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Original Article

Comprehensive pan-cancer analysis of LAMA3: implications for prognosis and immunotherapy

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Abstract: Objectives: Laminin subunit alpha 3 (LAMA3) has been implicated in various cellular processes relevant to cancer progression, including cell proliferation, migration, and adhesion. In this study, we explored the expression, prognostic significance, and functional role of LAMA3 across multiple cancer types. Methodology: The in silico analyses involve using various bioinformatics tools and databases, such as The Cancer Genome Atlas (TCGA), TIMER2.0, GEPIA2, UALCAN, Kaplan-Meier (KM) plotter, GENT2, Human Protein Atlas (HPA), OncoDB, Gene Set Cancer Analysis (GSCA), and TISIDB. The in vitro analyses include cell culture, gene knockdown, and assays for cell proliferation, colony formation, and wound healing. Results: Pan-cancer analysis revealed significant variations in LAMA3 expression, with upregulation observed in cancers such as pancreatic adenocarcinoma (PAAD) and stomach adenocarcinoma (STAD), and downregulation in breast cancer (BRCA) and colon adenocarcinoma (COAD). Prognostic analyses indicated high LAMA3 expression correlated with poor overall survival (OS) in PAAD and STAD, whereas low expression was associated with adverse outcomes in BRCA. Validation analysis confirmed differential expression and localized LAMA3 primarily to the endoplasmic reticulum. Analysis of clinical features in BRCA, PAAD, and STAD showed consistent expression trends across different stages, races, and age groups. Additionally, mutational and copy number variations (CNVs) analyses revealed prevalent heterozygous amplifications and deletions in LAMA3 across BRCA, PAAD, and STAD. Promoter methylation was inversely correlated with LAMA3 expression in BRCA, PAAD, and STAD, although survival outcomes were unaffected. Protein-protein interaction (PPI) and gene enrichment analyses indicated LAMA3's involvement in ECM-receptor interactions and PI3K-Akt signaling, pathways critical in cancer. Finally, functional assays following LAMA3 knockdown in HT-29 cells demonstrated reduced cell proliferation, colony formation, and wound healing, implicating LAMA3 in tumor growth and metastasis. Conclusion: Overall, these findings suggest that LAMA3 plays a multifaceted role in tumorigenesis and holds potential as a prognostic biomarker and therapeutic target in multiple cancers.

Keywords: Cancer, LAMA3, pan-cancer analysis, diagnosis, treatment

Introduction

Cancer remains a major global health challenge, contributing significantly to mortality and morbidity despite notable progress in early detection, advanced treatment methods, and research breakthroughs [1-3]. It is a highly heterogeneous disease characterized by aberrant cell proliferation, invasion into surrounding tissues, and eventual metastasis to distant organs

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[4-6]. With millions of new cases diagnosed annually and an ever-growing impact on public health, the need for novel therapeutic targets and predictive biomarkers is more urgent than ever. Efforts to better understand the molecular drivers of cancer progression have revealed key components of the tumor microenvironment, particularly those involved in cell adhesion and signaling, as essential factors contributing to tumor growth and metastasis [7-9].

Laminins, a family of glycoproteins integral to the structure and function of the basement membrane, have emerged as critical players in cancer biology [10, 11]. These proteins are involved in maintaining tissue architecture, mediating cell adhesion, and regulating migration, proliferation, and differentiation. Laminin subunit alpha 3 (LAMA3), a component of the laminin-332 complex, has garnered considerable interest in modulating cell behavior within the tumor microenvironment [12, 13]. Alterations in LAMA3 expression have been associated with increased tumor aggressiveness, metastatic potential, and poor clinical outcomes across several cancer types, underscoring its significance in oncogenesis [14, 15].

Previous studies have identified LAMA3 as a pivotal factor in various malignancies. In lung adenocarcinoma, elevated LAMA3 expression has been linked to enhanced metastatic capabilities and poor survival rates [16]. Similarly, in breast cancer, LAMA3 overexpression correlates with higher tumor grade, increased invasion, and worse prognosis [17]. In colorectal cancer, LAMA3 has been implicated in promoting tumor cell migration and invasion, contributing to disease progression [18]. Furthermore, studies in other cancer types, such as ovarian, esophageal, and head and neck cancers, have also highlighted LAMA3's role in fostering a more invasive tumor phenotype [19-21]. These findings suggest that LAMA3 may play a universal role in facilitating cancer progression, irrespective of tissue origin.

Despite its established importance in individual cancer types, a systematic pan-cancer analysis of LAMA3 across diverse cancers has not yet been conducted. Investigating LAMA3's expression, mutation profiles, and its correlation with clinical outcomes across multiple cancers could provide a more comprehensive understanding of its role in cancer biology. This study aims to address this gap by performing a detailed pan-cancer analysis of LAMA3, utilizing extensive publicly available datasets such as The Cancer Genome Atlas (TCGA) [22, 23] to explore its potential as a universal biomarker and therapeutic target. By examining its expression patterns, mutation landscape, and association with patient survival across different tumor types, this research seeks to determine whether LAMA3 could serve as a predictive biomarker or therapeutic target for improving cancer treatment outcomes across a broad spectrum of malignancies.

Methodologies

Gene expression analysis of LAMA3 in pan-cancer

The mRNA expression of LAMA3 in normal tissues and cancer tissues from TCGA was obtained using the 'Gene_DE' module of the TIMER2.0 database (http://timer.comp-genomics.org/timer/) [24]. Additionally, transcriptomic data for LAMA3 across pan-cancer cohorts were downloaded from the UCSC database (https://xenabrowser.net/) [25], which includes data from TCGA (http://cancergenome.nih.gov) and the Genotype-Tissue Expression (GTEx; https://gtexportal.org/home/) databases. Tumor abbreviations are listed in **Table 1**.

Prognostic significance of LAMA3 in pan-cancer

KM Plotter and GENT2 are valuable bioinformatics tools for analyzing gene expression and survival data across various cancers. KM Plotter (http://kmplot.com) enables researchers to assess the prognostic significance of genes by providing Kaplan-Meier survival curves for specific cohorts [26, 27]. GENT2 (http://gent2.appex.kr/) facilitates the exploration of gene expression across normal and tumor tissues [28]. In this study, both KM plotter and GENT2 databases were used to evaluate the prognostic significance of LAMA3 in pan-cancer.

Expression validation and subcellular localization analysis

GEPIA2 and the Human Protein Atlas (HPA) are essential bioinformatics resources for exploring gene expression in normal and cancer tissues. GEPIA2 (http://gepia2.cancer-pku.cn/) provides user-friendly access to RNA sequenc-

Table 1. Full names and abbreviations of cancers

Cancer Type	Full Name
ACC	Adrenocortical carcinoma
BLCA	Bladder Urothelial Carcinoma
BRCA	Breast cancer
CESC	Cervical squamous cell carcinoma and endocervical adenocarcinoma
CHOL	Cholangiocarcinoma
COAD	Colon adenocarcinoma
COADREAD	Colon adenocarcinoma/Rectum adenocarcinoma Esophageal carcinoma
DLBC	Lymphoid Neoplasm Diffuse Large B-cell Lymphoma
ESCA	Esophageal carcinoma
GBM	Glioblastoma multiforme
GBMLGG	Glioma
HNSC	Head and Neck squamous cell carcinoma
KICH	Kidney Chromophobe
KIPAN	Pan-kidney cohort (KICH+KIRC+KIRP)
KIRC	Kidney renal clear cell carcinoma
KIRP	Kidney renal papillary cell carcinoma
LAML	Acute Myeloid Leukemia
LGG	Brain Lower Grade Glioma
LIHC	Liver hepatocellular carcinoma
LUAD	Lung adenocarcinoma
LUSC	Lung squamous cell carcinoma
MESO	Mesothelioma
OV	Ovarian serous cystadenocarcinoma
PAAD	Pancreatic adenocarcinoma
PCPG	Pheochromocytoma and Paraganglioma
PRAD	Prostate adenocarcinoma
READ	Rectum adenocarcinoma
SARC	Sarcoma
STAD	Stomach adenocarcinoma
SKCM	Skin Cutaneous Melanoma
STES	Stomach and Esophageal carcinoma
TGCT	Testicular Germ Cell Tumors
THCA	Thyroid carcinoma
THYM	Thymoma
UCEC	Uterine Corpus Endometrial Carcinoma
UCS	Uterine Carcinosarcoma
UVM	Uveal Melanoma

ing (RNA-seq) data from TCGA and GTEx projects [29, 30]. The HPA (https://www.proteinatlas.org/) offers comprehensive protein expression data across tissues and cell types, including immunohistochemistry-based expression data [31]. In this work, GEPIA2 and HPA were used to validate LAMA3 expression in the extended cohorts of cancer patients. Moreover, HPA was also used to analyze the subcellular localization of the LAMA3 protein.

Correlation analysis of LAMA3 expression with clinical features of cancer patients

UALCAN (http://ualcan.path.uab.edu) is an interactive web resource that provides easy access to cancer omics data from TCGA [32]. This resource enables analysis of gene expression, survival data, promoter methylation, and protein expression across cancers, aiding biomarker discovery and tumor biology insights.

This study used UALCAN to explore LAMA3 expressional correlation with clinical features in cancer patients.

Mutational and copy number variations (CNVs) analysis of LAMA3

OncoDB and Gene Set Cancer Analysis (GSCA) are robust databases designed for cancer research. OncoDB (https://oncodb.org) provides integrated multi-omics data, including gene expression, mutation analysis, and survival outcomes across diverse cancer types [33]. GSCA (http://bioinfo.life.hust.edu.cn/GSCA) focuses on gene set analysis, offering insights into pathway-level alterations and their clinical implications [34]. In this work, OncoDB was used to perform mutational analysis while GSCA was utilized to conduct CNVs analysis of LAMA3 across distinct cancers.

Promoter methylation analysis of LAMA3

In this study, the promoter methylation analysis of LAMA3 was conducted using the UALCAN [32] and GSCA [34] databases.

Protein-protein interaction (PPI) network and enrichment analysis of LAMA3

GeneMANIA [35] (http://genemania.org/) and STRING [36] (https://string-db.org/) databases were used for exploring gene interactions and functions and identifying co-expressed genes. The combined outcomes were summarized in a Venn diagram. Moreover, DAVID (https://david.ncifcrf.gov/) tool [37] was utilized to perform gene enrichment analysis of LAMA3-interacting partners.

