Overexpressed PLAU and its potential prognostic value in head and neck squamous cell carcinoma

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Background. Metastasis is a major event for survival and prognosis in patients with head and neck squamous cell carcinomas (HNSCC). A primary cause of metastasis is the proteolytic degradation of the extracellular matrix (ECM). The plasminogen activator urokinase (PLAU) is involved in the transformation of plasminogen to plasmin leading to hydrolyzation of ECM-related proteins. However, the role of PLAU expression in HNSCC is unclear and worth to be investigated. **Methods.** PLAU expression profiles and clinical parameters from multiple HNSCC datasets were used to investigate the relationship of PLAU expression and HNSCC survival. GO and PPI network were established on PLAU-related downstream molecular. The stroma score was deconvoluted for analysis of PLAU's association with the immune environment. ROC analysis was applied to show the performance of PLAU in predicting HNSCC prognosis. **Results.** PLAU mRNA was significantly elevated, as opposed to its methylation, in HNSCC tumor samples over normal specimens (all p<0.01). Univariate and multivariate cox analysis showed PLAU could be an independent indicator for HNSCC prognosis. Combining with neck lymph node status, the AUC of PLAU in predicting 5-years overall survival reached to 0.862. GO enrichment analysis showed the major biological process (extracellular matrix organization and the P13K-Akt signaling pathway) may involve to the possible mechanism of PLAU's function on HNSCC prognosis. Furthermore, PLAU expression was positively correlated with stroma cell score, M1 type macrophages, and negatively associated with CD4 + T cell, Tregs cell, and follicular helper T cell. **Conclusions:**PLAU might be an independent biomarker for predicting outcomes of HNSCC patients. The elevated expression of PLAU was associated with HPV positivity and neck node status. PI3K-Akt pathway and aberrant proportions of immune cells might underly the mechanism of PLAU's oncogene role in HNSCC.

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2 head and neck squamous cell carcinoma

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22 Abstract

23 **Background.** Metastasis is a major event for survival and prognosis in patients with head and
24 neck squamous cell carcinomas (HNSCC). A primary cause of metastasis is the proteolytic
25 degradation of the extracellular matrix (ECM). The plasminogen activator urokinase (PLAU) is
26 involves in the transformation of plasminogen to plasmin leading to hydrolyzation of ECM
27 related proteins. However, the role of PLAU expression in HNSCC is unclear and worth to be
28 investigated.
29 **Methods.** PLAU expression profiles and clinical parameters from multiple HNSCC datasets
30 were used to investigate the relationship of PLAU expression and HNSCC survival. GO and PPI
31 network were established on PLAU-related downstream molecular. The stroma scores were
32 deconvoluted for analysis of PLAU's association with the immune environment. ROC analysis
33 was applied to show the performance of PLAU in predicting HNSCC prognosis. **Results.** PLAU
34 mRNA was significantly elevated, as opposed to its methylation, in HNSCC tumor samples over
35 normal specimens (all p<0.01). Univariate and multivariate cox analysis showed PLAU could be
36 an independent indicator for HNSCC prognosis. Combining with neck lymph node status, the
37 AUC of PLAU in predicting 5-years overall survival reached to 0.862. GO enrichment analysis

38 showed the major biological process (extracellular matrix organization and the P13K-Akt
39 signaling pathway) may involve to the possible mechanism of PLAU's function on HNSCC
40 prognosis. Furthermore, PLAU expression was positively correlated with stroma cell score, M1
41 type macrophages, and negatively associated with CD4 + T cell, Tregs cell, and follicular helper
42 T cell.

43 **Conclusions.** PLAU might be an independent biomarker for predicting outcomes of HNSCC
44 patients. The elevated expression of PLAU was associated with HPV positivity and neck node
45 status. PI3K-Akt pathway and aberrant proportions of immune cells might underly the
46 mechanism of PLAU's oncogene role in HNSCC.

47 **Keywords.** The plasminogen activator urokinase (PLAU), head and neck squamous cell
48 carcinomas (HNSCC), The Cancer Genome Atlas (TCGA), Gene Expression Omnibus
49 (GEO),bioinformatics analysis, Survival

