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Research Article

Urokinase-type plasminogen activator and related microRNAs in hepatocellular carcinoma; a bioinformatic based study

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Abstract

Objectives: Hepatocellular carcinoma (HCC) is one of the most prevalent cancers worldwide. Urokinase-type plasminogen activator (uPA), which is encoded by the PLAU gene, is a serine protease involved in the degradation of the extracellular matrix. Increasing evidence indicates that PLAU is overexpressed in various cancers and is associated with poor prognosis, making it a potential biomarker for cancer. However, its potential role in HCC remains unclear. Therefore, this study aimed to investigate the role of PLAU and related microRNAs in HCC using multiple bioinformatics tools.

Methods: PLAU expression was evaluated using the TNMplot and GEPIA2 databases. Promoter methylation levels were assessed through UALCAN. Survival analysis (overall survival (OS) and recurrence-free survival (RFS) rates), was conducted using the Kaplan-Meier Plotter. Protein-protein interaction networks were examined with STRING. Target miRNAs were identified using TargetScan 8.0. Differential expression, survival analysis, and co-expression of miRNAs were investigated using ENCORI.

Results: PLAU expression was significantly upregulated in liver hepatocellular carcinoma (LIHC) compared to normal tissues ($p<0.05$). Promoter methylation level of PLAU was significantly increased (hypermethylation) in LIHC tissues ($p=5.43\times10^{-12}$). Elevated PLAU expression was not associated with OS ($p=0.16$) and RFS ($p=0.28$) rates. hsa-miR-181a-5p, hsa-miR-181b-5p, hsa-miR-181c-5p, and hsa-miR-181d-5p were positively correlated with PLAU in LIHC tissue ($p<0.05$). The hsa-miR-181a-5p and hsa-miR-181b-5p were up-regulated in LIHC ($p<0.05$).

Conclusion: In conclusion, our study highlights the potential role of PLAU and its related miRNAs (hsa-miR-181a-5p and hsa-miR-181b-5p) in HCC. However, elevated PLAU expression did not correlate with survival rates, indicating its involvement in tumor development but no prognostic significance. Further applicable studies are needed on this subject.

Keywords: Bioinformatic analysis, hepatocellular carcinoma, DNA methylation, microRNA, PLAU, prognosis, urokinase-type plasminogen activator

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Hepatocellular carcinoma (HCC), which constitutes approximately 90% of primary liver cancers, is one of the most prevalent cancers globally, ranking sixth in incidence and third in cancer-related mortality [1, 2]. HCC remains a significant global health concern, with rising incidence rates observed in both developed and developing countries [3, 4]. The pathogenesis of HCC involves a complex array of molecular alterations, such as cell cycle dysregulation, immune modulation, DNA methylation changes, epithelial-mesenchymal transition (EMT), and microRNA (miRNA) dysregulation [5]. HCC is characterized by poor overall survival and a high recurrence rate [4]. Despite notable advances in surgical interventions,

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targeted therapies, and imaging techniques, the overall survival rates remain low. Early-stage cases may benefit from surgical approaches such as hepatic resection, liver transplantation, and local/regional therapies, while options for advanced stages remain limited, with median survival of 6 to 8 months. Therefore, understanding the mechanisms underlying HCC and identifying new biomarkers is crucial for improving early diagnosis, prognosis, and treatment [4–6].

Urokinase-type plasminogen activator (uPA), encoded by the PLAU gene, is a serine protease that converts inactive plasminogen into active plasmin. This process plays a crucial role in the degradation of the extracellular matrix (ECM) and the basement membrane [7]. Such degradation facilitates cancer cell invasion and serves as a critical initial step in tumor progression. Numerous studies have demonstrated that uPA is integral to various stages of tumor progression, including tumor cell proliferation, migration, angiogenesis, and EMT [7–9]. Furthermore, research has shown that PLAU expression, and consequently uPA levels, are significantly elevated in tumor cells, with higher PLAU expression strongly correlating with poor prognosis [7, 8, 10–12]. Additionally, PLAU levels have been found to be markedly increased in HCC; however, the number of studies on this topic remains limited [13–16].

MiRNAs are small non-coding RNA molecules that regulate target gene expression by binding to specific mRNAs, serving as key modulators of post-transcriptional gene silencing. They play a crucial role in the initiation and progression of cancer and are considered potential biomarkers for cancer diagnosis and treatment [17, 18]. The role of PLAU in HCC, particularly its interaction with miRNAs, remains poorly understood. To date, no research has explored the relationship between PLAU and its associated miRNAs in HCC. Therefore, this study aimed to investigate the role of PLAU and related miRNAs in HCC using various bioinformatics tools.

Materials and Methods

Statement of ethics

Data for this study were retrieved from various publicly available databases; therefore, ethical approval was not necessary.

The analysis of differential gene expression of PLAU using the tnmplot database

The TNMplot database (http://www.tnmplot.com/, accessed on December 12, 2024) is an online tool designed for analyzing differential gene expression in tumor, normal, and metastatic tissues. This resource comprises 56,938 unique samples collected from the Gene Expression Omnibus (GEO), the Genotypic-Tissue Expression (GTEx), the Cancer Genome Atlas (TCGA), and the Therapeutically Applicable Research to Generate Effective Treatments (TARGET) databases [19]. We utilized the TNMplot database to investigate the differential gene expression of PLAU in various tumor tissues and normal tissues derived from the TCGA (adjacent normal) and GTEx (healthy normal) datasets.

The analysis of PLAU gene expression in LIHC using the GEPIA2 database

Gene Expression Profiling Interactive Analysis, version 2 (GEPIA2, http://gepia2.cancer-pku.cn, accessed on December 12, 2024), is a comprehensive bioinformatics tool designed for analyzing gene expression data derived from the TCGA and GTEx databases [20]. We used the GEPIA2 platform to analyze the expression levels of PLAU in liver hepatocellular carcinoma (LIHC) tumor tissues compared to adjacent normal tissues from TCGA and health normal tissues from the GTEx database, using the "Match TCGA normal and GTEx data" option. Additionally, PLAU expression was examined across different LIHC subtypes.