Immune infiltration and drug sensitivity analysis of LAMA3

The GSCA database [34] offers gene set analysis for cancer research, including insights into pathways, drug sensitivity, and immune infiltration. In this study, the GSCA database was utilized to perform immune infiltration and drug sensitivity analysis of LAMA3.

Correlation analysis of LAMA3 with immune subtypes and immune-related genes

TISIDB (http://cis.hku.hk/TISIDB) is a comprehensive database for studying tumor-immune interactions [38]. This study utilized the TISIDB database to explore correlations of LAMA3 with immune subtypes and immune-related genes.

LAMA3 knockdown in HT-29 cells

HT-29 cells, a human colorectal adenocarcinoma cell line obtained from ATCC (Catalog Number: HTB-38), USA, were selected due to their relevance in studying epithelial tumor biology and extracellular matrix components, including laminins like LAMA3. LAMA3 was targeted for knockdown using specific siRNA (Catalog Number: AM16708) from Thermo Fisher Company. The transfection was performed using Lipofectamine RNAiMAX (Catalog Number: 13778150) according to the manufacturer's instructions. After 48 hours, RT-qPCR was conducted to assess the knockdown efficiency, utilizing TaqMan Gene Expression Assays (Catalog Number: 4448892) for LAMA3 and GAPDH as the internal control.

Cell proliferation, colony formation, and wound healing assays

Cell proliferation was evaluated using the CellTiter 96® AQueous One Solution Assay (G3580) by seeding HT-29 cells in a 96-well plate, followed by treatment and absorbance measurement at 490 nm after 1-4 hours. Colony formation was assessed by seeding cells at a low density in 6-well plates, growing for 7-14 days, fixing with 4% formaldehyde, and staining with crystal violet (C581, Thermo Fisher); colonies were quantified using ImageJ software. Wound healing was analyzed using the IncuCyte™ System (9600) by creating standardized wounds in 12-well plates, treating cells, imaging over time, and measuring wound closure with ImageJ software.

Statistics

All gene expression data were normalized using Log2 transformation. The Wilcoxon test assessed statistical significance in gene expression analysis, while the Student's t-test examined the correlation between LAMA3 expression and clinicopathological data. Spearman's test was employed to explore the relationship between the two variables. A p-value of < 0.05 was considered statistically significant. Data analysis was performed using Origin 2021 or R Studio.

Results

Gene expression analysis of LAMA3 in pan-cancer

The TIMER2.0 database was utilized to examine LAMA3 expression across pan-cancer and normal tissues. The analysis revealed that LAMA3 expression differed significantly in 15 types of tumors. Among these, LAMA3 was upregulated in 10 cancers - including CHOL, ESCA, HNSC, KIRC, KIRP, LIHC, PAAD, PCPG, SKCM, and STAD-when compared to normal tissues. Conversely, it was downregulated in 5 cancers, specifically BRCA, COAD, GBM, LUAD, and PRAD (**Figure 1A**). Since some tumors in TIMER2.0 lacked normal tissue data, we integrated TCGA with GTEx data to investigate LAMA3 expression across 34 tumor types, finding it upregulated in 19 tumors and downregulated in 11 relative to their corresponding normal tissues (**Figure 1B**).

Prognostic significances of LAMA3 in pan-cancer

Next, the KM plotter tool was used to analyze the prognostic significance of LAMA3 in pan-cancer. The results show that low LAMA3 expression correlated with poor OS in BRCA patients (**Figure 2**). In contrast, high LAMA3 expression was associated with worse survival in both PAAD and STAD (**Figure 2**). To further verify these results, we then employed the GENT2 database. The forest plots in Supplementary Figure 1A-C present a meta-analysis of hazard ratios across different datasets, consolidating evidence for LAMA3's influence on survival outcomes. For BRCA, PAAD, and STAD, the pooled data in forest plots from multiple studies further support the findings of **Figure 2**, with HRs consistently greater than 1.0, indicating a trend toward poor prognosis with high LAMA3 expression in these cancers (Supplementary Figure 1A-C). These results collectively suggest that LAMA3 expression has adverse associations with OS in BRCA, PAAD, and STAD. Since the dysregulation of LAMA3 was significantly associated with poor OS in BRCA, PAAD, and STAD, while no similar associations were observed in other cancers, the next part of our study focused primarily on these three cancers. This allowed us to further explore the mechanistic and clinical implications of LAMA3 dysregulation, with the goal of uncovering its role in tumor progression and its potential therapeutic significance in these specific cancer types.

Gene expression validation analysis and exploring subcellular localization of LAMA3 protein

To validate LAMA3 expression in additional cohorts, we used the GEPIA2 and HPA databases. In **Figure 3A**, analysis of LAMA3 mRNA expression with the GEPIA2 tool showed significantly lower expression in BRCA samples compared to controls, while expression was higher in PAAD and STAD samples. **Figure 3B** presents LAMA3 protein expression in these cancers using immunohistochemistry images from the HPA database. The HPA analysis revealed lower staining in BRCA tissue compared to controls, but higher staining in PAAD and STAD tissues relative to controls, consistent with the mRNA expression findings (**Figure 3B**). Furthermore, **Figure 3C** illustrates the subcellular localization of the LAMA3 protein, as observed in U-251 MG and U2OS human cell lines, also based on data from the HPA. LAMA3 is primarily localized in the endoplasmic reticulum, with distinct fluorescent staining patterns confirming its presence in this cellular compartment (**Figure 3C**). Together, these findings confirmed the intracellular distribution of LAMA3 protein within the endoplasmic reticulum, suggesting its involvement in tumor biology across these cancers.

Relationship between LAMA3 and clinical features

Relationship between LAMA3 and clinical features, including cancer stage, race, gender, and age, for BRCA, PAAD, and STAD were explored using the UALCAN database. In BRCA, LAMA3 expression was significantly lower in all cancer stages compared to normal tissue (**Figure 4A**). Similarly, LAMA3 expression was lower in BRCA patients across racial groups and in both males and females when compared to normal controls (**Figure 4A**). In terms of age, the expression remains consistently lower across all age groups (**Figure 4A**). In PAAD, LAMA3 expression showed significantly higher expression levels across all cancer stages relative to normal tissues (**Figure 4B**). Racial analysis indicated higher LAMA3 expression in all racial groups, with slight variations. Male and female PAAD patients exhibited higher LAMA3 expression

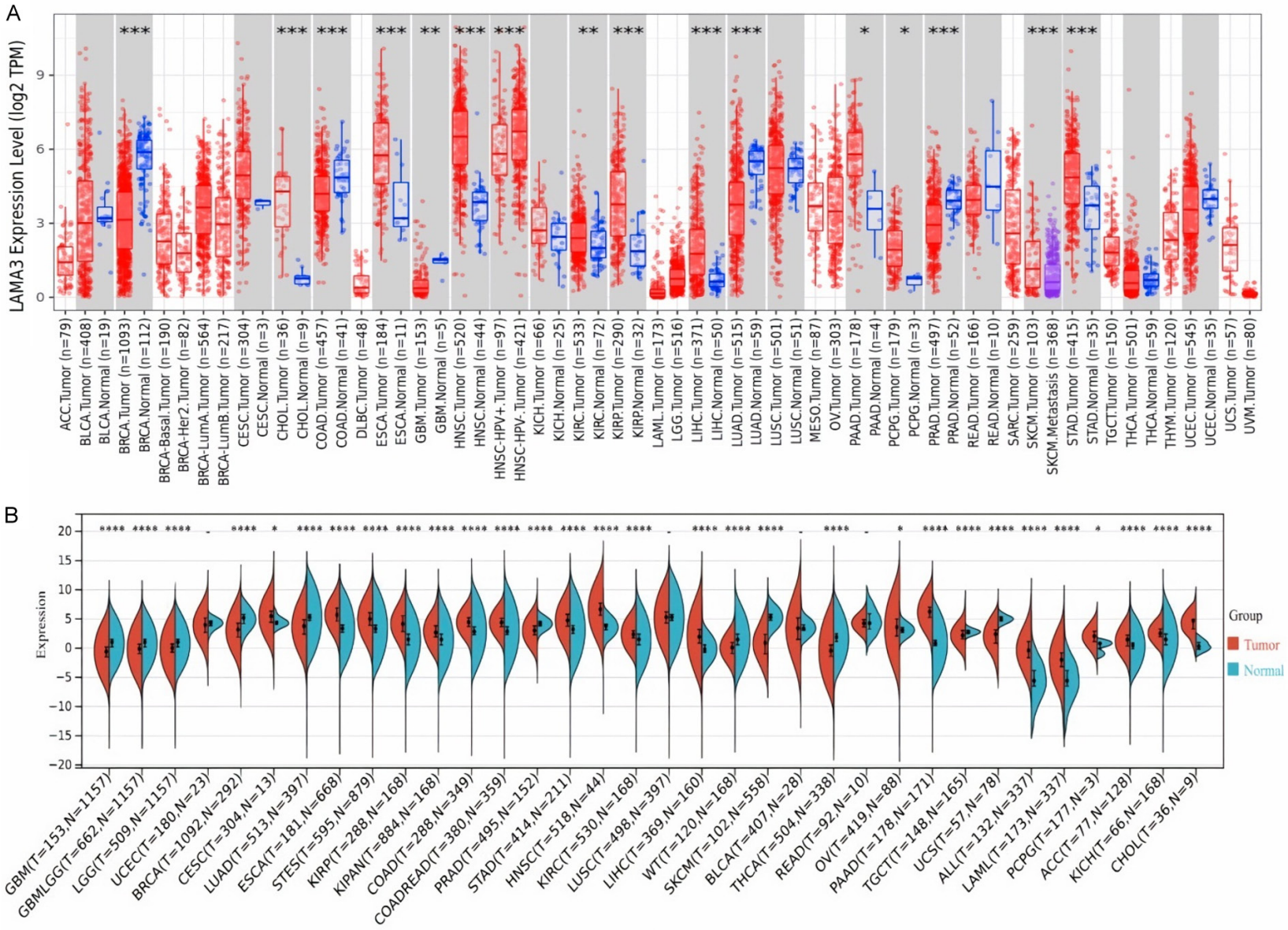


Figure 1. Differential expression of laminin subunit alpha 3 (LAMA3) in pan-cancer. A. LAMA3 expression levels (log2 TPM) in pan-cancer and matched normal tissues as analyzed using the TIMER2.0 database. B. Violin plot of LAMA3 expression across 34 tumor types from The Cancer Genome Atlas (TCGA) combined with Genotype-Tissue Expression (GTEx) datasets. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