Introduction

51 Head and neck squamous cell carcinomas (HNSCC) are among the most aggressive
52 malignancies and over 50% of patients present with locally advanced or metastatic disease(Torre
53 et al. 2015). More than 830,000 patients are diagnosed and over 430,000 patients die from this
54 disease worldwide annually (Cramer et al. 2019). This disease is characterized by low survival
55 rates, high recurrence rates, and/or regional lymph node that become metastatic (Siu et al. 2019).
56 Although the examination and treatment has been improving in recent decades, the overall 5-year
57 survival rate of HNSCC patients doesn't increase remarkably (Yang et al. 2019). Prognosis
58 prediction is crucial for physicians to offer consultants and personized treatment. However,
59 clinical parameters such as TNM classification are the main sources physicians generally relying
60 on for predicting patient outcome and making therapeutic decision, which is inaccurate in many
61 situation(Kowalski et al. 2005; Moertel et al. 1995).It's well accepted that molecular biomarkers
62 may facilitate the prognosis prediction for SCCHN patients(Kang et al. 2015; Leemans et al.
63 2018). Currently, there is no matured biomarkers is approved for HNSCC prognosis prediction.
64 Therefore, it is expected and worth that the identification of novel biomarkers assisting with
65 patient care and survival improvement.

66 Metastasis is one of the major events leading to unfavorable survival time for HNSCC patients
67 (Chen et al. 2018). The mechanism of HNSCC metastasis is unknown, accumulated evidence
68 show that ECM reconstruction may involve providing a physical and biochemical niche for
69 humor cell metastasis(Hanahan & Weinberg 2011; Murphy & Courtneidge 2011).

70 PLAU belonging to the S1 serine peptidase of Clan PA, also named Urokinase-type plasminogen
71 activator (uPA) is a proteinase involving in the transformation of plasminogen to plasmin(Ai et
72 al. 2020). And it could hydrolyze ECM remodeling related proteins and activates growth
73 factors(Danø et al. 2005). Some studies report that the expression level of PLAU is significantly
74 correlated to tumor cell lymph node and distant organ metastasis (Gutierrez et al. 2000).
75 Emerging evidence implies that PLAU plays a critical role in the initiation and development of
76 various cancers including breast cancer, colorectal cancer, and esophageal cancer(Li et al. 2017;
77 Lin et al. 2019; Novak et al. 2019).However, the role of PLAU needs to be explored further in

78 HNSCC. Here, we applied multiple datasets to evaluate the increased expression of PLAU in
79 HNSCC tumor samples as compared to adjacent tissues and confirm it as an independent
80 prognosis predictor of HNSCC patients in different angles and levels. The co-expression network
81 and scores of tumor immune microenvironment were established and analyzed in this study as
82 well, which could be interpreted to possible mechanism of PLAU's role in HNSCC patients.

Materials & Methods

Data collection and normalization

85 GSE25099 from 79 HNSCC patients(Peng et al. 2011), GSE13601 consisting of 37 HNSCC
86 patients(Estilo et al. 2009), GSE65858 from 270 HNSCC patients(Wichmann et al. 2015),
87 GSE136037 from 49 HNSCC patients(Alfieri et al. 2020), and The Cancer Genome Atlas
88 (TCGA) -HNSC cohorts including 546 HNSCC HTSeq-counts, methylation profiles and related
89 clinical information were downloaded. CalcNormFactors was used to calculate normalization
90 factors to scale the gene expression in TCGA dataset (Anders & Huber 2010; Le et al. 2019).
91 Youden index (sensitivity + specificity -1) was used to calculate the best cutoff of survival
92 analysis by R package ("SurvivalROC")(Huang et al. 2017; Luo & Xiong 2013). GSE65858 and
93 GSE136037 dataset from the GEO database were converted to transcripts per million
94 (TPM)(Zhao et al. 2020a). And the term "N-" means primary lesions in HNSCC patients without
95 neck lymph node metastasis, and "N+" means primary lesions in HNSCC patients with neck
96 lymph node metastasis. The normalized data provided from original studies in other datasets
97 were used in this study directly.

Survival analysis

99   Briefly, the expression of PLAU was categorized into low or high by 'Maximally Selected Rank
100 Statistics' (maxstat) method(Lausen & Schumacher 1992). The cutoff value of PLAU mRNA
101 expression in TCGA was 9532 in Table 1. Older or younger is classified based on its mean age.
102 The overall survival (OS), progression-free interval (PFI) or recurrence free survival (RSF)
103 curves were visualized by Kaplan-Meier plots. Univariate and multivariate Cox regression
104 analysis was applied to death hazard ratios calculation after the proportional hazard assumption
105 was tested.

Go analysis and co-expression network establishment

107 Go enrichment analysis was performed by Bioconductor package "clusterProfiler" (Yu et al.
108 2012). The method of KEGG enrichment analysis was performed same as the Go enrichment
109 analysis. The co-expression genes were screened using R packages ("limma").From the TCGA-
110 HNSCC databases, we used Pearson correlation coefficients (|Pearson correlation coefficient| >
111 0.5 and P-value <0.001) and the z-test to examine the correlation between PLAU expression
112 level and co-expression genes. STRING database and Cytoscape tool were used to construct the
113 protein-protein-interactions (PPI) of genes (Struk et al. 2019).

