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*Research article*

# TCGA and Bioinformatics Reveal the Molecular Network and Prognostic Implication of Laminin Beta 1 in Cholangiocarcinoma

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## Abstract

This study investigates the expression, prognostic relevance, and molecular network of laminin subunit beta 1 (LAMB1) in cholangiocarcinoma (CCA) using TCGA data and bioinformatics tools. LAMB1 mRNA expression in CCA tissues was analyzed using the TCGA-CHOL dataset via GEPIA2, and protein expression data was obtained from The Human Protein Atlas (HPA). Survival analysis was performed using Kaplan–Meier plots and the log-rank test. Correlated genes were identified via UALCAN and analyzed for protein–protein interactions using STRING, followed by Gene Ontology (GO) and KEGG pathway enrichment. TCGA data showed significant upregulation of LAMB1 mRNA in CCA, and HPA images revealed stronger protein staining in CCA than in normal tissues. Although LAMB1 expression showed no significant association with overall survival (OS) or disease-free survival (DFS) in TCGA-CHOL alone, analysis of the combined CHOL and liver hepatocellular carcinoma (LIHC) datasets revealed that patients with high LAMB1 expression had significantly shorter OS and DFS. Among 250 LAMB1-correlated genes, STRING analysis identified 51 interacting partners, with GO and KEGG analyses highlighting enrichment in cell proliferation, DNA repair, and extracellular matrix (ECM)-related pathways. Notably, agrin (AGRN), collagen type IV alpha 5 chain (COL4A5), integrin alpha-2 (ITGA2), and nidogen 1 (NID1) emerged as network neighbors of LAMB1, with high AGRN and ITGA2 expression correlating with poor prognosis. These genes participate in ECM–integrin crosstalk and mechanotransduction signaling, processes that facilitate tumor progression and resistance to therapy. Together with our previous findings that LAMB1 promotes drug resistance and malignant phenotypes in CCA cells, these results reinforce LAMB1 as a central component of ECM-associated signaling networks and support its potential as a prognostic biomarker and therapeutic target.

**Keywords:** laminin subunit beta 1; cholangiocarcinoma; TCGA database; prognostic biomarker; protein-protein interaction; bioinformatics

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## 1. Introduction

Cholangiocarcinoma (CCA) is a malignant tumor of the biliary tree that, despite its slow onset, is highly aggressive and associated with a poor prognosis and limited treatment options (Valle et al., 2016; Forner et al., 2019). Although CCA is classified as a rare cancer, its incidence and mortality rates have been steadily increasing worldwide, raising significant global health concerns (Banales et al., 2020). The reported global incidence and mortality rates range from 0.3 to 6 and 1 to 6 per 100,000 per year, respectively (Banales et al., 2016; Bertuccio et al., 2019). However, in certain Asian countries, including Thailand, China, and South Korea, the incidence exceeds 6 per 100,000 (Bertuccio et al., 2019). Given its aggressive nature, high mortality rate, and lack of effective treatment options, there is an urgent need to identify novel therapeutic targets for CCA. Advancing our understanding of the molecular mechanisms underlying its progression could pave the way for more effective treatment strategies and improved patient outcomes.

Laminin subunit beta 1 (LAMB1) is a member of the laminin protein family, a group of extracellular matrix glycoproteins composed of three polypeptide subunits: alpha, beta, and gamma (Aumailley et al., 2005; Aumailley, 2013). Laminins are primarily secreted by basement membrane-associated cells, such as epithelial and endothelial cells, and play a crucial role in maintaining the structural integrity of the basement membrane (Jayadev & Sherwood, 2017). Beyond serving as structural components, laminins actively regulate key cellular processes, including adhesion, migration, proliferation, and intracellular signaling (Patarroyo et al., 2002; Givant-Horwitz et al., 2004; Domogatskaya et al., 2012). Their functions are often cell type–specific, exerting their effects through interactions with surface receptors that initiate diverse signaling cascades. These interactions contribute to tissue development, homeostasis, and repair, while dysregulation of laminin expression has been implicated in various pathological conditions, including cancer and fibrosis.

Emerging evidence suggests that LAMB1 is upregulated in several cancer types and plays a significant role in tumor progression. In gastric cancer, high LAMB1 expression is associated with poor prognosis and promotes tumor growth and migration (Lee et al., 2021), supporting its potential as a diagnostic biomarker and therapeutic target (Ran et al., 2021). In brain cancer, elevated LAMB1 levels correlate with reduced patient survival, further supporting its role in disease severity (Zhao et al., 2025). Similarly, in nasopharyngeal carcinoma, LAMB1 overexpression contributes to an immunosuppressive tumor microenvironment and enhances cancer cell proliferation, migration, and invasion (Feng et al., 2025). In hepatocellular carcinoma, LAMB1, in conjunction with DEAD-box helicase 24 (DDX24), promotes cell migration and proliferation (Liu et al., 2022). Bioinformatics analyses of extracellular matrix (ECM) receptor pathways have also identified LAMB1 as a prognostic biomarker in lung adenocarcinoma (LUAD), where its expression correlates with patient survival and the tumor immune microenvironment (Liu et al., 2024). Furthermore, TCGA datasets reveal that LAMB1 expression is elevated in several additional cancer types, including diffuse large B-cell lymphoma (DLBC), head and neck squamous cell carcinoma (HNSC), kidney renal papillary cell carcinoma (KIRP), pancreatic adenocarcinoma (PAAD), and thymoma (THYM). However, in these malignancies, the functional significance of LAMB1 upregulation has not yet been confirmed by *in vitro* or *in vivo* studies.

Our recent study demonstrated that drug-resistant CCA cells exhibit increased migration and invasion abilities, with LAMB1 emerging as a key protein associated with these aggressive phenotypes. Notably, LAMB1 knockdown attenuated cell motility and reversed the drug-resistant phenotype of CCA cells, highlighting its potential role in tumor

progression and chemoresistance (Kerdkumthong et al., 2024). Building upon these findings, the present study aims to further investigate the prognostic and molecular relevance of LAMB1 in CCA by utilizing The Cancer Genome Atlas (TCGA) database and online bioinformatics tools to analyze its expression patterns, prognostic value, correlated genes, and potential pathways involved.