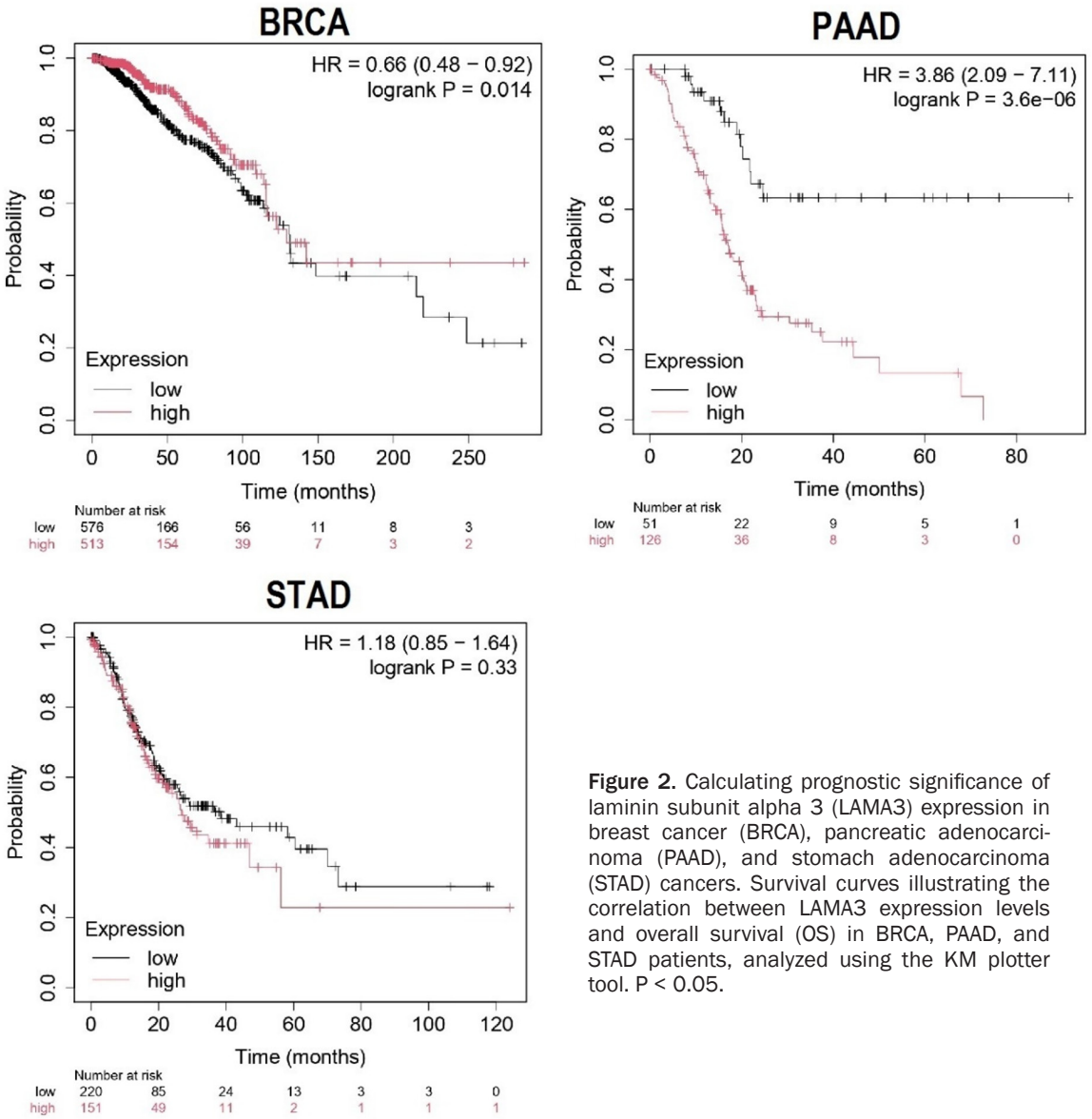


Figure 2. Calculating prognostic significance of laminin subunit alpha 3 (LAMA3) expression in breast cancer (BRCA), pancreatic adenocarcinoma (PAAD), and stomach adenocarcinoma (STAD) cancers. Survival curves illustrating the correlation between LAMA3 expression levels and overall survival (OS) in BRCA, PAAD, and STAD patients, analyzed using the KM plotter tool. $P < 0.05$.

compared to normal samples (**Figure 4B**). When comparing age groups, LAMA3 expression remained consistently overexpressed in PAAD across all age categories (**Figure 4B**). For STAD, LAMA3 expression was significantly upregulated across all cancer stages compared to normal samples (**Figure 4C**). In terms of racial distribution, higher expression levels were observed in all racial groups relative to normal tissues (**Figure 4C**). Both male and female STAD patients exhibited higher LAMA3 expression as compare to controls (**Figure 4C**). Additionally, expression remains higher across all age groups, with minor fluctuations but no age-dependent trends (**Figure 4C**).

Mutational and CNVs analysis of LAMA3

Mutational and CNV analyses of LAMA3 in BRCA, PAAD, and STAD were performed using the OncoDB and GSCA databases. **Figure 5A** illustrates the mutation landscape of LAMA3 in these cancers, revealing various mutation types, including missense mutations, nonsense mutations, frame-shift deletions, and frame-shift insertions (**Figure 5A**). The most prominent mutations identified in LAMA3 were D504N in BRCA, R507W in PAAD, and E1838K in STAD (**Figure 5A**). In **Figure 5B**, the CNV analysis of LAMA3 via the GSCA database is illustrated through pie charts for BRCA, PAAD, and

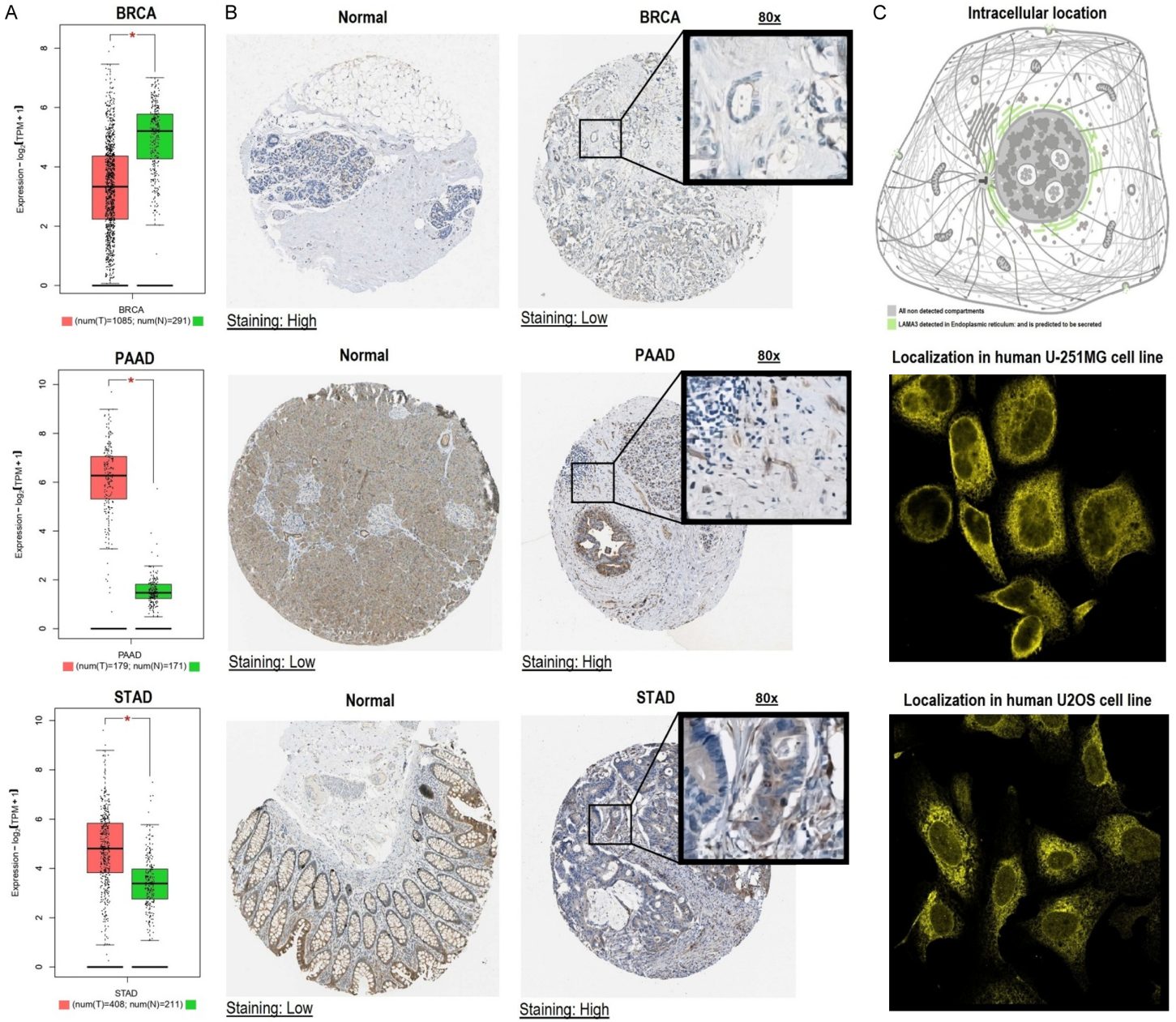

A
BRCA
Expression = log2[TPM+1]
BRCA
(num(T)=1085; num(N)=291)
PAAD
Expression = log2[TPM+1]
PAAD
(num(T)=179; num(N)=171)
STAD
Expression = log2[TPM+1]
STAD
(num(T)=408; num(N)=211)
B
Normal
Staining: High
BRCA
80x
Staining: Low
Normal
Staining: Low
PAAD
80x
Staining: High
Normal
Staining: Low
STAD
80x
Staining: High
C
Intracellular location
All non detected compartments
LAMA3 detected in Endoplasmic reticulum; and is predicted to be secreted
Localization in human U-251MG cell line
Localization in human U2OS cell line

Figure 3. Analysis of laminin subunit alpha 3 (LAMA3) expression levels and subcellular localization in breast cancer (BRCA), pancreatic adenocarcinoma (PAAD), and stomach adenocarcinoma (STAD). A. Box plots displaying LAMA3 mRNA expression in BRCA, PAAD, and STAD tissues compared to normal controls, using data from the GEPIA2 tool. B. Immunohistochemistry (IHC) images from the Human Protein Atlas (HPA) database depicting LAMA3 protein expression in normal and cancerous tissues for BRCA, PAAD, and STAD. C. Subcellular localization of LAMA3 protein in human U-251 MG and U2OS cell lines, based on fluorescent staining data from the HPA database. *P < 0.05.