The analysis of gene expression and promoter methylation of PLAU using the UALCAN database

The University of ALabama at Birmingham CANcer (UALCAN, http://ualcan.path.uab.edu, accessed on December 12, 2024) is an interactive and comprehensive web-based resource for the analysis of cancer OMICS data, including gene expression and promoter methylation profiles derived from TCGA datasets [21]. In this study, the UALCAN platform was utilized to evaluate PLAU gene expression and promoter methylation levels in LIHC tissues compared to adjacent normal tissues. Gene expression analysis was performed across various clinicical characteristics, including race, gender, age, weight, and nodal metastasis status.

The survival analysis of PLAU in LIHC using the kaplan-meier plotter database

Kaplan-Meier plotter (KM plotter, http://kmplot.com/analysis, accessed on December 12, 2024) is a web-based database designed to explore the relationship between gene expression and prognosis across 21 different types of cancer using clinical data [22]. We utilized this database to investigate the overall survival (OS) and relapse-free survival (RFS) rates of PLAU in LIHC tissue.

The analysis of the protein-protein interaction network and biological process (Gene Ontology) enrichment analysis of PLAU using the STRING database

The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING, https://string-db.org/, accessed on December 12, 2024) is a widely used online database for exploring and predicting protein-protein interaction (PPI). Its objective is to establish a comprehensive and objective global network that encompasses both physical and functional interactions between two or more proteins [23]. We utlized the STRING database to examine the PPI networks and the biological process (Gene Ontology) enrichment of PLAU.

The analysis of target miRNAs using the TargetScan 8.0 database

TargetScan 8.0 (https://www.targetscan.org/vert_80/, accessed on December 12, 2024) is a web resource utilized for predicting the target genes of miRNAs [24]. TargetScan

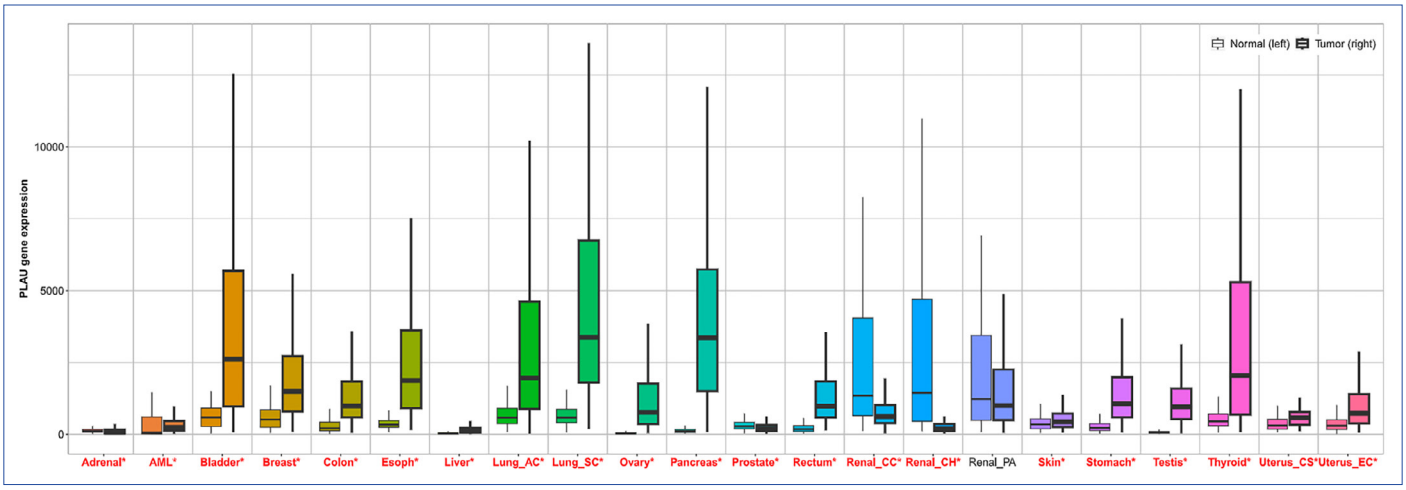

Figure 1. Box plots illustrating the differential PLAU expression analysis in normal (left) and tumor (right) tissues in TNMplot database. Significant differences are indicated in red, with *p<0.05.

predicts the biological targets of miRNAs by identifying conserved 8mer, 7mer, and 6mer sites that align with the seed region of each miRNA [25]. Furthermore, it provides predictions that encompass poorly conserved sites and nonconserved miRNAs. The tool also identifies sites with seed region mismatches that are compensated by conserved 3' pairing [26]. In mammals, predictions are prioritized based on their estimated targeting efficacy, which is determined using a biochemical model of miRNA-mediated repression. This model has been extended to all miRNA sequences through the application of a convolutional neural network [24]. We used this database to identify the target miRNAs of PLAU.

The analysis of differential expression, survival analysis, and co-expression of miRNAs using the ENCORI database

The Encyclopedia of RNA Interactomes (ENCORI, https://rnasysu.com/encori/panCancer.php, accessed on December 12, 2024) Pan-Cancer analysis platform is a comprehensive tool developed to decode Pan-Cancer Networks of long noncoding RNAs (lncRNAs), miRNAs, pseudogenes, small nucleolar RNAs (snoRNAs), RNA-binding proteins (RBPs), and all protein-coding genes by analysing their expression profiles across 32 different cancer types [27]. We performed the this database to analyze differential expression, survival analysis, and co-expression of miRNAs in LIHC and normal tissues.