## 2. Materials and Methods

### 2.1 Gene expression analysis

The mRNA expression levels of LAMB1 and its positively correlated genes in CCA tissues were analyzed using TCGA data via the Gene Expression Profiling Interactive Analysis 2 (GEPIA2) web portal (http://gepia2.cancer-pku.cn) (Tang et al., 2019) and the University of Alabama at Birmingham Cancer (UALCAN) data analysis portal (https://ualcan.path.uab.edu) (Chandrashekar et al., 2022). For GEPIA2, box plots comparing CCA tissues (TCGA tumor cohort; n = 36) and normal tissues (TCGA normal cohort; n = 9) were generated using $\log_2$(TPM + 1)-transformed RNA-seq data, and differential expression significance was assessed via one-way ANOVA ($p<0.05$). For UALCAN, heatmap visualization of LAMB1 and 51 interacting genes was performed using primary tumor samples (n = 36) and normal tissues (n = 9) from the TCGA-CHOL dataset. Expression values were displayed as $\log_2$(TPM + 1) using a color gradient from blue (low expression; $\log_2$(TPM + 1) = 0) to white (intermediate) to red (high expression; $\log_2$(TPM + 1) = 15). Samples were grouped by tissue type (tumor vs. normal), and genes were displayed in the order provided in the input list. Protein expression levels of LAMB1 in CCA and normal bile duct tissues were obtained from The Human Protein Atlas (HPA) (https://proteinatlas.org/) (Pontén et al., 2008). Immunohistochemistry (IHC) images from all five CCA tumors and all three normal tissues available in HPA were used. Panels with the same antibody clone (HPA004132) were employed to ensure consistent staining quality and specificity. Representative images of all cases were presented, but no quantitative scoring was performed.

### 2.2 Survival and gene correlation analysis

The prognostic potential of LAMB1 and its correlated genes was evaluated using GEPIA2 to generate overall survival (OS) and disease-free survival (DFS) curves. Patients were divided into high- and low-expression groups based on the median expression value (top 50% vs. bottom 50%). The hazard ratio (HR) and 95% confidence interval (CI) were calculated using the Cox proportional hazards model, and survival curves were generated using GEPIA2 default parameters with a 95% CI. Statistical significance was determined using the log-rank test, with a threshold of $p<0.05$. The correlation between LAMB1 and its associated genes (AGRN, COL4A5, ITGA2, and NID1) was assessed using Pearson's correlation coefficient (R) in GEPIA2. Scatter plots were used to visualize the results, with a p-value <0.05 considered statistically significant.

### 2.3 Protein–protein Interaction analysis

The top 250 genes positively correlated with LAMB1 in CCA clinical samples were identified from UALCAN and further analyzed for protein-protein interactions (PPI) using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) (https://string-

db.org/) database version 12.0. The PPI network was constructed using full STRING network with a minimum required interaction score set to high confidence (0.700). All active interaction sources were enabled, including experiments, databases, co-expression, neighborhood, gene fusion, co-occurrence, and text mining. The maximum number of interactors was not limited, and a false discovery rate (FDR) correction was applied with a significance level of 0.05. In the constructed network, nodes represented proteins and edges indicated predicted functional interactions.

### 2.4 Gene Ontology and KEGG pathway analysis

Gene Ontology (GO) term enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed using the functional enrichment tool in STRING version 12.0, with LAMB1 and its 51 experimentally validated and predicted interacting genes from the PPI network used as the input dataset. The enrichment method was based on STRING's built-in hypergeometric test, and FDR correction was applied using the Benjamini–Hochberg procedure. A significance threshold of FDR < 0.05 was used for both GO and KEGG analyses. Annotation sources included GO Biological Process (BP), GO Molecular Function (MF), GO Cellular Component (CC), and KEGG pathways, with the whole human genome (*Homo sapiens*) set as the background. For GO analysis, BP, MF, and CC categories were analyzed separately. For KEGG pathway analysis, all pathway categories were enabled, and results were considered significant at FDR <0.05. Enriched terms and pathways were ranked according to their enrichment scores ($-\log_{10}$[FDR]) and presented with the corresponding gene counts and FDR values.

### 2.5 Statistical analysis

Statistical analyses were performed using the built-in analysis tools of GEPIA2, UALCAN, and STRING version 12.0, with the parameters described for each respective analysis above. Correlations between LAMB1 and other genes were analyzed using Pearson's correlation coefficient (R) in GEPIA2 and UALCAN, with a two-tailed p-value <0.05 considered statistically significant. Differences in OS and DFS outcomes were assessed using Kaplan–Meier survival analysis in GEPIA2, with patients dichotomized into high- and low-expression groups based on the median expression value. HR and 95% CI were calculated using the Cox proportional hazards model, and survival differences were assessed using the log-rank test ($p<0.05$). Differences in gene expression levels between normal and tumor tissues were evaluated using one-way ANOVA in GEPIA2 for multiple-group comparisons or Welch's t-test in UALCAN for two-group comparisons, depending on the dataset. All p-values were adjusted, where applicable, using the FDR method, and statistical thresholds were set as described in each subsection.

## 3. Results and Discussion

### 3.1 LAMB1 expression in CCA

LAMB1 mRNA expression was analyzed using TCGA CHOL dataset, which included tissue samples from 36 CCA patients and 9 normal tissues, through the GEPIA2 web portal. The results indicated a significant upregulation of LAMB1 in CCA tissues compared to normal tissues ($p<0.05$) (Figure 1A). For protein expression analysis, data from HPA showed that normal tissues exhibited low to undetectable levels of LAMB1, whereas CCA tissues

displayed varying degrees of brown staining, ranging from low to moderate to intense, indicating higher LAMB1 protein expression (Figure 1B). These findings suggest that LAMB1 is upregulated in CCA at both the mRNA and protein levels.

These findings reinforce prior evidence that aberrant overexpression of laminin subunits drives invasion, metastasis, and extracellular matrix (ECM) remodeling across multiple malignancies (Lee et al., 2021; Ran et al., 2021; Liu et al., 2022; Feng et al., 2025; Zhao et al., 2025). In addition to CCA, LAMB1 expression is elevated in several other cancer types, including PAAD, HNSC, DLBC, KIRP, and THYM, as observed in TCGA datasets. However, only the role of LAMB1 in PAAD has been further investigated *in vitro*.