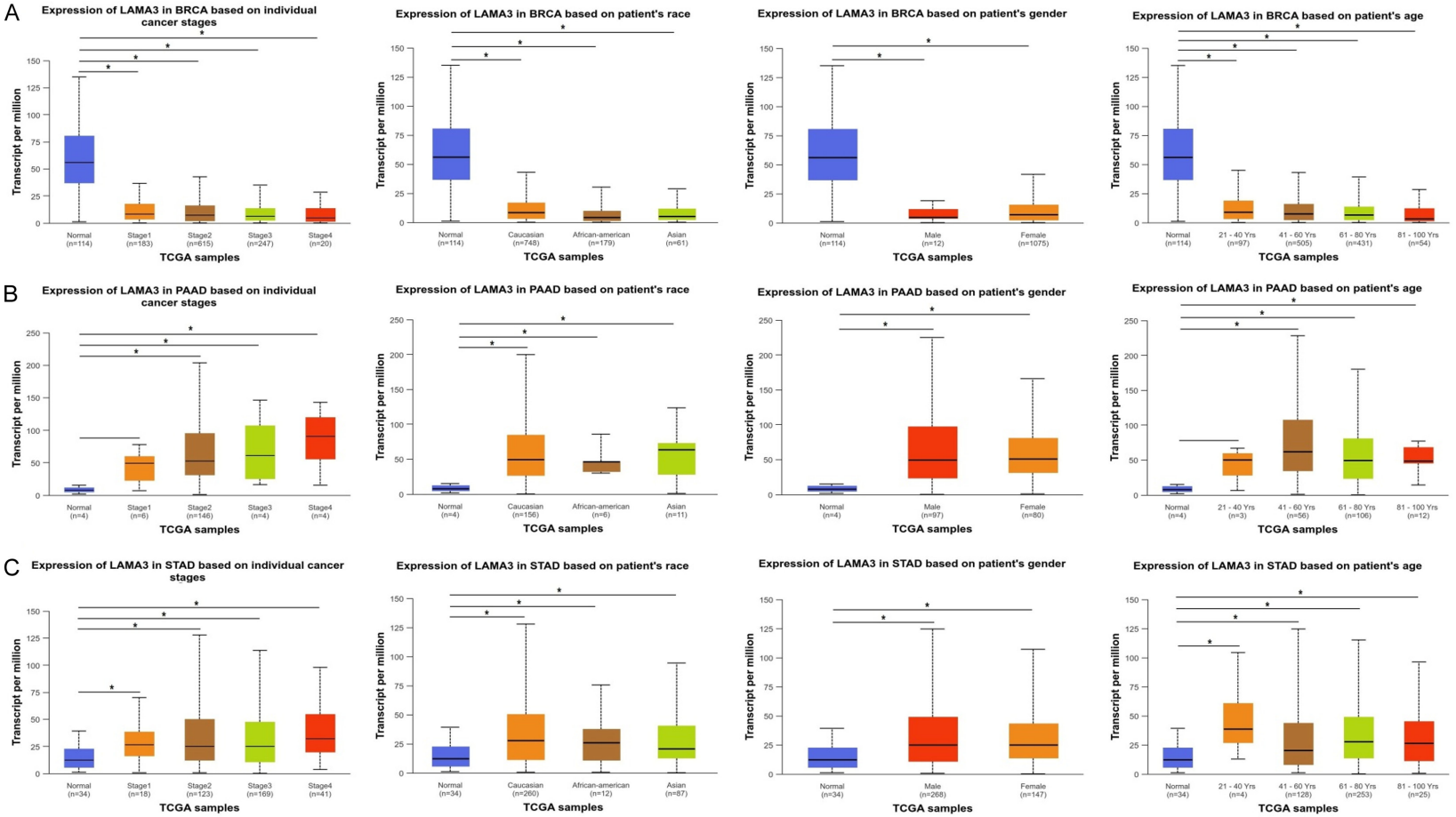
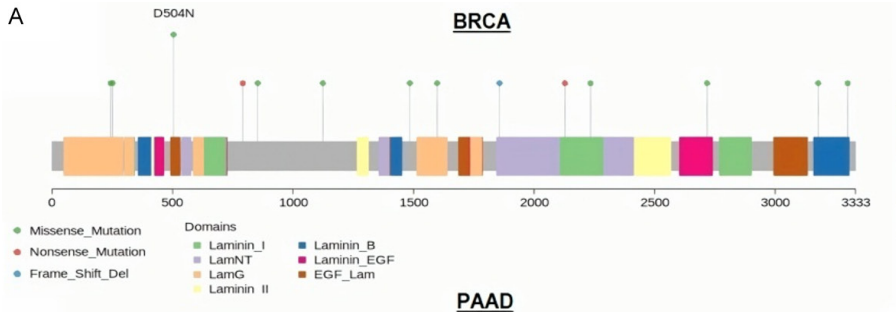


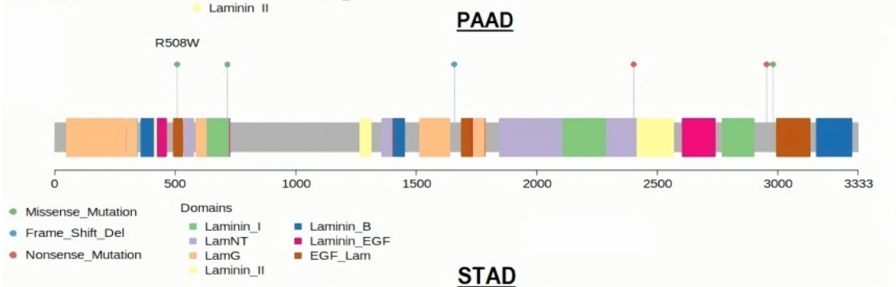
Figure 4. Relationship between laminin subunit alpha 3 (LAMA3) expression and clinical features in breast cancer (BRCA), pancreatic adenocarcinoma (PAAD), and stomach adenocarcinoma (STAD) patients. A. The expression of LAMA3 in BRCA. B. PAAD. C. STAD. *P < 0.05.

A



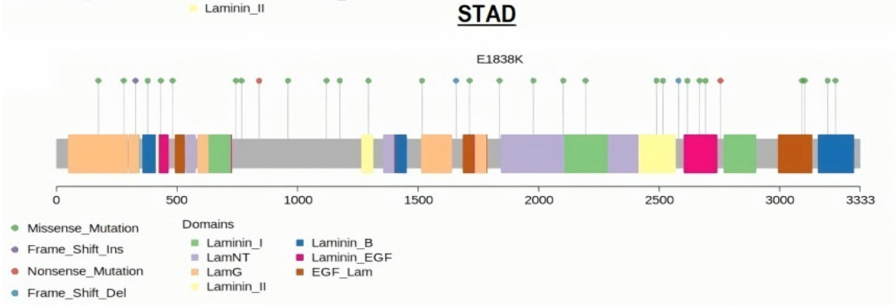
LAMA3 mutation subtypes in BRCA

Gene	Cancer type	Mutation type	num of patients with mutation	mutation frequency	Total patients
LAMA3	BRCA	Missense_Mutation	12	1.2%	1017
		Silent	4	0.4%	
		Nonsense_Mutation	2	0.2%	
		Splice_Site	2	0.2%	
		Frame_Shift_Del	1	0.1%	



LAMA3 mutation subtypes in PAAD

Gene	Cancer type	Mutation type	num of patients with mutation	mutation frequency	Total patients
LAMA3	PAAD	Missense_Mutation	3	0.6%	496
		Nonsense_Mutation	2	0.4%	
		Silent	2	0.4%	
		Frame_Shift_Del	1	0.2%	



LAMA3 mutation subtypes in STAD

Gene	Cancer type	Mutation type	num of patients with mutation	mutation frequency	Total patients
LAMA3	STAD	Missense_Mutation	26	5.9%	438
		Silent	6	1.4%	
		Nonsense_Mutation	2	0.5%	
		Frame_Shift_Del	2	0.5%	
		Frame_Shift_Ins	1	0.2%	

B

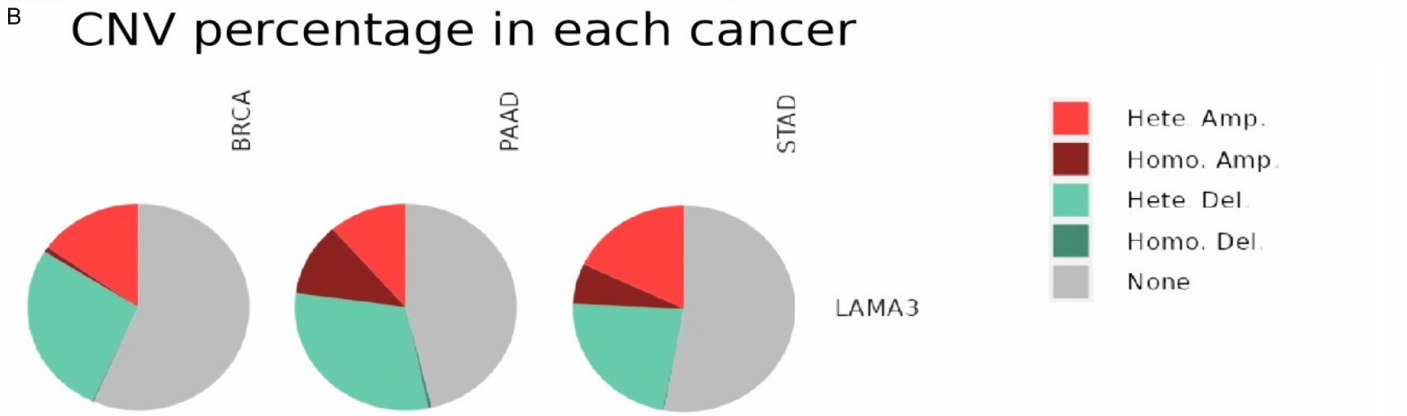


Figure 5. Mutation and copy number variation analysis of laminin subunit alpha 3 (LAMA3) in breast cancer (BRCA), pancreatic adenocarcinoma (PAAD), and stomach adenocarcinoma (STAD). A. Mutation landscape of LAMA3 in BRCA, PAAD, and STAD. B. Copy number variations (CNVs) analysis of LAMA3 in BRCA, PAAD, and STAD, visualized with pie charts.