Statistical analysis

All statistical analyses were conducted using the default or recommended settings of each database. In the TNMplot database, the Mann–Whitney U test was used, and $p<0.05$ was considered statistically significant. In the GEPIA2 database, differential expression analysis was performed using |log2 fold change| >1 and p-value <0.01 as cut-off criteria. The data were log2 (TPM+1) transformed, and a one-way ANOVA was applied for comparisons. Additionally, pathological stage analysis was conducted using the same platform. In the UALCAN database, a Student's t-test was employed with statistical significance defined as $p<0.05$. In the Kaplan–Meier Plotter, survival analysis was performed using log-rank p-values to compare high and low expression groups in LIHC, with auto-selected best cutoff, and significance was set at $p<0.05$. In the STRING database, $p<0.05$ was considered statistically significant for PPI network analysis, and FDR<0.05 was used for biological process enrichment analysis. In the ENCORI database, expression levels were presented as log2(RPM+0.01).

Results

The differential gene expression of the PLAU in various tumor tissues

We conducted a pan-cancer analysis of the TNMplot database to evaluate the expression of PLAU across 22 different tumor types. The results demonstrated that PLAU was significantly expressed in 21 out of the 22 tumor tissues analyzed. As shown in Figure 1, PLAU expression was significantly upregulated in the adrenal, acute myeloid leukemia (AML), bladder, breast, colon, esophagus, liver, lung adenocarcinoma (lung-AC), lung squamous cell carcinoma (lung-SC), ovary, pancreas, rectum, skin, stomach, testis, thyroid, and both subtypes of uterine carcinoma (uterus-CS and uterus-EC) when compared to normal tissues. In contrast, PLAU expression was downregulated in prostate, renal clear cell carcinoma (renal-CC), and renal chromophobe carcinoma (renal-CH) tumor tissues ($p<0.05$). There was no statistically significant difference in PLAU expression in renal papillary adenocarcinoma (renal-PA) ($p>0.05$).

The gene expression level of PLAU in LIHC

We examined the expression levels of PLAU in LIHC tissues (n=369) compared to normal tissues (n=160) using GEPIA2. The results indicated that PLAU expression was significantly upregulated in LIHC compared to normal tissues ($p<0.01$) (Fig. 2a). Box plot of the subtypes revealed that PLAU levels were upregulated in LIHC tissues compared to normal tissues in both iCluster_1 and iCluster_2 ($p<0.01$). However, no signifi-

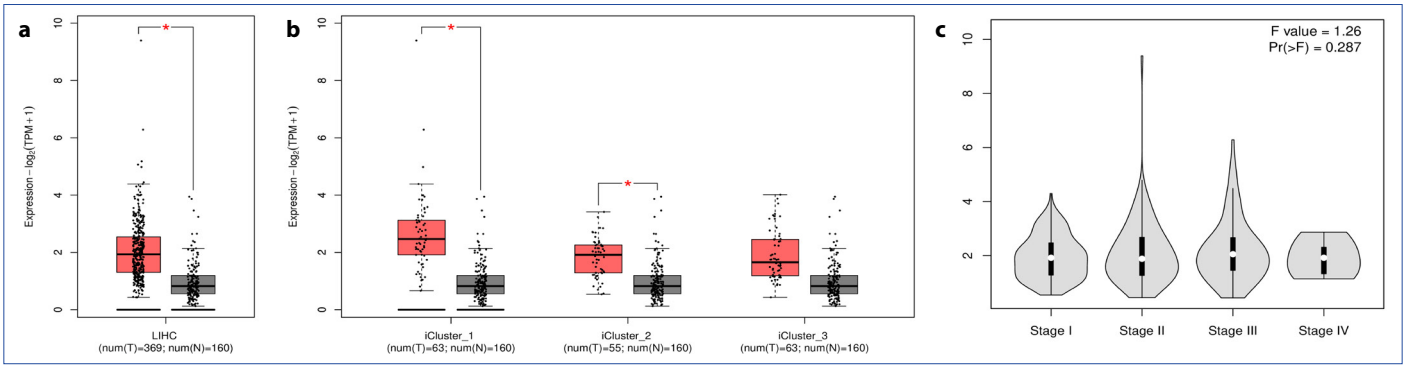

Figure 2. PLAU expression in LIHC tissue. (a) The box plot illustrating PLAU expression levels in LIHC (red) compared to normal tissues (gray) in GEPIA2 database. (b) The box plot showing PLAU expression levels at different iCluster groups in GEPIA2 database (c) The violin plot depicting PLAU expression levels at different stages of LIHC in GEPIA2 database.

Significant differences are indicated in red, with *p<0.01. LIHC: Liver hepatocellular carcinoma.