In CCA, the laminin–integrin axis is a dominant stromal signal shaping tumor aggressiveness. LAMB1 is absent in normal bile ducts but frequently expressed in CCA tissues, co-localizing with integrin β4 (ITGB4) to define a highly aggressive disease subset with poor survival outcomes (Islam et al., 2021). Laminin-mediated signaling promotes CCA cell migration more effectively than other ECM substrates, with ITGB4 or laminin receptor (LAMR) acting as critical mediators depending on the cell line. Other laminin subunits, such as γ2 (LAMC2), are strongly overexpressed in intrahepatic CCA (iCCA), enhancing epidermal growth factor receptor (EGFR) translation in the endoplasmic reticulum and predicting poor prognosis, while conferring sensitivity to EGFR tyrosine kinase inhibitors (Zhang et al., 2024).

Moreover, LAMB1 upregulation is observed in murine models of combined hepatocellular carcinoma and intrahepatic cholangiocarcinoma (cHCC-ICC), supporting its role in primary liver tumors with mixed hepatocyte–cholangiocyte features (Xu et al., 2022). Importantly, functional studies demonstrate that LAMB1 knockdown reduces CCA cell invasion and migration while increasing chemosensitivity, highlighting a direct role in promoting malignancy and therapy resistance (Kerdkumthong et al., 2024). Mechanistically, the DEAD-box RNA helicase DDX24 has been reported to stabilize LAMB1 mRNA in hepatocellular carcinoma, promoting its expression and enhancing cell proliferation and migration (Liu et al., 2022). By analogy, in CCA and LIHC, high LAMB1 expression may contribute to poor patient survival by sustaining tumor growth, invasion, and resistance to apoptosis, potentially via similar RNA stabilization mechanisms.

## 3.2 Prognostic value of LAMB1

The association between LAMB1 mRNA expression and patient survival was evaluated using the GEPIA2 platform. In the TCGA-CHOL dataset (CCA patients only; high-expression, n = 18; low expression, n = 18), Kaplan–Meier survival analysis with the log-rank test revealed no significant difference in OS or DFS between the two groups (Figure 2A). However, when analyzing the combined CHOL and liver hepatocellular carcinoma (LIHC) datasets, we found that patients with high LAMB1 expression had significantly worse OS and DFS compared to those with low LAMB1 expression ($P<0.05$) (Figure 2B). In contrast, no significant difference in OS or DFS was observed between the high- and low-expression groups in the LIHC dataset alone (Figure 2C).

The limited sample size in TCGA-CHOL likely reduces statistical power; combining datasets increases sample size and captures shared microenvironmental features of primary liver cancers, allowing stronger detection of prognostic trends. High LAMB1 expression likely drives reduced survival in CHOL and LIHC patients by facilitating ECM remodeling, integrin-mediated signaling, and mechanotransduction, which collectively enhance tumor invasiveness and therapy resistance (Islam et al., 2021; Liu et al., 2022). Stabilization of LAMB1 by DDX24 may amplify these effects, further contributing to poor

outcomes. These results suggest that LAMB1 contributes to aggressive liver malignancy phenotypes, with prognostic potential enhanced when evaluated in conjunction with ITGB4 or other laminin axis components (Islam et al., 2021; Xu et al., 2022; Zhang et al., 2024). Functional studies confirm that LAMB1 knockdown suppresses CCA aggressiveness and enhances chemosensitivity, reinforcing its biological relevance (Kerdkumthong et al., 2024).

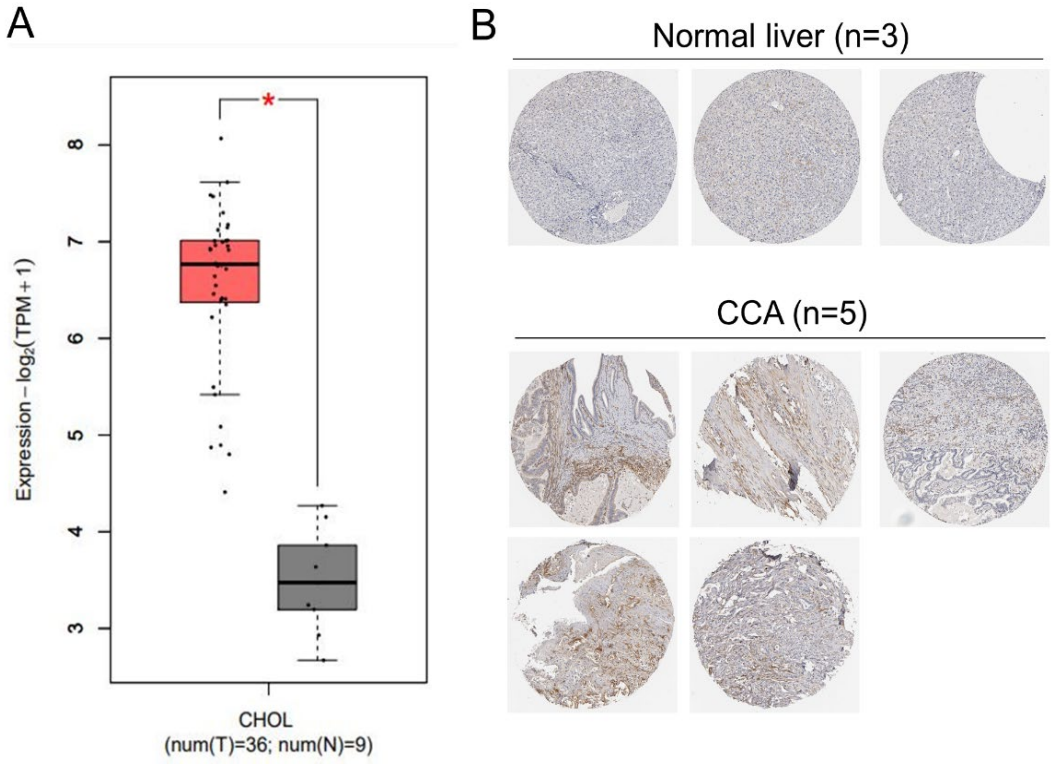


**Figure 1.** LAMB1 expression in CCA patients' tissues. (A) mRNA expression of LAMB1 in CCA patients' tissues, analyzed using TCGA data via the GEPIA2 website. Red box: LAMB1 mRNA expression levels in tumor tissues (n=36); grey box: LAMB1 mRNA expression levels in normal bile duct tissues (n=9). Significant differences between normal versus tumor are indicated by *p<0.05. (B) Representative images of all available normal (n=3) and CCA (n=5) tissues, each with unique patient ID, showing LAMB1 protein expression as determined by immunohistochemistry (IHC) staining and obtained from the Human Protein Atlas (HPA) database.