STAD, categorizing CNVs types into heterozygous amplification, homozygous amplification, heterozygous deletion, homozygous deletion, and no variation. This CNVs data showed that heterozygous deletion and heterozygous amplification were the most prominent CNVs in LAM3 across BRCA, PAAD, and STAD (**Figure 5B**).

Promoter methylation analysis of LAMA3

The promoter methylation analysis of LAMA3 in BRCA, PAAD, and STAD was conducted using the UALCAN and GSCA databases. **Figure 6A** presents boxplots comparing promoter methylation levels of LAMA3 between normal and primary tumor tissues for each cancer type, with data obtained from TCGA samples via UALCAN. The analysis shows a significantly higher promoter methylation level of LAMA3 in BRCA, while PAAD and STAD exhibit significantly lower methylation levels. In **Figure 6B**, the GSCA database was used to display the correlation between LAMA3 promoter methylation and mRNA expression levels. A negative Spearman correlation was observed across all three cancer types, suggesting that abnormal promoter methylation is linked to altered LAMA3 expression. In **Figure 6C**, the impact of LAMA3 methylation levels on patient survival has been shown through survival difference plots for different survival endpoints: Disease-Free Interval (DFI), Disease-Specific Survival (DSS), Overall Survival (OS), and Progression-Free Survival (PFS) using the GSCA database. However, no significant associations were observed between LAMA3 methylation levels and survival outcomes across BRCA, PAAD, and STAD, as indicated by the absence of significant hazard ratios and *p*-values above 0.05 (**Figure 6B**). Together, these results suggest that while promoter methylation of LAMA3 is associated with reduced expression in BRCA, PAAD, and STAD, it may not significantly impact patient survival in these cancers.

PPI network construction and gene enrichment analysis

Firstly, the PPI networks of LAMA3 interacting partners were constructed using Genemania and STRING databases. **Figure 7A** shows the PPI network of LAMA3 constructed using Genemania. This network revealed significant associations of LAM3 with 21 other proteins. **Figure 7B** presents the PPI network of LAMA3 created using the STRING database, another tool for examining protein interactions. Similar to Genemania, STRING also demonstrates a dense network of interactions with 50 other binding partners. Next, a Venn diagram analysis was conducted to highlight overlapping proteins between PPIs constructed via Genemania and STRING databases. The common proteins between both databases include ITGA3, LAMA5, and PLEC, among others, which reinforces the reliability of these interactions (**Figure 7C**).

Gene enrichment analysis of the common proteins was conducted via the DAVID tool to gain further insights. **Figure 7D** shows gene enrichment analysis for the common proteins, indicating high enrichment in cellular component terms related to the ECM, such as laminin and integrin complexes, basement membrane, and cell junctions. **Figure 7E** provides insights into molecular function enrichment, with significant enrichment in binding activities, particularly collagen and laminin binding. **Figure 7F** further expands on biological processes enriched among the common proteins, with terms related to cellular adhesion and motility, such as hemidesmosome assembly and cell-substrate adhesion. **Figure 7G** shows pathway enrichment, with terms like ECM receptor interaction and PI3K-Akt signaling pathway, both of which are important in cancer biology. Pathways associated with cell adhesion, focal adhesion, and actin cytoskeleton regulation were also highlighted, which may imply that dysregulation of LAMA3 and its interactors could play a role in cancer progression or metastasis.

Immune infiltration and drug sensitivity analysis of LAMA3

The correlations of LAMA3 with immune infiltration and drug sensitivity in BRCA, PAAD, and STAD were explored using the GSCA database. **Figure 8A** indicates a mild negative correlation with immune infiltrates across multiple cell types in BRCA, although most correlations do

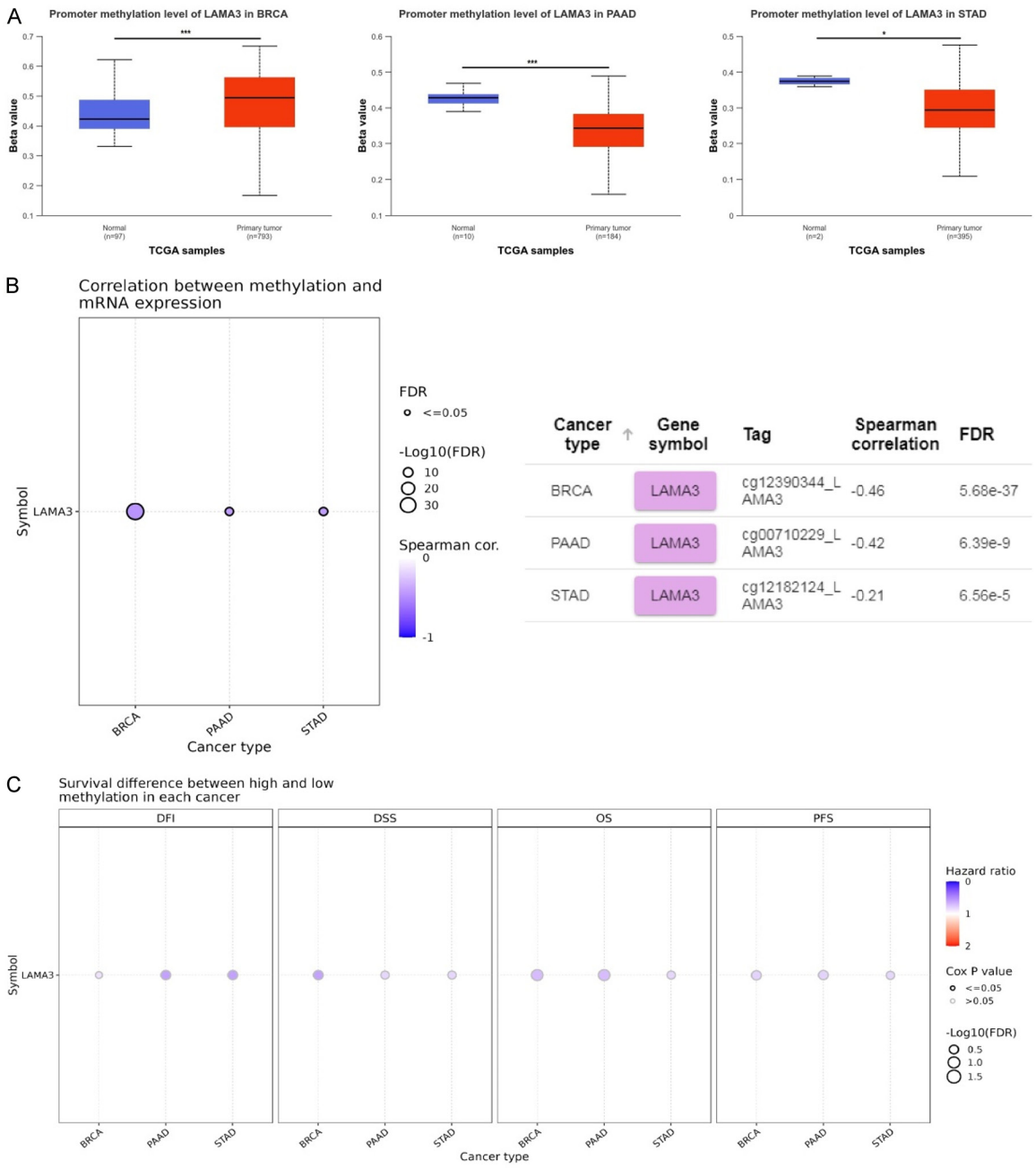


Figure 6. Promoter methylation analysis of laminin subunit alpha 3 (LAMA3) and its correlation with mRNA expression and patient survival in breast cancer (BRCA), pancreatic adenocarcinoma (PAAD), and stomach adenocarcinoma (STAD). A. Promoter methylation levels of LAMA3 in BRCA, PAAD, and STAD via UALCAN. B. Correlation between promoter methylation of LAMA3 and its mRNA expression levels in BRCA, PAAD, and STAD, analyzed using the GSCA database. C. Impact of LAMA3 methylation levels on patient survival outcomes, including Disease-Free Interval (DFI), Disease-Specific Survival (DSS), Overall Survival (OS), and Progression-Free Survival (PFS) in BRCA, PAAD, and STAD, using data from the GSCA database. $P < 0.05$.

not reach statistical significance (FDR > 0.05). In PAAD (**Figure 8B**), there were more prominent positive correlations between LAMA3 expression and dendritic cells (DC) and monocytes, suggesting that higher LAMA3 expression might be associated with increased infiltration of these cell types, though significance varies across cell types. Similarly, in STAD (**Figure 8C**), positive correlations were observed, particularly with dendritic cells and