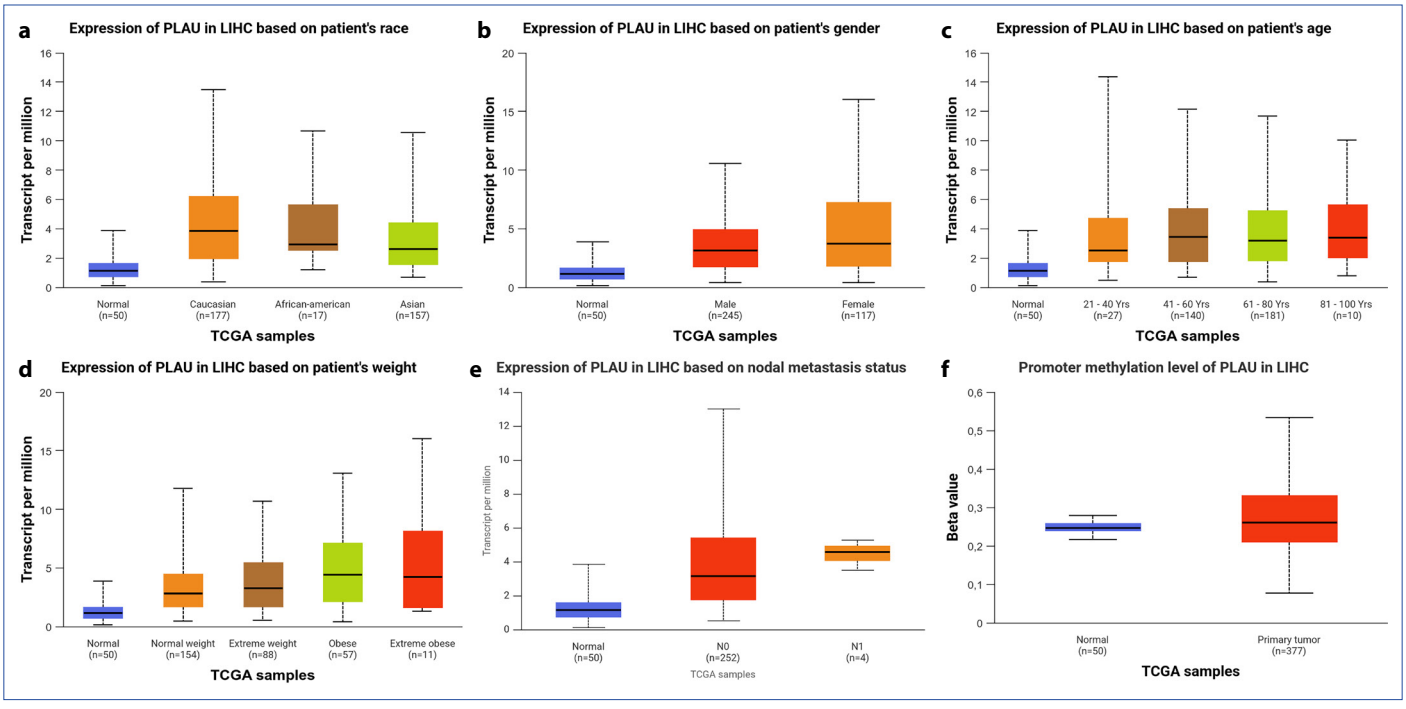

Figure 3. Box plots illustrating the gene expression and promoter methylation levels of PLAU in LIHC tissues compared to adjacent normal tissues using the UALCAN database. PLAU expression levels are shown based on (a) race, (b) gender, (d) age, (d) weight, and (e) nodal metastasis status. (f) Promoter methylation levels of PLAU.

TCGA: The cancer genome atlas.

cant difference was observed in iCluster_3 (Fig. 2b). Additionally, violin plot of the pathological stages showed no statistically significant differences among stages I, II, III, and IV of LIHC (F=1.26; Pr(>F)=0.287) (Fig. 2c).

The gene expression and promoter methylation level of PLAU in LIHC

The expression of PLAU in LIHC was analyzed based on patients' race, gender, age, weight and nodal metastasis status using TCGA data via the UALCAN platform. The results indicated that PLAU expression was significantly upregulated in tumor tissues compared to adjacent normal tissues in Caucasian ($p=4.1\times10^{-15}$) and African-American (p=0.016) patients, while no statistically significant difference was observed in Asian patients (p=0.089). Additionally, there were no significant differences in PLAU expression among racial groups (Caucasian vs. African-American: p=0.991; Caucasian vs. Asian: p=0.341; African-American vs. Asian: p=0.355) (Fig. 3a). PLAU expression was significantly upregulated in both male (p=0.0362) and female ($p=7.05\times10^{-11}$) patients compared to adjacent normal tissues. However, there was no statistically significant difference in PLAU expression between male and female patients (p=0.342) (Fig. 3b). Age-stratified analysis showed significant upregulation of PLAU expression in the 21–40

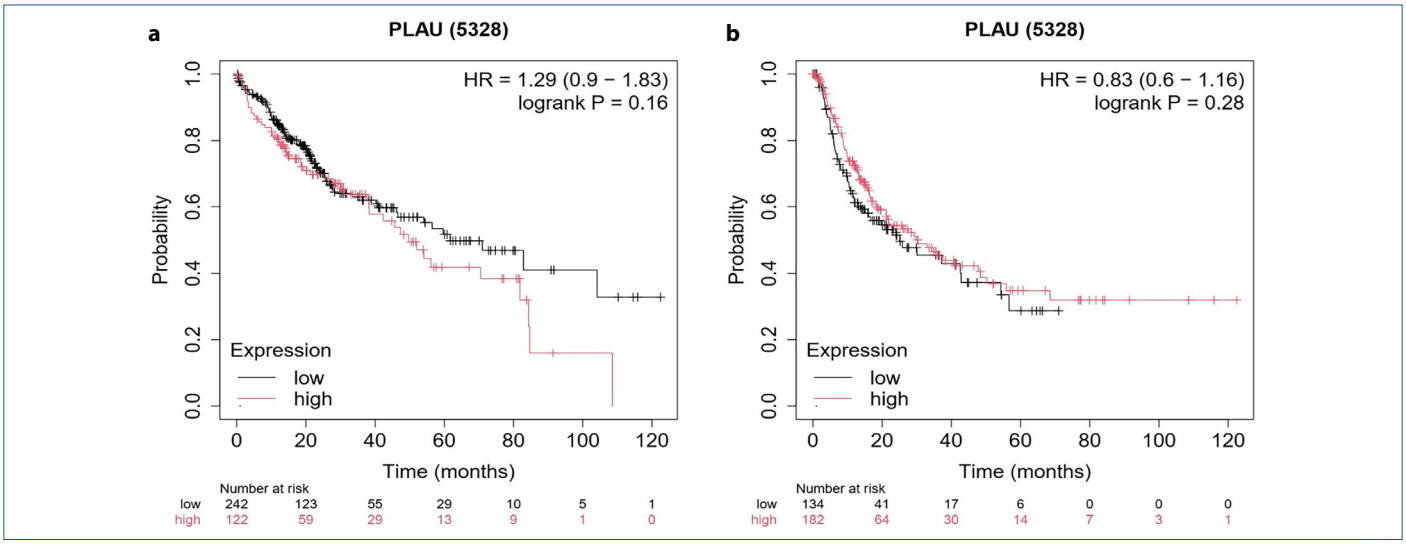

Figure 4. The survival analysis of PLAU in LIHC in the Kaplan-Meier plotter database. (a) Overall survival rates. (b) Relapse-free survival rates. HR: Hazard ratio; LIHC: Liver hepatocellular carcinoma.