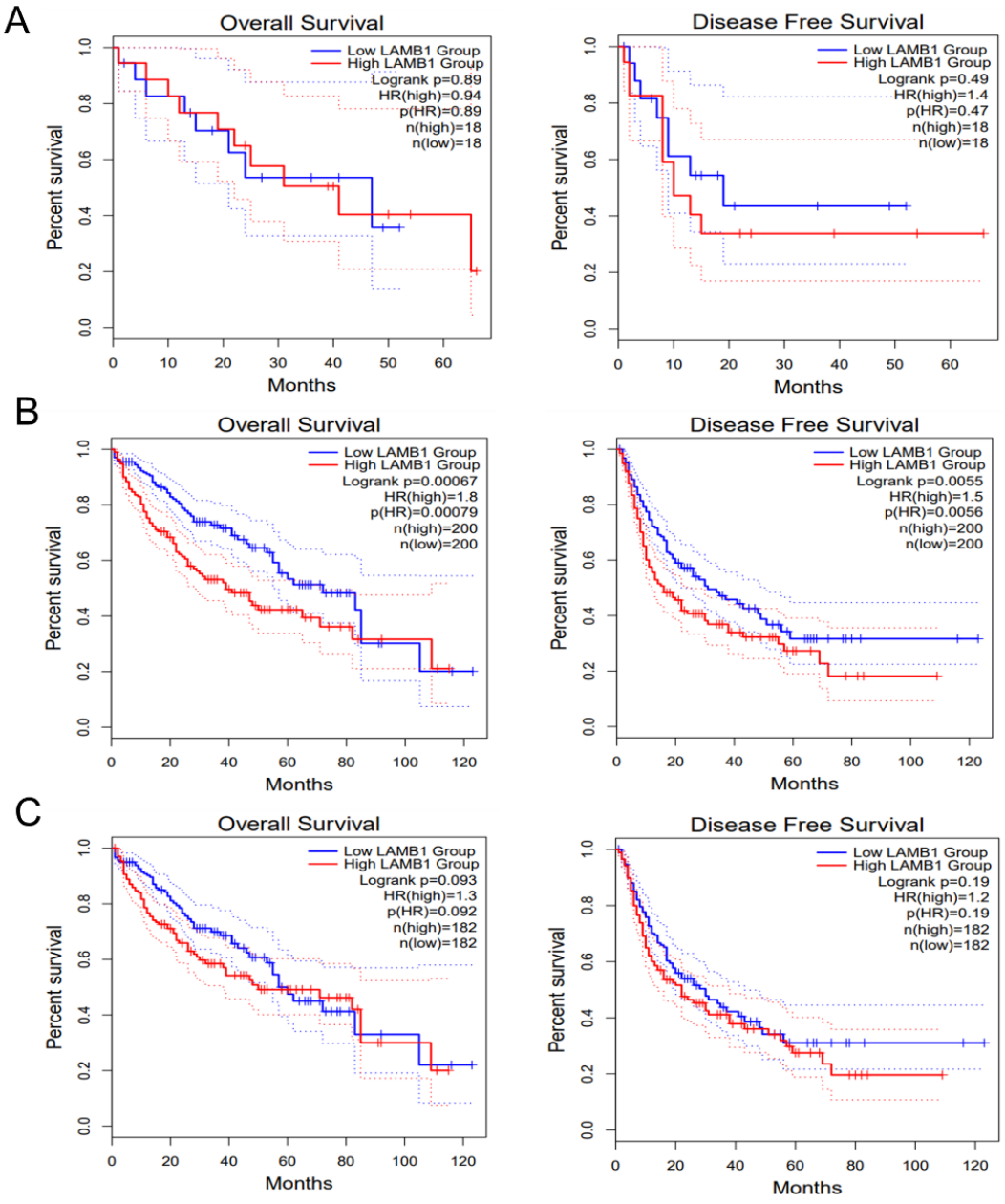


**Figure 2.** Survival analyses of LAMB1 in CCA and hepatocellular carcinoma patients. (A) Overall survival (OS) and disease-free survival (DFS) in CCA patients (CHOL dataset). (B) OS and DFS in combined CCA and hepatocellular carcinoma patients (CHOL and LIHC datasets). (C) OS and DFS in hepatocellular carcinoma patients (LIHC dataset). OS and DFS were visualized using Kaplan–Meier plots. Red line: high expression group; Blue line: low expression group. Log-rank test was used, with a *p* value of less than 0.05 considered statistically significant.

### 3.3 Genes positively correlated with LAMB1 in CCA and their functional network

To identify genes whose expression is positively correlated with LAMB1 in CCA patient tissues, we retrieved the top 250 correlated genes based on TCGA data from the UALCAN website. This gene list, including LAMB1, was then analyzed using the STRING online tool for PPI network analysis. The analysis revealed 52 connected nodes, connected by 155 edges, with an average node degree of 5.96 and a local clustering coefficient of 0.569. The expected number of edges was 81, and the PPI enrichment p value was 1.91e-13 (Figure 3A). Additionally, the expression profiles of these 51 genes in CCA patient samples and normal tissues were visualized using a heatmap generated via UALCAN (Figure 3B), with red indicating high expression and blue representing low expression.