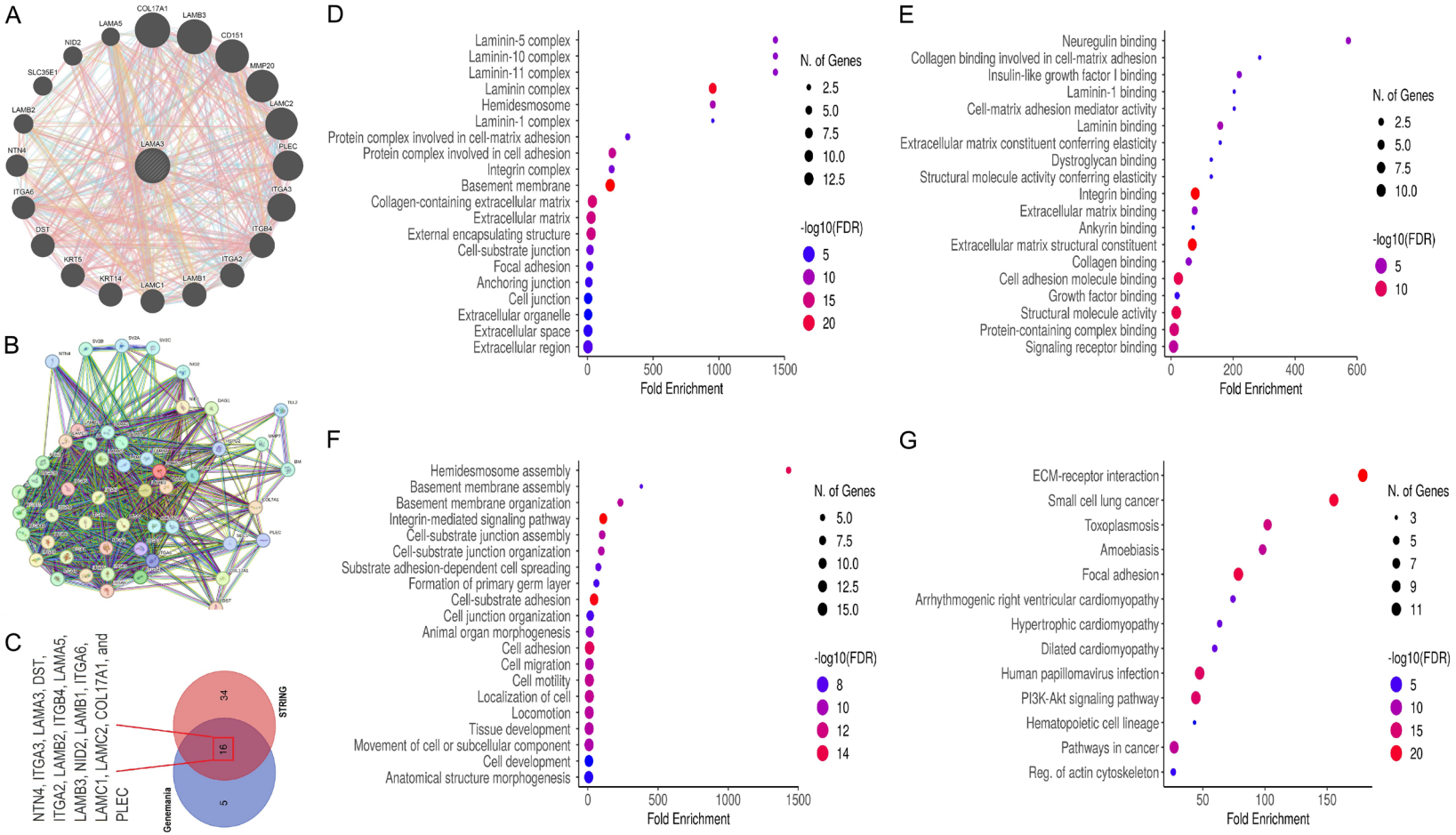


Figure 7. Protein-protein (PPI) interaction network and enrichment analysis of laminin subunit alpha 3 (LAMA3) and its interacting partners. A. PPI interaction network of LAMA3 and its interacting partners, constructed using the Genemania database. B. PPI network of LAMA3 created using the STRING database. C. Venn diagram comparing PPI results from Genemania and STRING databases. D. Gene enrichment analysis of the common interacting proteins, with cellular component terms. E. Molecular function enrichment analysis. F. Biological process enrichment analysis. G. Pathway enrichment analysis. $P < 0.05$.

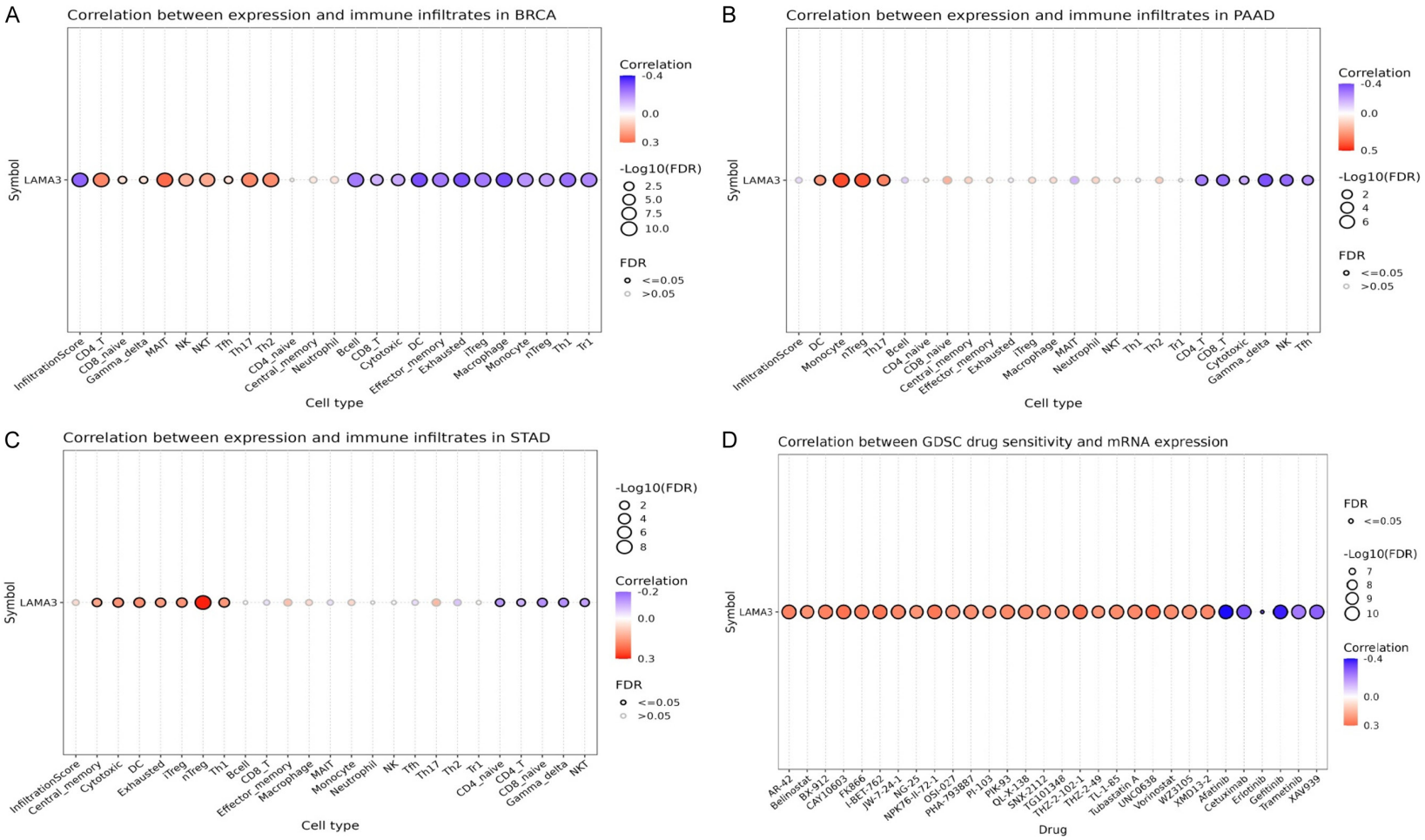


Figure 8. Correlation analysis between laminin subunit alpha 3 (LAMA3) expression, immune cell infiltration, and drug sensitivity in breast cancer (BRCA), pancreatic cancer (PAAD), and stomach cancer (STAD). A. Correlation between LAMA3 expression and immune infiltrates in BRCA. B. Correlation between LAMA3 expression and immune infiltrates in PAAD. C. Correlation between LAMA3 expression and immune infiltrates in STAD. D. Correlation between LAMA3 mRNA expression and drug sensitivity for various compounds in the Genomics of Drug Sensitivity in Cancer (GDSC) database. $P < 0.05$.

some T cell types, but these correlations also show limited statistical significance. Furthermore, **Figure 8D** presents a correlation analysis between LAMA3 mRNA expression and drug sensitivity across various drugs from the Genomics of Drug Sensitivity in Cancer (GDSC) database. Here, the results suggest a generally positive correlation with most drugs, implying that higher LAMA3 expression may be associated with increased resistance to various drugs, including AR-42, Belinostat, BX-912, CAY10603, and FK866 (**Figure 8D**), etc.

Correlations of LAMA3 with immune subtypes and immune-related genes

Correlations of LAMA3 with immune subtypes and immune-related genes were explored using the TISDB database. In **Figure 9A**, The Kruskal-Wallis test indicates statistically significant differences in LAMA3 expression among the immune subtypes in each cancer type, as shown by the *p*-values. For BRCA, LAMA3 expression varies slightly across subtypes, but no particular subtype shows an extreme deviation in expression levels (**Figure 9A**). In PAAD, LAMA3 expression is relatively high in C1 and C2 subtypes and lower in other subtypes (**Figure 9A**). Similarly, in STAD, expression differences are observed across subtypes, though no single subtype shows a pronounced difference (**Figure 9A**). Furthermore, **Figure 9B** shows heatmaps depicting the correlation of LAMA3 expression with immune-related genes categorized as immune inhibitors, immune stimulators, and major histocompatibility complex (MHC) genes in BRCA, PAAD, and STAD. In the immune inhibitors section, LAMA3 exhibits varying correlation patterns with genes like PDCD1, CD274, and CTLA4, which are known to play critical roles in immune suppression (**Figure 9B**). For immune stimulators, LAMA3's expression is positively correlated with genes like TNFRSF4 and CD86, potentially indicating an association with immune activation markers in these cancers (**Figure 9B**). In the MHC category, there is a varied correlation with genes such as HLA-A and HLA-B across cancer types, suggesting LAMA3 may have a differential impact on antigen presentation (**Figure 9B**).

LAMA3 gene knockdown and functional assays

To gain further insight into the function impact, the LAMA3 gene was knocked down in HT-29 cells. RT-qPCR results shows that knockdown of LAMA3 in si-LAMA3-HT-29 cells leads to a significant reduction in LAMA3 expression (**Figure 10A**) and a marked decrease in cell proliferation compared to control cells (**Figure 10B**). Colony formation assay images (**Figure 10C**) revealed fewer colonies in the si-LAMA3-HT-29 cells than in the control, with quantitative analysis confirming a significant reduction in colony number (**Figure 10D**), suggesting that LAMA3 is crucial for cell proliferation and colony formation in HT-29 cells. Wound healing assay images (**Figure 10E**) demonstrated slower migration in LAMA3 knockdown cells over a 24-hour period. Quantification (**Figure 10F**) showed a significantly lower wound healing percentage in the si-LAMA3-HT-29 cells, consistent with the time-course graph (**Figure 10G**), which indicates a reduced wound healing rate over time compared to controls. Overall, these findings suggest that LAMA3 promotes cell proliferation, colony formation, and migration in HT-29 cells and its knockdown impairs these processes, highlighting its potential role in tumorigenesis.

Discussion

Cancer, a multifaceted disease characterized by uncontrolled cellular growth, invasion, and metastasis, remains one of the leading causes of death worldwide [39-42]. The complexity of cancer stems not only from the diversity of tumor types but also from the numerous genetic, epigenetic, and environmental factors that influence tumor behavior and patient prognosis [43]. Consequently, understanding molecular players like Laminin subunit alpha-3 (LAMA3) across different cancer types is crucial to uncover shared and distinct mechanisms of tumorigenesis. LAMA3, part of the laminin protein family, is known to influence cell adhesion, migration, and interaction with the extracellular matrix (ECM), making it a potential modulator of cancer progression [10, 44, 45]. A pan-cancer analysis of LAMA3 can offer comprehensive insights into its role across diverse malignancies, which is essential for identifying broad-spectrum therapeutic targets and enhancing personalized cancer treatment.

