggests that ANLN may play a crucial role as a regulator of proliferation in LIHC, highlighting its potential as a therapeutic target or diagnostic marker in this cancer type.

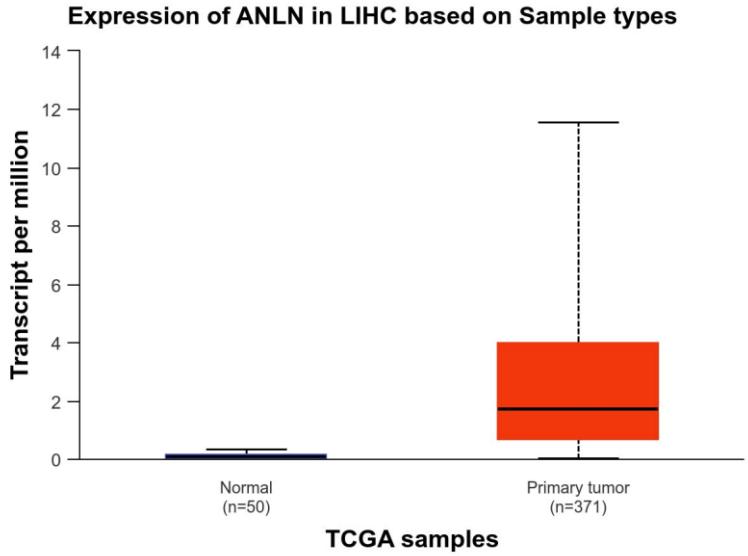

Figure 1: Expression of ANLN gene in LIHC and control samples.

Expression analysis of ANLN in LIHC based on different parameters

Continuing our analysis, we delved into the expression patterns of ANLN in LIHC while considering various parameters such as patient's race, age, gender, and different stages of cancer. Firstly, we scrutinized ANLN expression across different stages of LIHC and observed variations in expression levels. However, notably, we found significant overexpression of ANLN across all stages of LIHC (see Figure 2A). Following this, we explored ANLN expression in LIHC with respect to patient's race. Our analysis revealed significant overexpression of ANLN in LIHC

samples from different racial backgrounds compared to normal samples (see Figure 2B). Subsequently, we investigated ANLN expression in LIHC based on patient's gender. Remarkably, we observed considerable overexpression of ANLN in LIHC regardless of gender (see Figure 2C). Furthermore, we examined ANLN expression in LIHC across different age groups. Although we detected variations, there was a consistent trend of overexpression of ANLN across different age groups (see Figure 2D). These findings underscore the significance of ANLN in LIHC and highlight its potential as a valuable biomarker for diagnosis and therapeutic targeting.

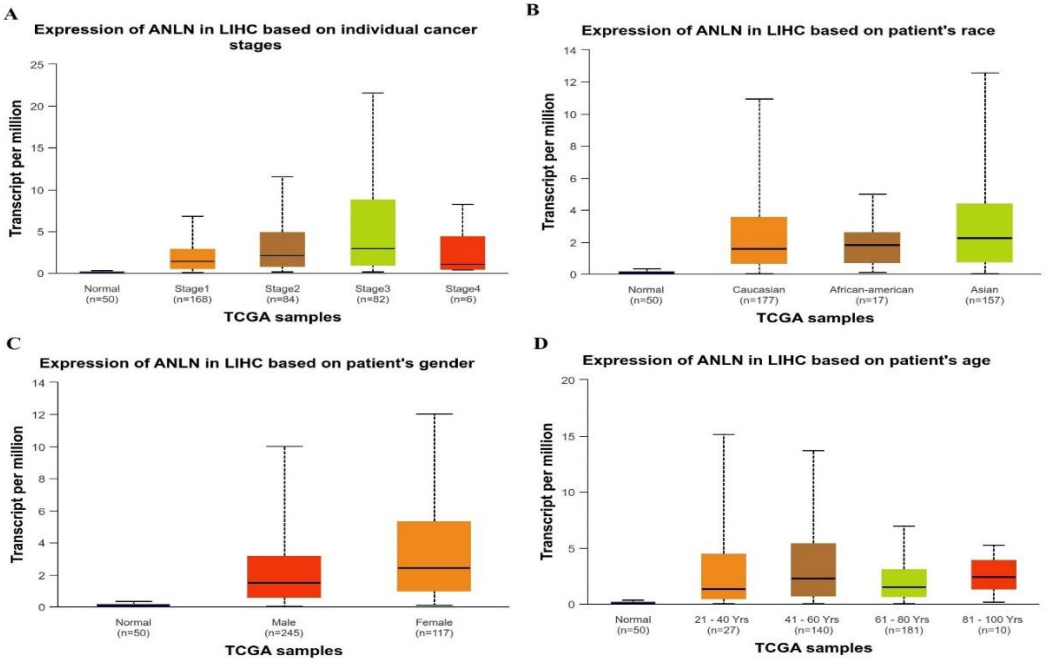

Figure 2: Expression of ANLN gene in LIHC samples divided by various clinical parameters and control samples.