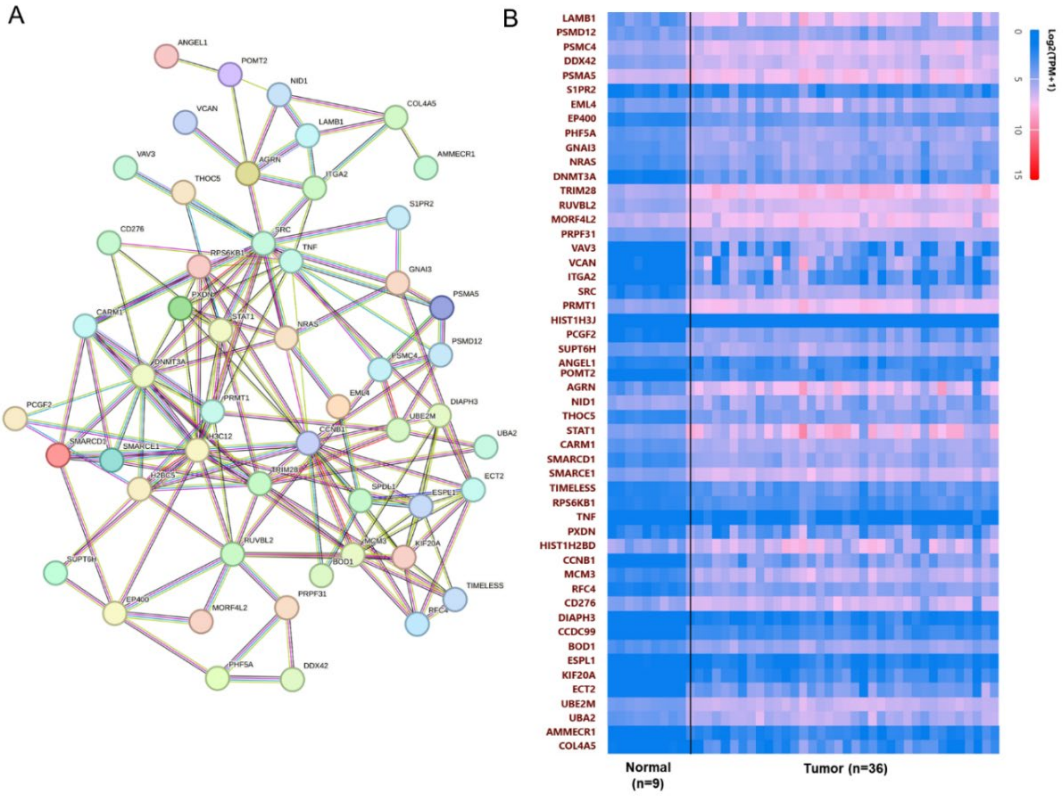


**Figure 3.** Protein-Protein Interaction (PPI) analysis and expression of LAMB1 positively correlated genes. (A) The top 250 LAMB1 positively correlated genes were input into STRING, with unconnected nodes filtered out. The 52 connected nodes (proteins, represented by circles) and 155 edges (predicted or known interactions, represented by lines) are shown. (B) Heatmap analysis using UALCAN shows the mRNA expression of LAMB1 and its 51 associated genes in CCA patients' tissues and their adjacent normal tissues, based on TCGA data.

Central interactors included AGRN, COL4A5, ITGA2, and NID1, frequently upregulated in CCA tissues. The functional roles of these proteins suggest multiple mechanisms by which LAMB1 may promote tumor progression: AGRN and ITGA2 have been directly studied in CCA, with AGRN supporting cell adhesion and migration (Zhong et al., 2018) and ITGA2 mediating collagen I-dependent proliferation and invasion, consistent with the collagen I–ITGA2 axis driving clonogenic growth in iCCA (Rattanasinchai et al., 2022). In contrast, COL4A5 and NID1 have not been directly studied in CCA, but both are known to promote malignancy in other cancers, with COL4A5 stabilizing basement membrane architecture and NID1 crosslinking ECM components (Rokavec et al., 2023; Sun et al., 2023; Xue et al., 2025; Zeng et al., 2020). These findings are consistent with previous integrative gene expression analyses in CCA, which identified upregulation of extracellular matrix–associated genes and highlighted their roles in tumor–microenvironment interactions (Zhong et al., 2018).

Experimental evidence shows that ITGB4 silencing reduces laminin-induced migration in some iCCA cell lines, although receptor plasticity may allow compensation via LAMR (Islam et al., 2021). Our knockdown results further demonstrate that reducing LAMB1 levels suppresses malignant behaviors and enhances chemosensitivity (Kerdkumthong et al., 2024). Collectively, these data support LAMB1 as a central regulator of ECM-associated signaling networks in CCA.

### 3.4 Gene classification and pathway analysis

GO and KEGG pathway analyses of the LAMB1-associated gene network were performed using the built-in features of STRING. For GO analysis, genes were categorized based on their biological processes (BP) (Figure 4A), molecular functions (MF) (Figure 4B), and cellular components (CC) (Figure 4C). In the BP category, 79 GO terms were identified, with significant enrichment in pathways related to the positive regulation of cell proliferation, DNA repair, and chromosome organization (Figure 4A). In the MF category, five GO terms were identified, with predominant enrichment in binding functions, including laminin binding, chromatin binding, carbohydrate binding, and protein binding. Functions associated with extracellular matrix (ECM) structural components were also noted (Figure 4B). For CC, 43 GO terms were identified, with enrichment in protein-DNA binding complexes and the basement membrane (Figure 4C).

The enrichment of LAMB1-associated genes in BP terms such as positive regulation of cell proliferation, DNA repair, and chromosome organization suggests that LAMB1 may support tumor growth, genomic stability, and survival in CCA (Lee et al., 2021; Liu et al., 2022; Feng et al., 2025). MF enrichment in laminin, protein, chromatin, and carbohydrate binding reflects the diverse roles of these genes in ECM interactions, integrin-mediated signaling, and transcriptional regulation (Chakraborty et al., 2017; Islam et al., 2021; Hynes et al., 2022). CC enrichment, including protein–DNA binding complexes and basement membrane, emphasizes LAMB1's structural and signaling function in the ECM, facilitating mechanotransduction, cell migration, and invasion (Sasaki et al., 2004; Hohenester & Yurchenco, 2013).

Furthermore, 52 significant KEGG pathways were identified, highlighting dominant pathways such as the proteasome, cancer-related pathways, and ECM-receptor interactions. Additionally, various signaling and endocrine resistance pathways were observed (Figure 4D).