Given LAMA3's involvement in cell-ECM interactions and prior associations with various cancers, it is hypothesized that LAMA3 may play a widespread role in oncogenesis across multiple cancer types. However, its expression patterns

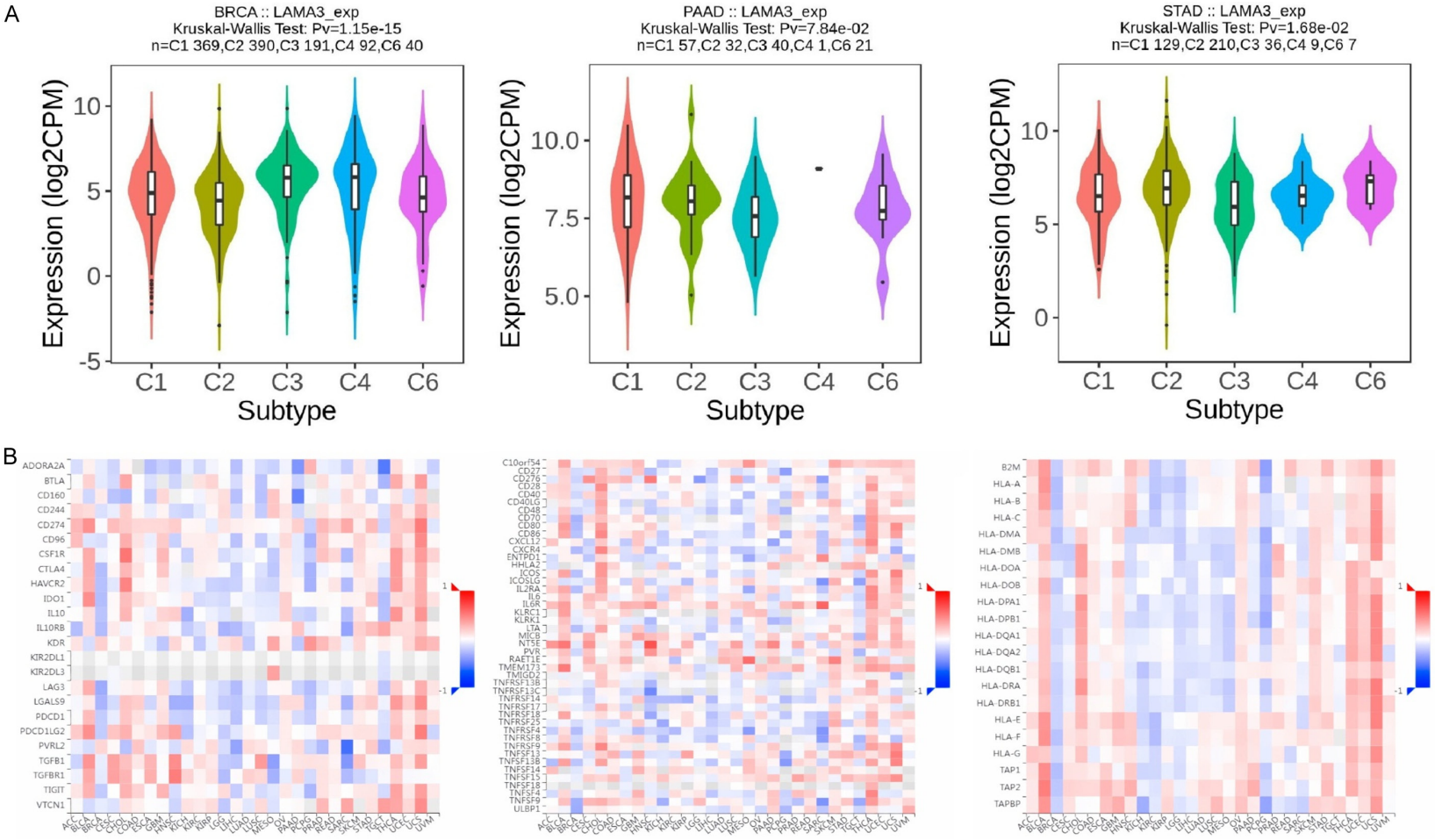


Figure 9. Correlation of laminin subunit alpha 3 (LAMA3) expression with immune subtypes and immune-related genes in breast cancer (BRCA), pancreatic cancer (PAAD), and stomach cancer (STAD). A. Violin plots showing the distribution of LAMA3 expression (log2 counts per million, CPM) across different immune subtypes (C1, C2, C3, C4, C6) in BRCA, PAAD, and STAD. B. Heatmaps showing the correlation of LAMA3 expression with immune-related genes, grouped into immune inhibitors, immune stimulators, and major histocompatibility complex (MHC) genes. $P < 0.05$.

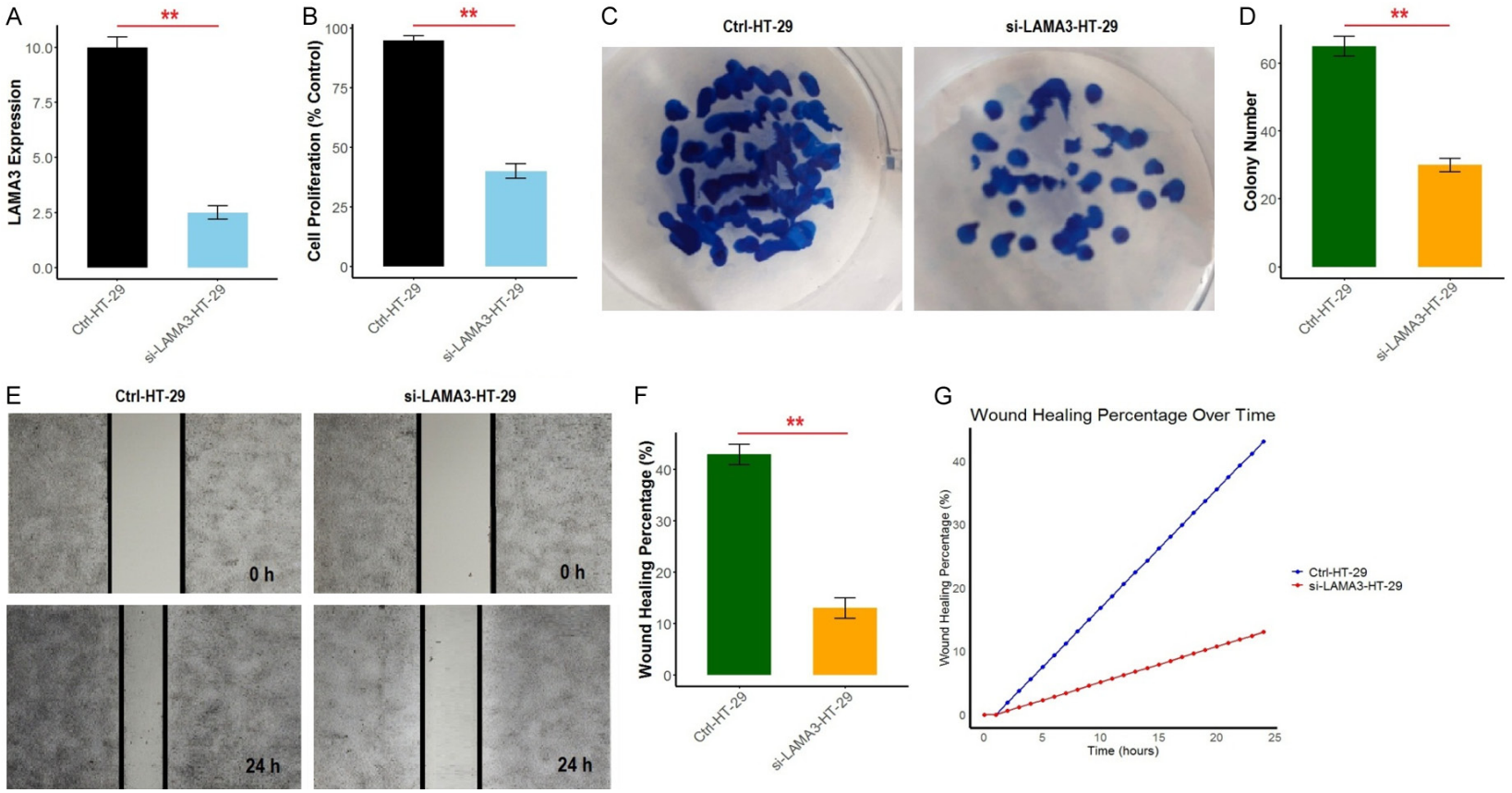


Figure 10. Knockdown of laminin subunit alpha (LAMA3) reduces cell proliferation, colony formation, and migration in HT-29 cells. A. Real-time quantitative PCR (RT-qPCR) results show a significant reduction in LAMA3 expression in si-LAMA3-HT-29 cells compared to control cells (Ctrl-HT-29). B. Cell proliferation assay reveals a significant decrease in proliferation in si-LAMA3-HT-29 cells relative to controls. C. Representative images from the colony formation assay demonstrate fewer colonies in si-LAMA3-HT-29 cells compared to controls. D. Quantification of colony numbers confirms a significant reduction in colony formation upon LAMA3 knockdown. E. Representative images from the wound healing assay at 0 hours and 24 hours show slower migration in si-LAMA3-HT-29 cells. F. Quantitative analysis of wound healing percentage indicates a significantly lower wound closure rate in si-LAMA3-HT-29 cells compared to controls after 24 hours. G. Time-course analysis of wound healing percentage over a 24-hour period highlights the reduced migration rate in LAMA3 knockdown cells. **P < 0.01.

and prognostic value in many cancers are not well-documented, necessitating a pan-cancer investigation. By utilizing data from extensive cancer databases, this study offers a comprehensive view of LAMA3's expression and functional roles across cancers, elucidating its potential as a pan-cancer biomarker and target. Such a broad analysis allows us to capture tumor-specific differences and commonalities in LAMA3 regulation, thus refining our understanding of its role in cancer biology.