($p=1.48\times10^{-3}$), 61–80 ($p=1.20\times10^{-12}$), and 81–100 (p=0.040) year groups. No significant upregulation was observed in the 41–60 group (p=0.087). Additionally, no significant differences were found among age groups (all p>0.05) (Fig. 3c). Regarding weight groups, PLAU expression was significantly upregulated in tumors from patients classified as extreme weight ($p=6.38\times10^{-8}$), obese ($p=2.38\times10^{-6}$), and extreme obese (p=0.027), whereas no significant upregulation was observed in the normal weight group (p=0.079). Additionally, no significant differences were found among the tumor weight groups (all p>0.05) (Fig. 3d). PLAU expression was significantly upregulated in patients without regional lymph node metastasis (N0) compared to adjacent normal tissues (p=0.032). However, no significant differences were observed in patients with limited lymph node involvement (N1, defined as metastasis in 1 to 3 axiallry lymph nodes) compared to adjacent normal tissues (p>0.05), nor between the N0 and N1 groups (p>0.05) (Fig. 3e). Additionally, promoter methylation levels of PLAU were investigated in LIHC using the UALCAN database. The results demonstrated that promoter methylation was significantly increased (hypermethylation) in LIHC tissues (n=377) compared to adjacent normal tissues (n=50), with median beta values of 0.26 and 0.247, respectively ($p=5.43\times10^{-12}$) (Fig. 3f).

The survival analysis of PLAU in LIHC

We examined the association between PLAU expression and the OS and RFS rates in LIHC. The analysis revealed that PLAU expression was not significantly associated with OS in LIHC patients (HR=1.29, 95% CI: 0.90–1.83, p=0.16) (Fig. 4a). The median survival rates for cohorts with low and high PLAU expression were 61.7 months and 49.7 months, respectively. PLAU expression was not significantly associated with RFS in LIHC patients (HR=0.83, 95% CI: 0.60–1.16, p=0.28) (Fig. 4b). The median survival rates for cohorts with low and high PLAU expression were 25.14 months and 30.4 months, respectively.

The analysis of protein-protein interactions and biological process enrichment of PLAU

The protein-protein interactions and biological process enrichment of PLAU were analyzed using the STRING database. The resulting PPI network comprises 11 nodes and 41 edges, with an average node degree of 7.45, an average local clustering coefficient of 0.856, and an expected number of edges of 12. The PPI enrichment p-value was 9.26×10–11. The results demonstrated that PLAU interacts with serine protease inhibitor 1 (SERPIN1), serine protease inhibitor 2 (SERPIN2), serine protease inhibitor A5 (SERPIN5), serine protease inhibitor EB2 (SERPINEB2), plasminogen activator urokinase receptor (PLAUR), plasminogen (PLG), matrix metalloproteinase-9 (MMP9), vitronectin (VTN), cathepsin B (CTSB), insulin like growth factor 2 receptor (IGF2R) (Fig. 5a). Furthermore, the biological process enrichment analysis revealed that the interactions are associated with several biological processes, including the regulation of blood coagulation, fibrinolysis, plasminogen activation, and proteolysis (Fig. 5b).

The analysis of target miRNA

The miRNAs associated with PLAU were analyzed using the TargetScan 8.0 database. We identified five conserved miRNAs: hsa-miR-181a-5p, hsa-miR-181b-5p, hsa-miR-181c-5p, hsa-miR-181d-5p, and hsa-miR-4262.

The analysis of differential expression, survival, and co-expression of miRNAs

ENCORI analysis was conducted to compare the differential expression, survival analysis, and co-expression of hsa-miR-181a-5p, hsa-miR-181b-5p, hsa-miR-181c-5p, hsa-miR-181d-5p, and hsa-miR-4262 between LIHC (n=370) and normal tissues (n=50). The results indicated that hsa-miR-181a-5p and hsa-miR-181b-5p were significantly upregulated in LIHC

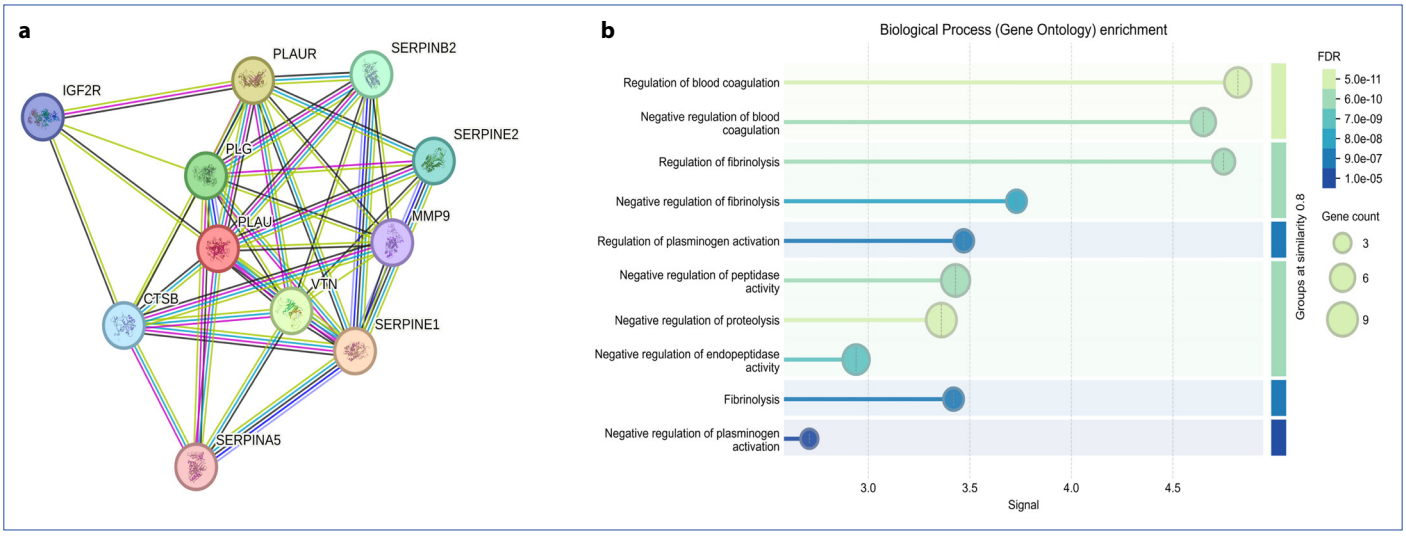

Figure 5. Interaction network of PLAU in the STRING database. (a) Protein-protein interactions of PLAU. (b) Biological process (Gene Ontology) enrichment analysis for PLAU.