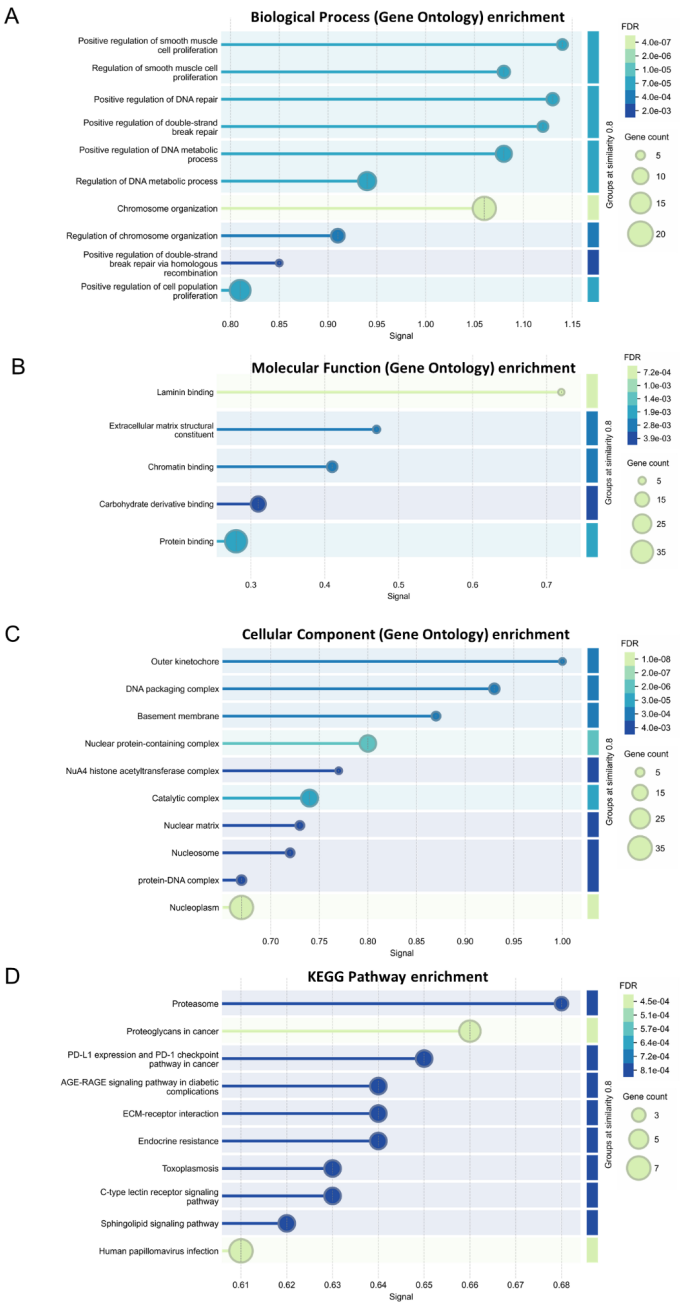


**Figure 4.** Gene Ontology (GO) and KEGG Pathway Enrichment Analysis. (A) GO biological process enrichment. (B) GO molecular function enrichment. (C) GO cellular component enrichment. (D) KEGG pathway enrichment. The shades from mint to blue represent different levels of False Discovery Rate (FDR), and the size of the circle represents the gene count. The enrichment process clusters terms/pathways by similarity (≥ 0.8) and sorts them by the strength of the signal.

The KEGG enrichment analysis suggests that LAMB1-associated networks contribute to tumor proliferation, invasion, metastasis, and therapy resistance. Pathways such as ECM–receptor interactions and cancer-related signaling highlight the role of LAMB1 and its interactors in mediating cellular adhesion, integrin signaling, and mechanotransduction. Proteasome and endocrine resistance pathways indicate potential involvement in protein homeostasis and therapeutic response (Islam et al., 2021; Liu et al., 2022; Feng et al., 2025). Mechanistic studies support these findings: LAMB1–ITGB4 signaling promotes migration and correlates with poor survival (Islam et al., 2021), LAMC2-mediated EGFR translation sustains proliferative signaling (Zhang et al., 2024), and LAMB1 contributes to focal adhesion formation in cHCC-ICC (Xu et al., 2022). Collectively, these observations align with the established role of laminins in basement membrane assembly, where LAMB1 serves as a scaffold coordinating ECM components and integrin-mediated signaling (Sasaki et al., 2004; Hohenester & Yurchenco, 2013). Enrichment in cell proliferation, DNA repair, and chromosome organization underscores LAMB1's role in tumor growth and genomic stability (Pickup et al., 2014; Lee et al., 2021). KEGG enrichment in ECM and endocrine resistance pathways further supports its contribution to invasion, metastasis, and chemoresistance (Feng et al., 2025; Zhao et al., 2025).

## 3.5 Expression and prognostic value of LAMB1 closely associated genes

From the STRING analysis, we narrowed our focus to the first-degree interacting genes of LAMB1, which included AGRN, COL4A5, ITGA2, and NID1 (Figure 5A). Based on GO classification, these genes, along with LAMB1, primarily function as basement membrane components and are involved in laminin binding. KEGG pathway analysis further highlighted their significant role in ECM–receptor interactions. Gene expression analysis in CCA patients using GEPIA2 revealed that AGRN, COL4A5, and ITGA2 were upregulated in CCA tissues compared to normal tissues (Figure 5B-D). However, NID1 expression did not show a significant difference between the two sample types (Figure 5E).

Co-overexpression of AGRN and COL4A5 with LAMB1 suggests a coordinated ECM remodeling program. AGRN enhances integrin- and growth factor-mediated signaling via YAP/TAZ and Lrp4/MuSK receptor pathways, converting ECM rigidity into oncogenic transcriptional programs (Chakraborty et al., 2017; Chakraborty & Hong, 2018; Mokhtari et al., 2025). COL4A5 maintains BM integrity, and LAMB1 mediates adhesion and migration through ITGB4 and LAMR, correlating with lymphatic invasion and poor prognosis (Islam et al., 2021; Xu et al., 2022). RNA-binding proteins, such as DDX24 may further modulate LAMB1 and its associated genes, reinforcing ECM-driven oncogenic signaling, as observed in HCC, where DDX24 binds LAMB1 mRNA to enhance stability and promote proliferation and migration (Liu et al., 2022; Ma et al., 2024).

Kaplan–Meier survival analysis using the combined CHOL and LIHC datasets demonstrated that high AGRN and ITGA2 expression was associated with shorter OS and DFS in patients (Figures 6A, 6C). Notably, NID1 overexpression correlated with shorter OS but not with DFS (Figure 6D). In contrast, COL4A5 expression showed no significant correlation with either OS or DFS (Figure 6B).

These distinctions reflect the functional specialization of LAMB1-associated genes: ITGA2 promotes invasion via collagen adhesion and mechanosignaling (Oh et al., 2014; Huang et al., 2020), AGRN amplifies oncogenic signaling, NID1 maintains structural BM support, and COL4A5 provides biomechanical ECM stability. Together, LAMB1 acts as a hub, coordinating ECM remodeling and pro-survival signaling. Functional studies confirm that LAMB1 knockdown disrupts invasion, migration, and chemoresistance,

emphasizing the central role of LAMB1 and its first-degree interactors in promoting CCA malignancy (Kerdkumthong et al., 2024).