Our results showed LAMA3's differential expression across cancer types, revealing that it is upregulated in certain cancers, including CHOL, ESCA, LIHC, PAAD, and STAD while being downregulated in others, such as BRCA and LUAD. This upregulation in tumors like PAAD and STAD aligns with prior studies, which have shown LAMA3 to be overexpressed in cancers, contributing to poor patient survival [46-49]. The findings of our study present intriguing yet seemingly contradictory results regarding LAMA3 expression and its prognostic significance in BRCA and LUAD when compared to prior literature. In BRCA, we observed that low LAMA3 expression correlated with poor OS, contrasting earlier reports linking high LAMA3 expression to increased tumor grade, invasion, and worse prognosis [17]. Similarly, while previous studies associate elevated LAMA3 levels with enhanced metastatic potential and poor survival outcomes in LUAD [16, 70], our analysis did not identify a significant prognostic impact in this cancer type. These discrepancies emphasize the complex and context-dependent roles of LAMA3 in cancer biology. Differences in cohort composition, molecular subtypes, and data sources likely contribute to these variations. For example, BRCA and LUAD are highly heterogeneous cancers, with subtype-specific molecular landscapes that may influence the functional impact of LAMA3 dysregulation. Moreover, our use of large-scale datasets from TIMER2.0, GEPIA, UALCAN, KM plotter, and GENT2 offers a broader, population-level perspective, while earlier studies often relied on smaller, more focused cohorts or experimental models. It is also plausible that LAMA3 plays dual roles in tumor progression, where its expression may confer context-specific effects depending on the tumor microenvironment and interactions with other signaling pathways. To address these complexities, further investigations, including subtype-specific analyses and functional validation studies, are warranted to elucidate the precise role of LAMA3 in BRCA and LUAD.

The prominent mutations identified across LAMA3 in this study, such as D504N in BRCA and R507W in PAAD, are novel findings that have not been extensively discussed in previous studies. However, CNV patterns, particularly heterozygous amplifications and deletions, align with reports suggesting that LAMA3 undergoes frequent CNV changes in various cancers [50-52]. The methylation analysis showed that LAMA3 promoter methylation varied across cancers, with BRCA exhibiting significantly higher levels, while PAAD and STAD displayed lower levels. Notably, this inverse relationship between promoter methylation and mRNA expression aligns with the commonly observed mechanism [53, 54], where hypermethylation silences gene expression, particularly in tumor suppressor genes. Previous research has also linked methylation with immune evasion [55-59], suggesting that in cancers like BRCA, higher methylation may suppress LAMA3, altering ECM composition and potentially affecting immune cell access to the tumor.

The correlations with immune cells, such as dendritic cells and monocytes in PAAD and STAD, align with studies indicating that LAMA3 influences immune infiltration in tumors. For instance, Wu et al. [60] found that LAMA3 expression correlated with immune cell types in gastric cancer, supporting this study's finding of mild but positive correlations in immune infiltration. Interestingly, while this study shows a trend of increased drug resistance with high LAMA3 expression, previous studies have similarly suggested that LAMA3 can modulate drug sensitivity, potentially through ECM-mediated drug barriers. For instance, LAMA3 has been implicated in enhancing cell survival and resistance to chemotherapy by influencing ECM composition [46, 61]. Specifically, LAMA3 overexpression has been shown to alter integrin signaling and ECM remodeling, which can reduce the efficacy of chemotherapeutic agents by creating a protective barrier around tumor cells [62]. In line with our findings, LAMA3 was found to contribute to the resistance of cancer cells to several chemotherapeutic drugs, such as

paclitaxel and cisplatin, by modulating the ECM and integrin signaling pathways [63]. However, the specific drugs noted here, such as Belinostat and FK866, require further validation, as research on LAMA3's role in resistance to these agents remains limited. Belinostat, a histone deacetylase inhibitor, and FK866, an NAD+ biosynthesis inhibitor, have both been explored in cancer therapy [64], but their relationship with LAMA3 expression is not well established. A recent study by Walter et al. (2022) found that LAMA3 overexpression in colorectal cancer cells conferred resistance to certain targeted therapies, but the exact mechanisms through which LAMA3 modulates resistance to agents like Belinostat and FK866 need further investigation [65]. The potential involvement of LAMA3 in resistance to these agents underscores the need for more comprehensive studies to validate these findings and explore how ECM dynamics influenced by LAMA3 contribute to drug resistance across different cancer types.

Our findings indicate that LAMA3 plays a crucial role in promoting cell proliferation, colony formation, and migration in HT-29 cells, as evidenced by the significant reduction in these processes following LAMA3 knockdown. The reduction in LAMA3 expression led to decreased cell proliferation and fewer colonies formed, which aligns with previous studies showing that LAMA3 is involved in promoting tumor growth and metastatic potential in various cancer types [66, 67]. For example, similar studies in BRCA have demonstrated that LAMA3 overexpression enhances cell proliferation and migration, promoting tumorigenesis and poor prognosis [68]. Our wound healing assay results, showing impaired migration in LAMA3 knockdown cells support these findings, suggesting that LAMA3 is critical for the invasive behavior of cancer cells, consistent with observations in other cancer models, including pancreatic and gastric cancers, where LAMA3 has been associated with cell motility and metastasis [46]. In contrast, while studies have indicated a positive correlation between LAMA3 expression and cancer progression, some reports suggest a more complex role depending on the cancer context. For instance, in lung and ovarian cancers, the relationship between LAMA3 and tumor progression has been reported as tissue-specific, with certain studies showing that LAMA3 expression might be less influential in some cancer types [69, 70]. These discrepancies highlight the need for further investigation into the exact mechanisms by which LAMA3 modulates cancer cell behavior across different cancer types. Nonetheless, our results provide compelling evidence that LAMA3 is an important factor in HT-29 cell proliferation and migration, emphasizing its potential as a therapeutic target in gastrointestinal cancers.

This study is limited by the lack of in vivo validation, restricting our understanding of LAMA3's functional role across diverse cancer contexts. The research does not fully address tumor heterogeneity or microenvironmental interactions, which may influence LAMA3's effects on immune response and drug sensitivity. Additionally, while functional assays were conducted in a single cell line, further studies across multiple models are needed to confirm the broader applicability of these findings.

Conclusion

This pan-cancer analysis positions LAMA3 as a multifaceted player in cancer biology, with its role varying by cancer type. Our findings reveal LAMA3's potential as a prognostic biomarker and a therapeutic target, particularly in cancers where its high expression promotes tumorigenesis, including BRCA, PAAD, and STAD. Future studies are warranted to further elucidate LAMA3's molecular mechanisms, especially regarding its influence on immune infiltration and drug resistance.

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Disclosure of conflict of interest

None.

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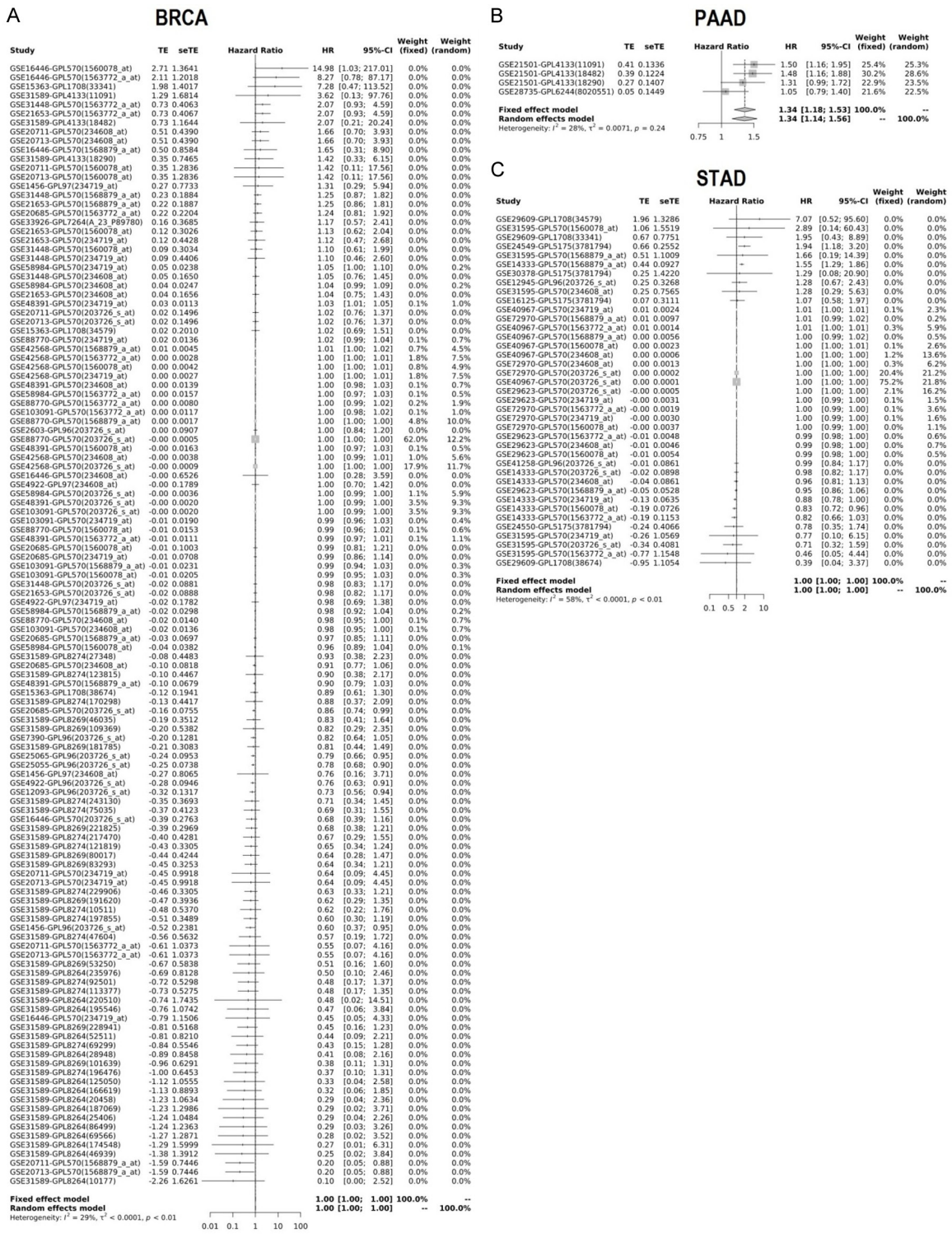

Supplementary Figure 1. Forest plots presenting meta-analyses of hazard ratios for LAMA3 expression from multiple datasets in the GENT2. (A) Breast cancer (BRCA), (B) Pancreatic adenocarcinoma (PAAD), and (C) Stomach adenocarcinoma (STAD). P < 0.05.