FDR: False discovery rate.

tissues compared to normal tissues ($p<0.05$) (Table 1; Fig. 6a). None of the miRNAs showed a statistically significant association with OS in LIHC tissues (Fig. 6b). According to the co-expression analysis, hsa-miR-181a-5p, hsa-miR-181b-5p, hsa-miR-181c-5p, and hsa-miR-181d-5p were positively correlated with PLAU in LIHC tissues ($p=3.60\times10^{-14}$, 1.64×10^{-17}, $p=1.47\times10^{-23}$, and $p=3.79\times10^{-21}$, respectively) (Fig. 6c).

Discussion

The role of PLAU in HCC, particularly its relationship with miRNAs, remains unclear. This study is the first to investigate PLAU's involvement in HCC and its interaction with miRNAs through bioinformatic analysis. The urokinase-type plasminogen activator is an extracellular proteolytic enzyme that plays a pivotal role in remodeling tumor microenvironment and the progression of cancer [8]. Recently, uPA has garnered significant attention due to its involvement in tumor growth, metastasis, and angiogenesis, as well as its overexpression in various cancers. Elevated levels of uPA have been linked to poor prognosis, highlighting its potential as a valuable diagnostic, prognostic, and therapeutic biomarker [7, 8, 10]. Numerous strategies have been developed to target the uPA system by modulating its expression and activity in cancer [7, 10, 28]. However, research on the role of PLAU in HCC remains limited [13–16]. In the present study, we first assessed the differential expression of PLAU across 22 different tumor types using the TNMplot database. Our findings demonstrated that PLAU was significantly expressed in the majority of tumor types (21 out of 22), with expression levels varying according to the specific cancer type. Consistent with previous research, our analysis confirmed that PLAU is consistently overexpressed in multiple cancers [12, 13, 15]. Subsequently, we analyzed PLAU expression using the GEPIA2 database to investigate its levels in LIHC tissue. The results revealed that PLAU expression was significantly upregulated in LIHC tissues compared to normal tissues, consistent with previous studies [13, 15]. Additionally, we examined PLAU expression across different iCluster groups using the GEPIA2 database. Significant differences were observed in iCluster_1 (proliferative/stem cell-like) and iCluster_2 (intermediate/immune-active), while no significant difference was found in iCluster_3 (non-proliferative/metabolic). This may suggest a potential subtype-specific role of PLAU in the tumor biology of LIHC. Furthermore, we assessed the expression of the PLAU gene across different stages of cancer using the same database. The results indicated that PLAU expression did not show a

Table 1. Differential expression table of pan-cancer analysis for miRNAs in LIHC

miRNAs	Fold change	p	False discovery rate
hsa-miR-181a-5p	1.37	0.044	0.14
hsa-miR-181b-5p	1.76	0.00091	0.0055
hsa-miR-181c-5p	1.19	0.94	0.96
hsa-miR-181d-5p	1.52	0.39	0.72
hsa-miR-4262	1.0	0.71	0.78