Immune cell environment analysis

115 Estimation of stromal and immune cells in malignant tumor tissues using Expression data
116 (ESTIMATE) is a tool for predicting tumor purity, and the presence of infiltrating stromal/immune
117 cells in tumor tissues using gene expression data (Yoshihara et al. 2013). We used R packages

118 ("estimate") to get immune-environment scores in HNSCC patients. R packages
119 ("CIBERSORT.R", https://cibersortx.stanford.edu/)(Newman et al. 2015) was used to
120 deconvoluted 22 common immune cell proportions in HNSCC patients. The correlation of PLAU
121 expression with immune environment and 22 immune cells were investigated by Pearson test.
122 **Software and statistical analyses**
123 GraphPad Prism 8 or R studio (version 3.5.3) was used to evaluate all data (Kamdem et al. 2019;
124 Le & Huynh 2019). Chi-squre or Fisher's exact test was performed to compare the   differences
125 expression of PLAU across different groups. P < 0.05 was considered statistically significant.
126 The detailed codes and other packages' information were included in the supplementary
127 materials.

Results

129 **PLAU mRNA is over-expressed in HNSCC**
130 To discover the expression of PLAU mRNA in HNSCC, we analyzed three independent patient
131 cohorts, which showed a consistent result that PLAU mRNA expression was elevated in HNSCC
132 tumors than normal tissues(all p < 0.01, Figure 1A-C). And the overexpression of PLAU mRNA
133 was confirmed in 10 different HNSCC cell lines as compared to 4 types of human keratinocyte
134 cell lines (p = 0.002, Figure 1D). Furthermore, PLAU mRNA in HPV positive HNSCC samples
135 was interposed between adjacent normal tissues and HPV negative tumors (Supplementary
136 Figure 1A). There was significantly less PLAU mRNA in HPV positive tumors than HPV
137 negative ones according to another patient cohort (Supplementary Figure 1B).
138 **Association of PLAU mRNA with neck lymph node status in HNSCC**
139 In order to study the role of PLAU mRNA may play in HNSCC patients, the relationship of
140 PLAU mRNA and clinical parameters was further characterized in TCGA-HNSC cohort. As
141 shown in Table 2, there was no significantly different expression of PLAU mRNA in different
142 age, gender, clinical stage, and tumor stage, but the difference of PLAU in HPV positivity and
143 neck node status was considered as significant(p = 0.001, p = 0.033, respectively). Higher
144 expression of PLAU was founded in patients with neck lymph node metastasis than patient
145 without neck lymph node metastasis in another independent cohort as well (Figure 1E).
146 **PLAU is an independent predictor of HNSCC prognosis**
147 Considering the above findings, we continued to analysis the possible correlation of HNSCC
148 survival time and PLAU expression. As shown in Table 3, age, clinical stage, tumor size, neck
149 lymph node status, HPV positivity and PLAU expression was considered to be significantly
150 associated with overall survival time in univariate cox analysis of 496 HNSCC patients.
151 Multivariate cox analysis indicated the hazard ratio of death was reached to 1.52 when high
152 PLAU expressed HNSCC patients compared to patients with low PLAU expression after
153 excluding the potential affections from age, tumor size, neck node metastasis and HPV positivity
154 (Figure 2A, p =0.012, 95%CI: 1.09-2.10).
155 The survival curves of PLAU expression were visualized by Kaplan-Meier plots (Figure 2B),
156 which implied that high PLAU expressed HNSCC patients had decreased overall survival
157 probability than patients with low PLAU expression (Figure 2B). The same finding could be

158 observed in another independent HNSCC cohort (Figure 2C). Additionally, high expression of
159 PLAU in HNSCC patients was founded to predict unfavorable outcomes in terms of PFI and
160 RSF (Figure 2D-E).

161 **Performance of PLAU expression in predicting 5-year overall survival outcomes of**
162 **HNSCC patients**
163 The predictive performance of PLAU expression in 5-year overall survival outcomes of HNSCC
164 patients was analyzed by ROC analysis. As shown in Figure 3, the areas under the ROC curve
165 (AUCs) of PLAU expression was 0.795, higher than HPV status(AUC: 0.531), neck node
166 status(AUC: 0.58) and tumor size stage(AUC: 0.532). Combining PLAU expression and neck
167 node status as an integrated factor (N+PLAU), the AUC was 0.862, with an 88.5% sensitivity,
168 64% specificity, 70.4% positive predictive value and 80.1% negative predictive value. Taken
169 together, PLAU itself or combined with neck node status may facilitate the overall survival
170 prediction for HNSCC patients.