Promoter methylation of ANLN in LIHC and normal control sample

We conducted an analysis of the promoter methylation levels of ANLN in LIHC and normal control samples using UALCAN. Our findings revealed that ANLN was hypo-methylated in LIHC samples compared to normal control samples (see Figure 3). This observation suggests a negative correlation between ANLN expression and promoter methylation in LIHC. Such a correlation unveils the therapeutic potential of ANLN in the pathogenesis of LIHC, indicating its role as a potential target for therapeutic interventions in this cancer type.

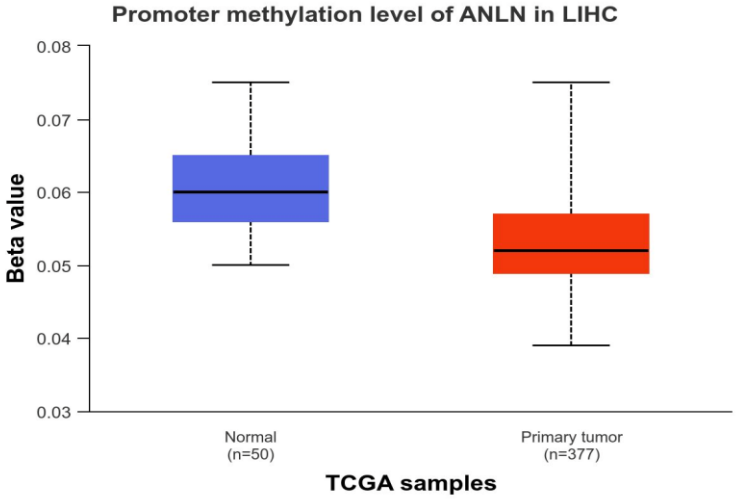

Figure 3: Promoter methylation level of ANLN gene in LIHC and control samples.

Promoter methylation of ANLN in LIHC divided based on different parameters

Concurrently, we investigated the promoter methylation level of ANLN in LIHC across different parameters including patient's race, age, gender, and different cancer stages. Initially, we focused on analyzing the promoter methylation level based on different cancer stages. Our analysis revealed significant hypo-methylation in the promoter methylation level of ANLN in patient samples from various stages of LIHC (see Figure 4A). Subsequently, we examined the hypo-methylation of ANLN promoter in LIHC samples from different racial backgrounds (see Figure 4B). Our findings indicated consistent hypo-methylation across LIHC samples irrespective of racial diversity. Next, we assessed the promoter methylation level of ANLN in LIHC based on patient's gender. Interestingly, we observed hypo-methylation in LIHC samples compared to normal samples across both genders (see Figure 4C). Moreover, we investigated the promoter methylation level of ANLN in LIHC across different age brackets (see Figure 4D). Consistently, hypo-methylation was observed in LIHC samples across various age groups. Overall, these results demonstrate a consistent pattern of hypo-methylation in the promoter methylation level of ANLN in LIHC, highlighting its potential significance in the pathogenesis of this cancer type.