miRNAs: microRNAs; LIHC: Liver hepatocellular carcinoma

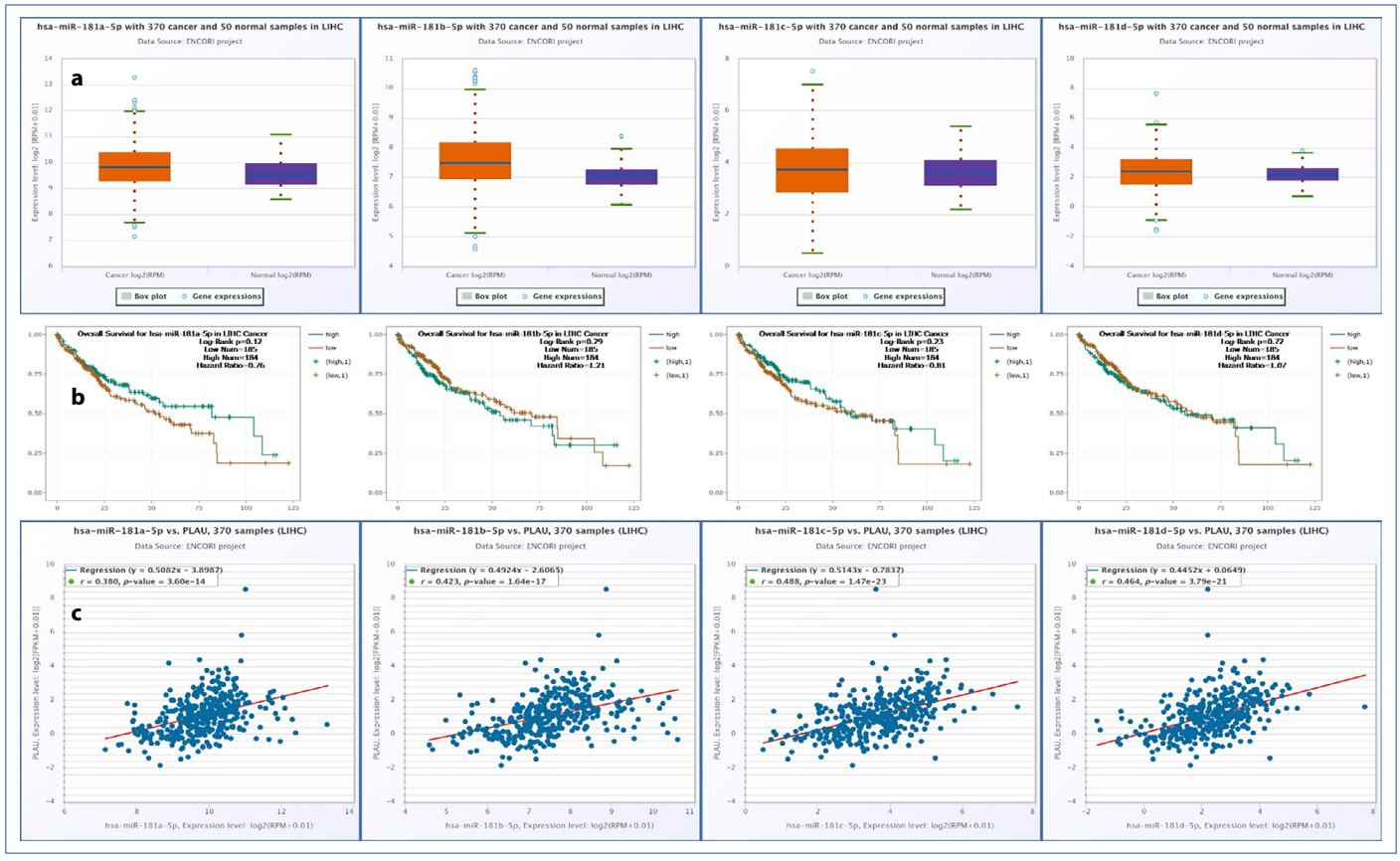

Figure 6. (a) The differential expression of hsa-miR-181a-5p, hsa-miR-181b-5p hsa-miR-181c-5p, and hsa-miR-181d-5p in LIHC in the ENCORI database. (b) Overall survival rates of hsa-miR-181a-5p, hsa-miR-181b-5p hsa-miR-181c-5p, and hsa-miR-181d-5p in LIHC in the ENCORI database. (c) Correlation between hsa-miR-181a-5p, hsa-miR-181b-5p hsa-miR-181c-5p, and hsa-miR-181d-5p expressions and PLAU expression in LIHC in the ENCORI database.

LIHC: Liver hepatocellular carcinoma.

statistically significant difference among stages I, II, III, and IV of LIHC. This suggests that PLAU expression remains relatively stable throughout disease progression, implying that its expression may not be stage-dependent. These findings underscore the need for further research into the functional role of PLAU in HCC subtypes. To our knowledge, this is the first study to explore this specific subject.

To further explore the clinical relevance of PLAU in LIHC, its expression was evaluated across various demographic and clinical subgroups. PLAU expression was significantly upregulated in Caucasian and African-American patients with LIHC, while no significant increase was observed in Asian patients. Moreover, no significant differences were found among the racial groups. These findings may suggest that PLAU plays a role in LIHC tumorigenesis in certain racial populations. PLAU expression was also significantly upregulated in both male and female patients with LIHC. However, no significant difference was observed between the sexes, suggesting that PLAU overexpression occurs independently of sex. Age-stratified analysis showed significant upregulation in the 21–40, 61–80, and 81–100 age groups, but not in the 41–60 group. Nonetheless, the lack of intergroup differences suggests that PLAU overexpression is not strongly age-dependent. Similarly, elevated PLAU expression in patients with extreme weight, obesity, and extreme obesity, but not in those with normal weight, was observed. However, the absence of significant variation among weight groups indicates a limited association with body weight. Notably, PLAU overexpression in patients without nodal metastasis (N0) suggests a potential role in early tumorigenesis. However, the absence of significant expression differences in N1 patients or between N0 and N1 groups, suggesting PLAU may not contribute to lymphatic spread in LIHC.

Numerous studies suggest that uPA may serve as a prognostic marker, with elevated PLAU expression associated with poor prognosis in HCC [13–15]. Wu et al. [15] reported that high PLAU expression was associated with poorer OS. Tsai et al. [14] found that elevated serum uPA levels were linked to poorer OS in HCC patients after resection. Furthermore, Niu et al. [13] demonstrated that high uPA expression correlated with poor prognosis, indicating its potential role as a prognostic biomarker in HCC. Despite these findings, our analysis using the KM Plotter database did not reveal a statistically significant correlation between PLAU expression and OS or RFS in LIHC patients. The discrepancies between our findings and

those of previous studies may be attributed to differences in sample sizes, methodologies, or the specific databases utilized for analysis. Additionally, the heterogeneity of PLAU expression across different tumor stages, etiologies, and molecular subtypes of HCC may influence prognostic outcomes. Further validation studies are necessary to clarify the prognostic value of PLAU expression in LIHC.

HCC is commonly associated with genetic and epigenetic aberrations [29]. DNA methylation, an important epigenetic modification, plays a critical role in regulating gene expression. Aberrant DNA methylation is a hallmark of cancer, closely linked to the onset, development, and progression of cancer, and it holds potential as a biomarker for diagnosis and prognosis [30, 31]. Specifically, the epigenetic modification of the PLAU gene through DNA methylation has been implicated in cancer development [7]. Numerous studies have demonstrated that the promoter region of PLAU undergoes hypomethylation, which is linked to increased PLAU expression and contributes to its oncogenic effects [28, 30, 32, 33]. Pakneshan et al. [32] found that DNA hypomethylation at the PLAU promoter correlates with elevated expression in aggressive breast cancer, suggesting its potential as an early bimarker. Similarly, Wu et al. [30] reported an inverse relationship between PLAU promoter methylation and gene expression in differentiated thyroid cancer. Additionally, Huo et al. [28] identified a link between PLAU overexpression and DNA hypomethylation in head and neck squamous cell carcinoma, highlighting its role as an independent diagnostic and prognostic biomarker. In the present study, we examined the methylation of the PLAU promoter using the UALCAN database to investigate its role in LIHC. Contrary to existing literature, we found that the PLAU promoter was hypermethylated in LIHC tissues. While hypermethylation is typically linked to gene silencing, our results indicated increased PLAU expression, contradicting the conventional view that DNA methylation always suppresses gene expression [8, 31, 34]. Recent studies have highlighted instances where promoter hypermethylation correlates with increased expression, suggesting a more complex role for DNA methylation [31, 35–37]. Spainhour et al. [35] analyzed data from the TCGA and found that promoter methylation exhibited a positive correlation with gene expression, contrary to the expected negative correlation. This growing evidence suggests a potential link between hypermethylation and increased transcriptional activity. Several hypotheses have been proposed to clarify the molecular mechanisms underlying gene activation from hypermethylated promoters. These mechanisms include the binding of repressive transcription factors, interactions with distal elements, and expression from alternative promoters [31]. Our findings also offer new insights into the intricate relationship between methylation and transcriptional regulation. Further research is necessary to clarify the molecular mechanisms involved in gene activation in hypermethylated promoters and to understand the functional consequences of this epigenetic modification.