171 **Hypomethylation of PLAU in HNSCC patients**
172 A growing body of literature reports that DNA methylation and mRNA expression most likely to
173 be negative correlated(Huang et al. 2015). Methylation profiles of 496 TCGA-HNSC patients
174 were analyzed to investigate the possible upstream reasons for elevated PLAU mRNA
175 expression in HNSCC tumors. As shown in Figure 4, the methylated level of PLAU was
176 successively and significantly decreased in adjacent normal tissues, HPV positive tumors and
177 HPV negative tumors (Figure 4A). The PLAU methylation and mRNA expression was
178 negatively correlated (Figure 4B, R = -0.43, p < 0.001). As expected, patients with
179 hypomethylated PLAU, indicating higher expression of PLAU mRNA, had worse overall
180 survival outcomes than patients with hypermethylated PLAU (Figure 4C).

181 **Network establishment for PLAU correlated genes in HNSCC**
182 To further understand the possible downstream reasons a total of 205 genes was correlated with
183 PLAU expression in TCGA-HNSCC patients(21 of 205 genes were negatively correlated with
184 PLAU and 184 genes were positively correlated with PLAU). The top 20 genes of positively or
185 negatively correlated with PLAU are shown in a Heatmap (Figure 5A). The interaction network
186 of these 205 genes was established based on STRING and Cytoscape (Figure 5B). Next, we
187 performed GO and KEGG enrichment analysis to understand the potential biological functions of
188 PLAU in HNSCC. GO analysis showed that the major biological process (extracellular matrix
189 organization), cellular component (extracellular matrix), and molecular functions (cell adhesion
190 molecule binding) may contribute to PLAU related biology (Figure 5C). KEGG pathway
191 analysis illuminated that the P13K-Akt signaling pathway, human papillomavirus infection,
192 proteoglycans in cancer, and focal adhesion as significantly enriched by the PLAU co-expressed
193 genes (Figure 5D).

194 **Distributions of tumor infiltrating immune cell in HNSCC patients with different PLAU**
195 **expression**
196 More and more evidence revealed the tumor immune microenvironment is a crucial factor in
197 tumor biology(Mao et al. 2020). To interpret the role of PLAU expression in HNSCC based on
198 immunity conception, the scores or proportions of tumor infiltrating cells were compared in

199   TCGA-HNSCC cohort. It shows that PLAU expression was positively correlated with stromal
200   score (Figure 6A). Further analysis found the expression level of PLAU was positively correlated
201   with M1 type macrophages, negatively associated with CD4 + T cell, Tregs cell, and follicular
202   helper T cell (Figure 6B-E) (All p<0.05).

Discussion

204   Our study, using multiple publicly available profiles in HNSCC cohorts and cell lines, confirmed
205   that PLAU mRNA was over-expressed and associated with neck node lymph metastasis in
206   HNSCC tumors. And, we showed that PLAU expression might be an independent prognosis
207   index for HNSCC patients, which consistent with many other cancer reports(Mahmood et al.
208   2018)including breast cancer, prostate cancer, ovarian cancer, sarcoma, melanoma, gastric
209   cancer, esophageal cancer, and colorectal cancer. Furthermore, the DNA methylation level and
210   mRNA expression level of PLAU was investigated in our work. As far as we know, this is the
211   first report to link PLAU methylation level with its mRNA expression in cancer samples. There
212   are many possible explanations, such as generic regulation, epigenetic modulation, or mRNA
213   decay, for aberrant expression of mRNA like PLAU. Our work implied that the increased
214   expression of PLAU in HNSCC tumors might be contributed by its hypomethylated levels to
215   some extent. Genetic mutations and epigenetic alterations have critical functions in modulating
216   oncogenes' transcription in human carcinomas(Wang et al. 2019b; Zhou et al. 2019). The
217   methylation values of DNA could be a prognosis biomarker in cancer(Teixeira et al. 2019),
218   which supported our finding that HNSCC patient with hypomethylated PLAU might have a
219   worse survival outcome.
220   PLAU is a gene encodes for urokinase plasminogen activator (uPA).The detailed mechanism that
221   underlying PLAU's role in HNSCC remain unclear. But it was indicated to involve in the
222   transformation of inactive plasminogen into active plasminogen, which plays an important role
223   in a series of transfer cascades(Choong & Nadesapillai 2003). Previous research has shown that
224   PLAU and type plasminogen activator (tPA) mediated the plasminogen activator (PA)
225   system(Mahmood et al. 2018). PLAU can increase cell proliferation through the activate growth
226   factors or adhesion molecules, for example, VEGF, TGF-β and the α5β1 integrins(Aguirre-Ghiso
227   et al. 2001; Duffy 2004; Ulisse et al. 2009). PLAU could increase cell adhesion and migration
228   during metastasis and proliferation of tumor cells(Zhao et al. 2020b), which may explain our
229   finding that elevated expression of PLAU in node metastasis tumors. Under hypoxia conditions,
230   PLAU expression can activate downstream Akt and Rac1 signaling pathways, thus promoting
231   EMT and cell invasion(Lester et al. 2007).In our GO and pathway enrichment assays, PI3K-Akt
232   pathway was enriched by PLAU co-expressed genes. Akt activation maybe the downstream
233   pathway of PLAU leading to HNSCC cell invasion and metastasis.
234   Growing evidence suggests that cells (such as macrophages, T cells, neutrophils, lymphoid cells
235   and so on) in the immune microenvironment are related to tumor escape and
236   progression(Hinshaw & Shevde 2019). Kipp Weiskopf et al. found that CD47 engaged signal-
237   regulatory protein alpha , which acts as an inhibitory receptor on macrophages to promote
238   immune evasion(Weiskopf et al. 2016). Recent data suggest that exposure to immune checkpoint