### 3.6 Gene correlation analysis

We conducted a gene correlation analysis between LAMB1 and four associated genes in CCA and hepatocellular carcinoma patients. The scatter plots revealed that all tested gene pairs—LAMB1 and AGRN, LAMB1 and COL4A5, LAMB1 and ITGA2, and LAMB1 and NID1—exhibited a significant positive correlation in the CHOL dataset, the LIHC dataset, and the combined CHOL+LIHC datasets (Figure 7). In the combined dataset, R values were slightly higher compared with the individual datasets, except for LAMB-NID1 correlation.

Pearson correlation analyses indicate strong positive associations between LAMB1 and ITGA2, NID1, AGRN, and COL4A5, reflecting a tightly co-regulated ECM network. LAMB1–ITGA2 interactions facilitate integrin-mediated signaling, LAMB1–NID1 interactions support basement membrane assembly, LAMB1–AGRN co-expression enhances mechanotransduction, and LAMB1–COL4A5 maintains ECM structural integrity (Sasaki et al., 2004; Hohenester & Yurchenco, 2013; Chakraborty et al., 2017; Jayadev et al., 2022). Overall, these results establish LAMB1 as both a structural and signaling hub within a coordinated ECM network, converting extracellular composition into intracellular signals to drive invasion, metastasis, and therapy resistance.

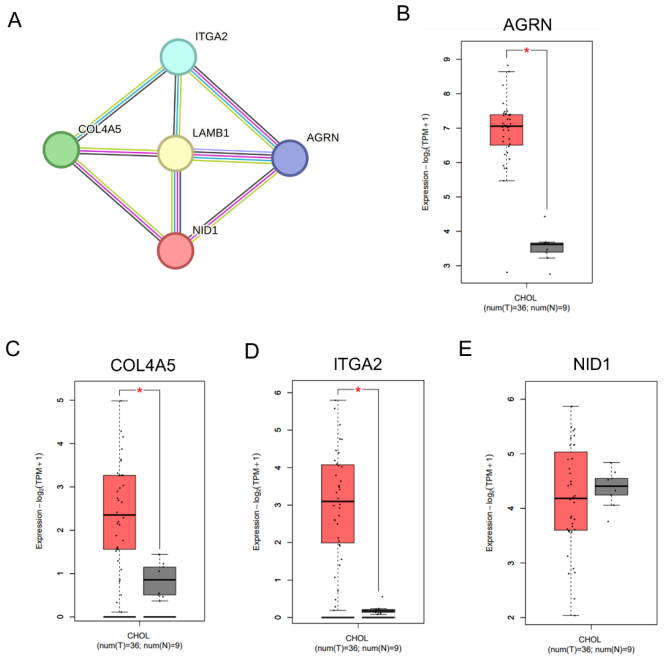


**Figure 5.** Expression of LAMB1-associated genes. (A) STRING network of LAMB1 and four closely associated genes. (B) Box plot showing the mRNA expression of AGRN, COL4A5, ITGA2, and NID1 in CCA patients' tissues versus normal tissues, based on TCGA data. Red box: mRNA expression levels in tumor tissues (n=36); grey box: mRNA expression levels in normal bile duct tissues (n=9).

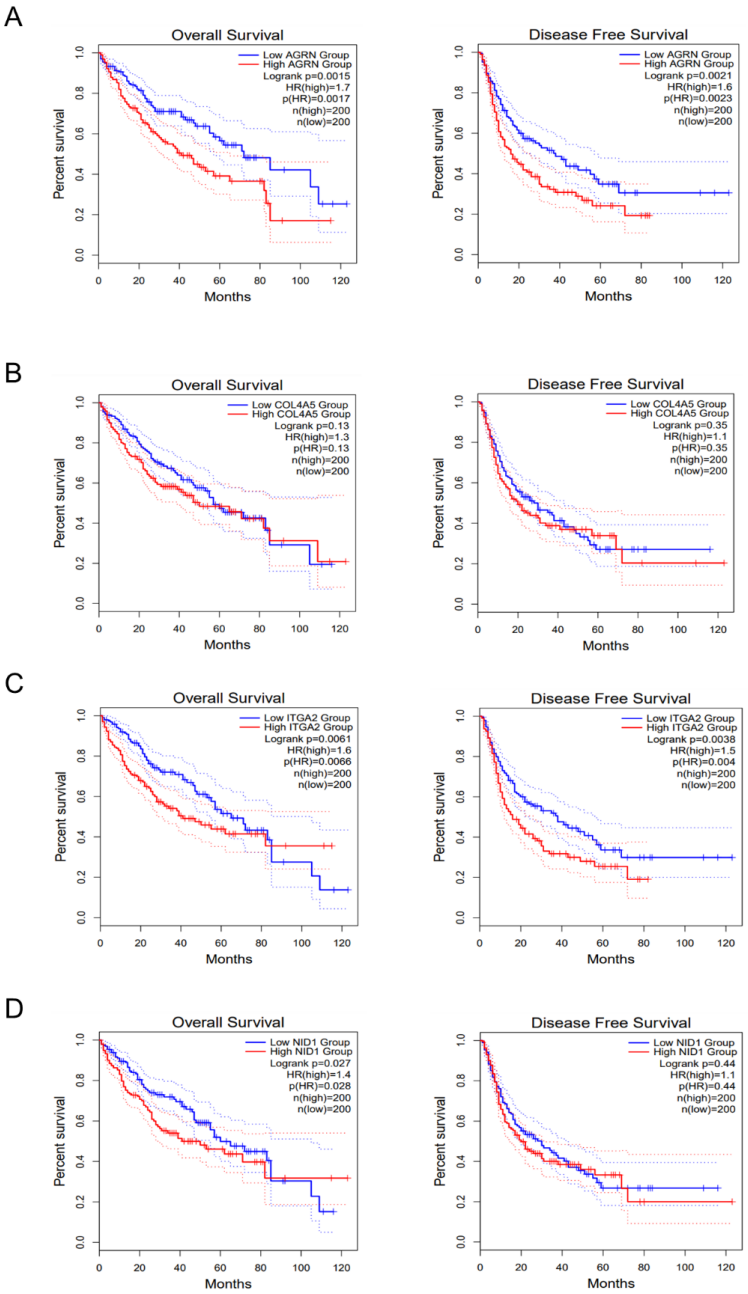


**Figure 6.** Survival analyses of four LAMB1-associated genes in CCA and hepatocellular carcinoma patients. Survival analyses were performed for (A) AGRN, (B) COL4A5, (C) ITGA2, and (D) NID1. Overall survival (OS) and disease-free survival (DFS) data from combined CHOL and LIHC patient cohorts were visualized using Kaplan-Meier plots. Red line: high expression group; Blue line: low expression group. Log-rank tests were performed, with a p-value of less than 0.05 considered significant.