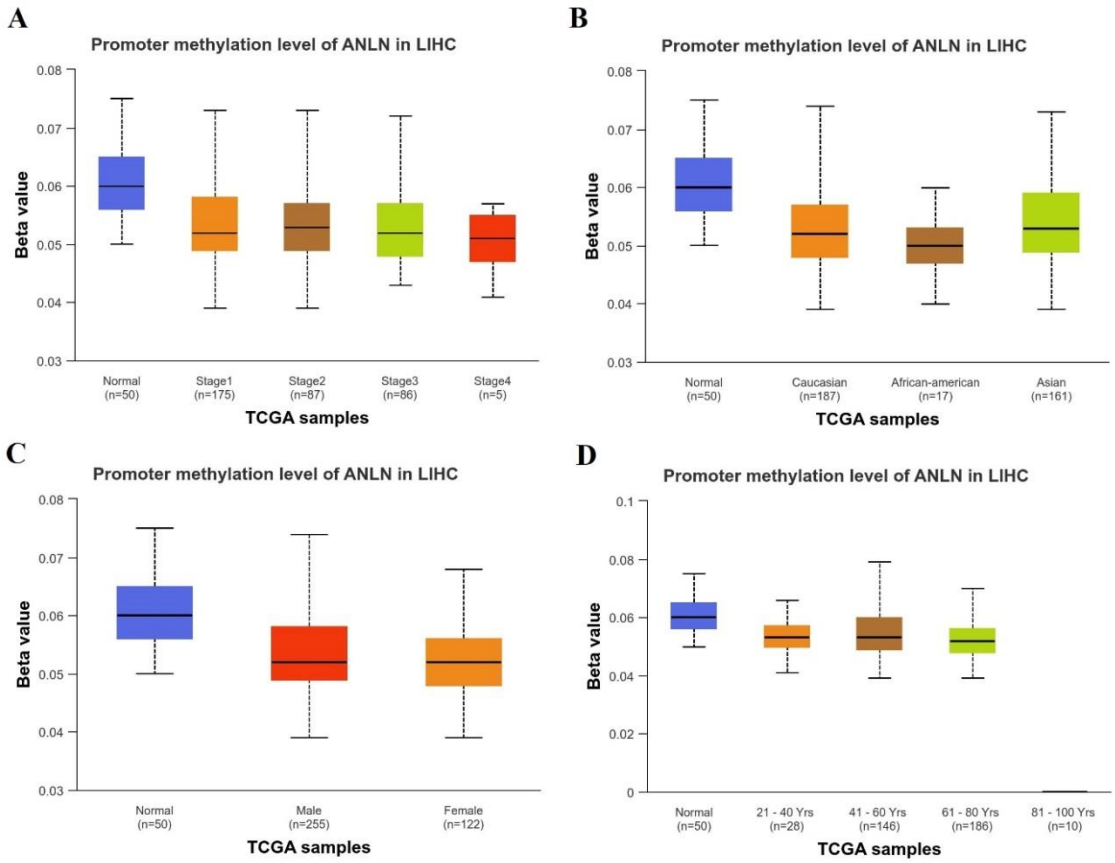

Figure 4: Promoter methylation level of ANLN gene in LIHC samples divided by various clinical parameters and control samples.

Genetic mutation analysis

We further investigated the genetic mutations of ANLN in LIHC patients using cBioPortal. Our analysis revealed that only 2.5% of LIHC samples exhibited genetic mutations in ANLN. The examined genetic mutations in LIHC included amplifications and missense mutations (see Figure 5). These findings suggest that while genetic mutations in ANLN are relatively rare in LIHC, the observed amplifications and missense mutations may play a fundamental role in the dysregulation of ANLN in LIHC.

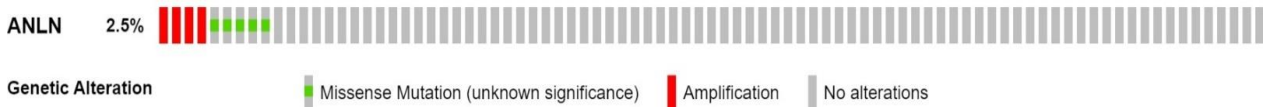

Figure 5: Oncoplot of TP53 mutations in LIHC patients.

Prognostic significance of ANLN in LIHC

Additionally, we utilized the KM plotter to assess the prognostic significance of ANLN in LIHC. Our analysis uncovered a significant correlation between ANLN overexpression and decreased overall survival (OS) rate (see Figure 6). The KM plotter curve indicated a poor prognosis associated with ANLN overexpression, with a hazard ratio (HR) of 2.38 and a p-value of 0.0000005. Based on these findings, ANLN may serve as a prognostic biomarker in LIHC, suggesting its involvement in the progression and development of this cancer type.

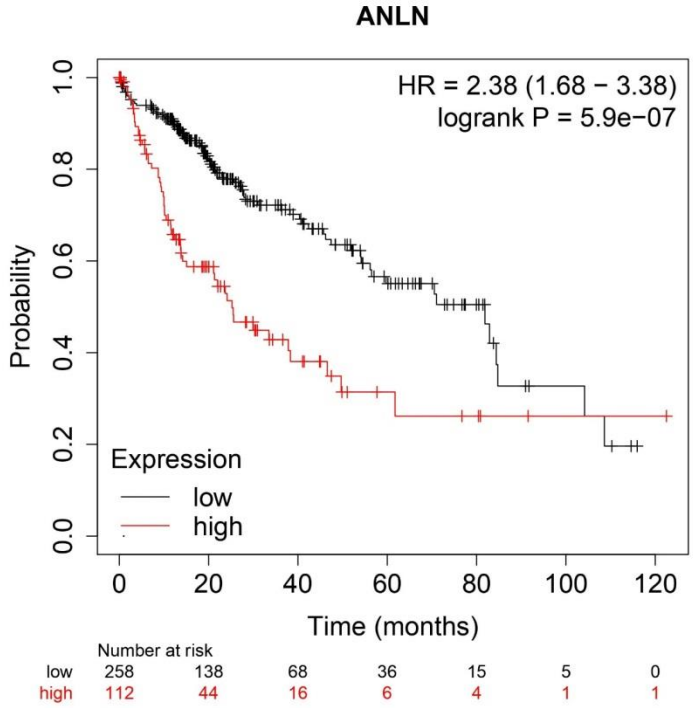

Figure 6: Survival curve of ANLN gene in LIHC patients.