The uPA is a key protease that converts plasminogen into plasmin, playing a crucial role in fibrinolysis and coagulation [38]. Our PPI networks and enrichment analysis revealed that PLAU interacts with several proteins, including SERPIN1, SERPIN2, SERPIN5, SERPINEB2, PLAUR, PLG, MMP9, VTN, CTSB, and IGF2R. These interactions are involved in blood coagulation, fibrinolysis, plasminogen activation, and proteolysis. These findings highlight PLAU as a central regulator of the plasminogen system, contributing to tumor progression through proteolytic activity and ECM degradation. The interaction between PLAU and coagulation-related proteins suggests a dynamic crosstalk between fibrinolysis and tumor microenvironment remodeling, supporting the hypothesis that dysregulated hemostasis contributes to cancer progression [39]. While uPA is not a direct coagulation factor, it plays an essential role in the fibrinolytic system. Dysregulation of coagulation and proteolysis has been strongly linked to cancer progression, with proteases promoting tumor invasion and metastasis [38]. Further research is needed to elucidate the precise roles of PLAU-related proteins and to explore whether targeting PLAU or its associated pathways could offer novel therapeutic strategies for cancer treatment.

The miRNAs regulate key cellular processes such as proliferation, differentiation, and apoptosis. Their dysregulation is linked to various diseases, including cancer, where they play a complex role in tumor development and progression [17, 40]. To further investigate the mechanisms underlying PLAU upregulation in LIHC tissue, we conducted a bioinformatics analysis to predict miRNAs targeting PLAU. Our analysis identified hsa-miR-181a-5p and hsa-miR-181b-3p as upregulated in LIHC, showing a significant positive correlation with PLAU expression. Notably, no prior studies have explored the relationship between these miRNAs and PLAU in HCC. Recently, there has been growing interest in the roles of the miR-181 family in cancer. Research suggests that the miR-181 family members can act as either oncogenes or tumor suppressors, depending on the cellular context, and influence major pathways by targeting multiple genes [40–44]. Hsa-miR-181a-5p, a highly conserved microRNA, regulates crucial tumor-related processes, including proliferation, apoptosis, autophagy, angiogenesis, EMT, and migration [40]. Extensive studies have reported both upregulated and downregulated expression levels of hsa-miR-181a-5p across various tumor types [43–47]. These conflicting findings highlight the complexity of miRNAs, as their functions can vary significantly across tumor types. Hsa-miR-181a-5p has also been studied in HCC. Korhan et al. [44] demonstrated that hsa-miR-181a-5p is downregulated in HCC and directly targets c-Met, thereby inhibiting cell motility, invasion, and branching morphogenesis. Similarly, Bi et al. [45] reported that hsa-miR-181a-5p is downregulated in HCC and inversely correlated with Early Growth Response Factor 1 (Egr1) expression. Notably, overexpression of hsa-miR-181a-5p suppressed Egr1, inhibiting the TGF-β1/Smad pathway and reducing proliferation. Conversely, Chang et al. [48] found that lncRNA-XIST enhances the expression of the tumor suppressor gene PTEN by inhibiting hsa-miR-181a-5p. Restoration of hsa-miR-181a-5p expression

was shown to promote HCC cell proliferation and invasion. Yadav et al. [49] demonstrated that free fatty acid-induced hsa-miR-181a-5p promotes apoptosis in hepatic cells by targeting and downregulating X-linked inhibitor of apoptosis protein and B-cell lymphoma 2, both of which are anti-apoptotic proteins. Numerous studies have shown that hsa-miR-181b-5p is overexpressed in various cancers, including HCC, where it promotes tumor progression through multiple signaling pathways [41, 42, 50]. Wang et al. [42] demonstrated that TGF-β signaling upregulates hsa-miR-181b in NASH-associated hepatocarcinogenesis by targeting tissue inhibitor of metalloproteinases 3 (TIMP3), leading to ECM degradation and tumor growth. These findings highlight the significance of the TGF-β/miR-181b/TIMP3 axis in hepatocarcinogenesis and its potential as a therapeutic target. Similarly, Yu et al. [50] found that cSMARCA5 suppresses HCC progression by sponging miR-181b-5p, thereby restoring TIMP3 expression. Our findings align with these studies. In conclusion, hsa-miR-181a-5p and hsa-miR-181b-5p play important roles in HCC progression by acting through multiple signaling pathways. Our study demonstrated that the upregulation of these miRNAs and their positive correlation with PLAU may be a shared mechanism promoting tumor progression. This highlights the potential of targeting PLAU and hsa-miR-181a/hsa-miR-181b as a therapeutic strategy in HCC. Further research is required to clarify the roles and regulatory mechanisms of hsa-miR-181a-5p and hsa-miR-181b-5p in HCC.

Conclusion

To our knowledge, our study is the first to examine the relationship between PLAU and miRNAs in HCC using several bioinformatic databases. This study indicates that PLAU may play a significant role in HCC development through epigenetic modification and miRNA interactions. The positive correlation with hsa-miR-181a-5p and hsa-miR-181b-5p suggest a complex regulatory network influencing tumor development. However, the lack of association with OS and RFS suggests that while PLAU may contribute to tumor development, its prognostic significance in HCC remains uncertain. Further investigation into their functional interplay and regulatory mechanisms is essential to understand their role in HCC pathogenesis better.

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