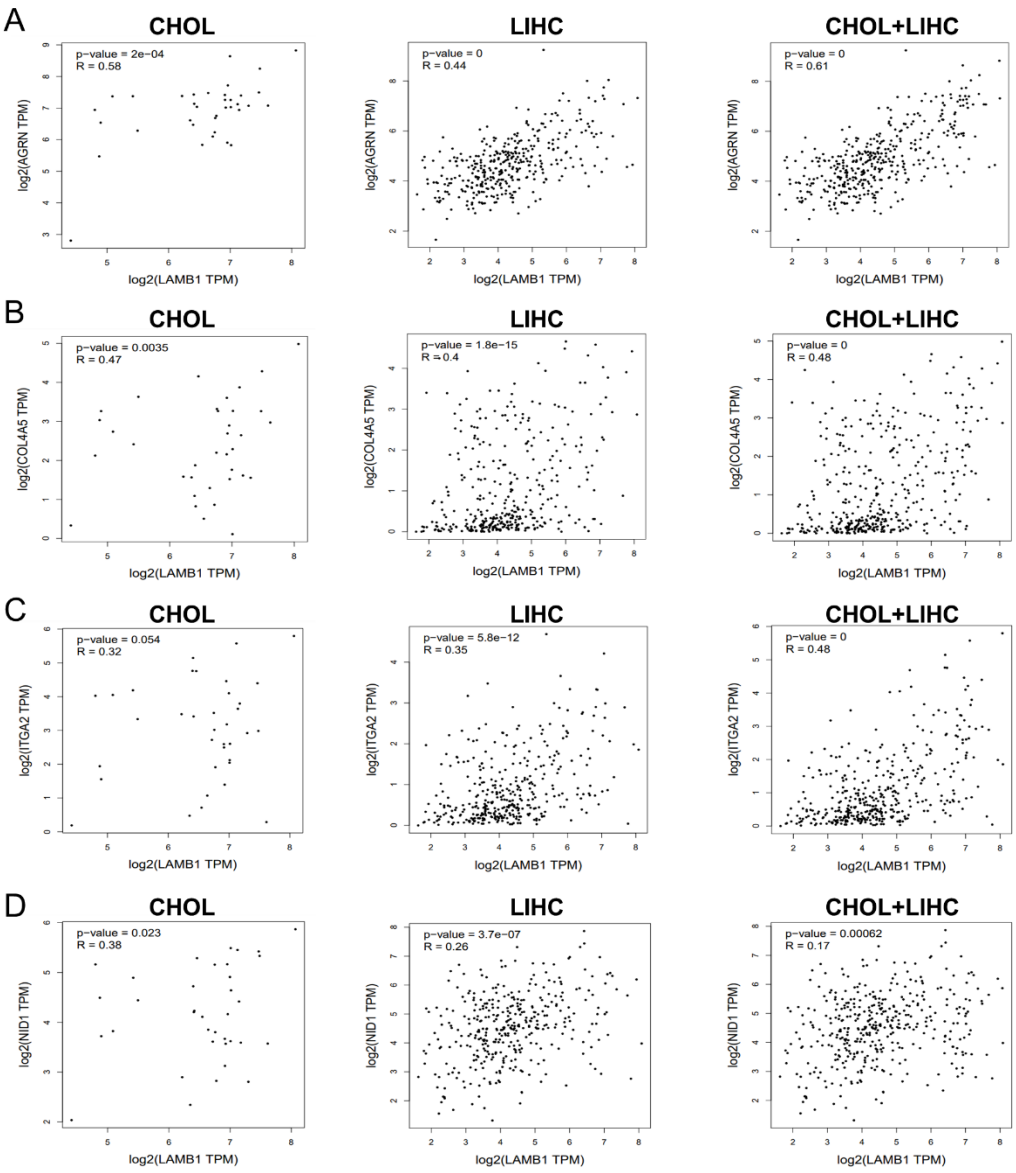


**Figure 7.** Correlation analysis of four LAMB1-associated genes in the CHOL, LIHC, and combined CHOL+LIHC datasets. Scatter plots showing the expression correlation between (A) LAMB1 and AGRN, (B) LAMB1 and COL4A5, (C) LAMB1 and ITGA2, and (D) LAMB1 and NID1. The left panel displays plots generated from the CHOL dataset, the middle panel from the LIHC dataset, and the right panel from the combined CHOL+LIHC datasets. Correlation was assessed using Pearson's correlation coefficient (R), with a p-value <0.05 considered statistically significant.

## 4. Conclusions

This study demonstrates that LAMB1 is significantly upregulated in CCA and is associated with poor patient prognosis, particularly when combined with hepatocellular carcinoma dataset. Bioinformatics analyses identified LAMB1-interacting genes involved in extracellular matrix organization, cell proliferation, and DNA repair, supporting its role in tumor progression. Notably, high expression of AGRN and ITGA2 was also linked to unfavorable outcomes. These findings, along with previous evidence of LAMB1's role in drug resistance, underscore its potential as a prognostic biomarker and therapeutic target in CCA. Further studies are warranted to validate these observations and investigate the mechanistic basis of LAMB1-mediated malignancy.

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## 6. Authors' Contributions

Sumalee Obchoei, Dissathut Yongpraderm conceptualized and designed research; Phonprapavee Tantimetta, Nicharas Chaimeteveth, Karnsinee Nounsuwan, Chatkanok Urairat analyzed data; Sumalee Obchoei, Phonprapavee Tantimetta, Thanakrit Rattanaarchanai illustrated the figures: Sumalee Obchoei, Phonprapavee Tantimetta, Nicharas Chaimeteveth, Thanakrit Rattanaarchanai wrote the paper; All authors reviewed the final manuscript.

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## 7. Conflicts of Interest

All authors declared that there are no conflicts of interest.

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