Discussion

In recent years, cancer has emerged as a significant medical challenge globally, with the number of cases surpassing 90 million worldwide (36-39). Consequently, researchers have increasingly turned to cancer analysis to enhance therapeutic strategies, improve diagnosis, identify new biomarkers, and gain a deeper understanding of gene mutations. While previous studies have examined ANLN in several cancers and noted its upregulation in some, its role in LIHC remains largely unexplored (40). To address this gap, we conducted a systematic analysis of ANLN in LIHC using public databases such as UALCAN, KM plotter, and cBioPortal. Through the comprehensive utilization of these resources, we investigated ANLN expression, promoter methylation levels, mutational analysis, and survival analysis in LIHC. We believe that the insights gained from this study have the potential to contribute significantly to the early prognostic, therapeutic, and diagnostic capabilities in LIHC, ultimately aiding in the development of more effective management strategies for this challenging cancer type.

Initially, our analysis focused on assessing ANLN expression levels in LIHC samples compared to normal control samples using the UALCAN database. We observed a significant overexpression of ANLN in LIHC samples, indicating its potential role in LIHC progression. This finding aligns with previous studies where ANLN was found to be overexpressed in other cancers such as cervical

squamous cell carcinoma and endocervical adenocarcinoma (CESE), head and neck squamous cell carcinoma (HNSC), and esophageal carcinoma (ESCA) (41). Moreover, we investigated ANLN expression across various parameters including patient's race, age, gender, and different cancer stages. Interestingly, we found consistent upregulation of ANLN expression across these parameters. These findings highlight a strong correlation between ANLN expression and LIHC progression, suggesting its potential utility as a prognostic biomarker. Overall, our analysis underscores the significance of ANLN in LIHC and suggests its potential as a prognostic indicator for this challenging cancer type..

Continuing our analysis, we explored the correlation between ANLN overexpression and promoter methylation levels to elucidate a possible cause of its up-regulation in LIHC. Our investigation revealed that ANLN was hypo-methylated in LIHC samples compared to normal samples. Subsequently, we extended our analysis to different parameters including patient's race, age, gender, and different cancer stages. Interestingly, we found consistent hypo-methylation of ANLN across these parameters. These findings provide valuable insights suggesting that the overexpression of ANLN may be influenced by hypo-methylation (42-44). This correlation between ANLN expression and promoter methylation levels highlights the potential regulatory role of methylation in ANLN expression in LIHC, further contributing to our understanding of the mechanisms underlying ANLN dysregulation in this cancer type.

Furthermore, we conducted mutational and survival analyses to further evaluate the role of ANLN in LIHC progression. For mutational analysis, we utilized cBioPortal and found that ANLN exhibited mutations in only 2.5% of LIHC cases, predominantly consisting of amplifications and missense mutations. While these mutations were relatively low in frequency, we speculate that they may contribute to ANLN overexpression at a subtle level. Additionally, we performed survival analysis using the KM plotter tool and discovered a significant correlation between ANLN overexpression and poor overall survival (OS) in LIHC patients. This association underscores the potential clinical implications of ANLN in the diagnosis and therapeutic strategies for LIHC. The convergence of findings from hypo-methylation, genetic mutations, and overexpression of ANLN highlights its potential significance in LIHC pathogenesis. However, further analyses are warranted to elucidate the precise mechanisms and clinical implications of ANLN dysregulation in LIHC.

Conclusion

In conclusion, our analysis indicates that ANLN overexpression in LIHC is closely associated with poor overall survival, promoter methylation levels, and genetic mutations. Through a systematic utilization of various public databases including UALCAN, TCGA, cBioPortal, and KM plotter, we have shed light on the diagnostic, prognostic, and potentially therapeutic roles of ANLN in LIHC. However, further research is warranted to validate and confirm these findings, as well as to elucidate the underlying mechanisms driving ANLN dysregulation in LIHC. These insights may ultimately contribute to the development of improved diagnostic tools and therapeutic strategies for LIHC patients.

Conflict of interest

None

Acknowledgement

None

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