239 inhibitors (ICI) increase tumor sensitivity to chemotherapy in HNSCC(Saleh et al. 2019).
240 Therefore, the investigation of the relationship between HNSCC and the immune
241 microenvironment may help us to both diagnose and treat more effectively. In our study, we
242 showed that PLAU expression is positively correlated with stromal score. The stromal-immune
243 score represents a prognosis stratification tool intended to be developed as reliable prognostic
244 signatures in gastric cancer (Wang et al. 2019a). Aberrance of macrophage function significantly
245 contributes to disease progressions, such as in the case of cancer, fibrosis, and diabetes
246 (Ngambenjawong et al. 2017). By analyzing the immune cell proportions, we identified PLAU
247 expression was positively correlated with M1 type macrophages, and   negative association with
248 CD4+ T cell, Tregs cell, and follicular helper T cell. These associations could explain the role of
249 PLAU in HNSCC prognosis from the immunological respective. In addition, we found that
250 PLAU expression was reduced in HPV positive HNSCC tumors as compared to HPV negative
251 ones. HPV positivity is well-accepted as a strong survival favorable factor in head and neck
252 cancer patients, which indirectly supports that low expression of PLAU predicts a better survival
253 in HNSCC patients. And PLAU activity could be a partial reason for HPV's role in HNSCC
254 tumors.
255 Back to clinical significance, the performances of PLAU and other independent prognosis
256 indicators in predicting HNSCC 5-year survival outcome were investigated. Although HPV
257 status, tumor size or neck node status is independent prognosis indicator in HNSCC, their AUC
258 is very low according to the ROC assays. However, the AUC of PLAU expression reach 0.795,
259 higher than other clinical parameters. Moreover, the combination of PLAU and neck node status
260 could predict HNSCC 5-year overall survival outcomes with an 88.5% sensitivity and 64%
261 specificity, which demonstrated the capability of PLAU expression in HNSCC prognosis.
262 Certainly, we need to be aware that our findings require the further validations from in vivo and
263 vitro experiments although the conclusion was confirmed across five independent cohorts.
264 Another limitation in this study is the platform used in different cohorts is different, which may
265 produce bias to the data analysis and bring hardness for the deep integrated analysis.

Conclusions

267 All in all, PLAU might be an independent biomarker for predicting outcomes of HNSCC
268 patients. The elevated expression of PLAU was associated with HPV positivity and neck node
269 status. PI3K-Akt pathway and aberrant proportions of immune cells might underly the
270 mechanism of PLAU's oncogene role in HNSCC.

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Figure 1

PLAU mRNA is over-expressed in HNSCC

(A, B) The expression of PLAU mRNA in normal and HNSCC tumor tissues was detected from the GEO databases. **(C)** Compared the difference expression of PLAU mRNA between tumor tissues and pair normal tissues in HNSCC. **(D)** The overexpression of PLAU mRNA was confirmed in HNSCC cell lines and human keratinocyte cell lines. **(E)** The difference expression of PLAU mRNA in neck lymph node status. "N-" means patients without neck lymph node metastasis, and "N+" means patients with neck lymph node metastasis.

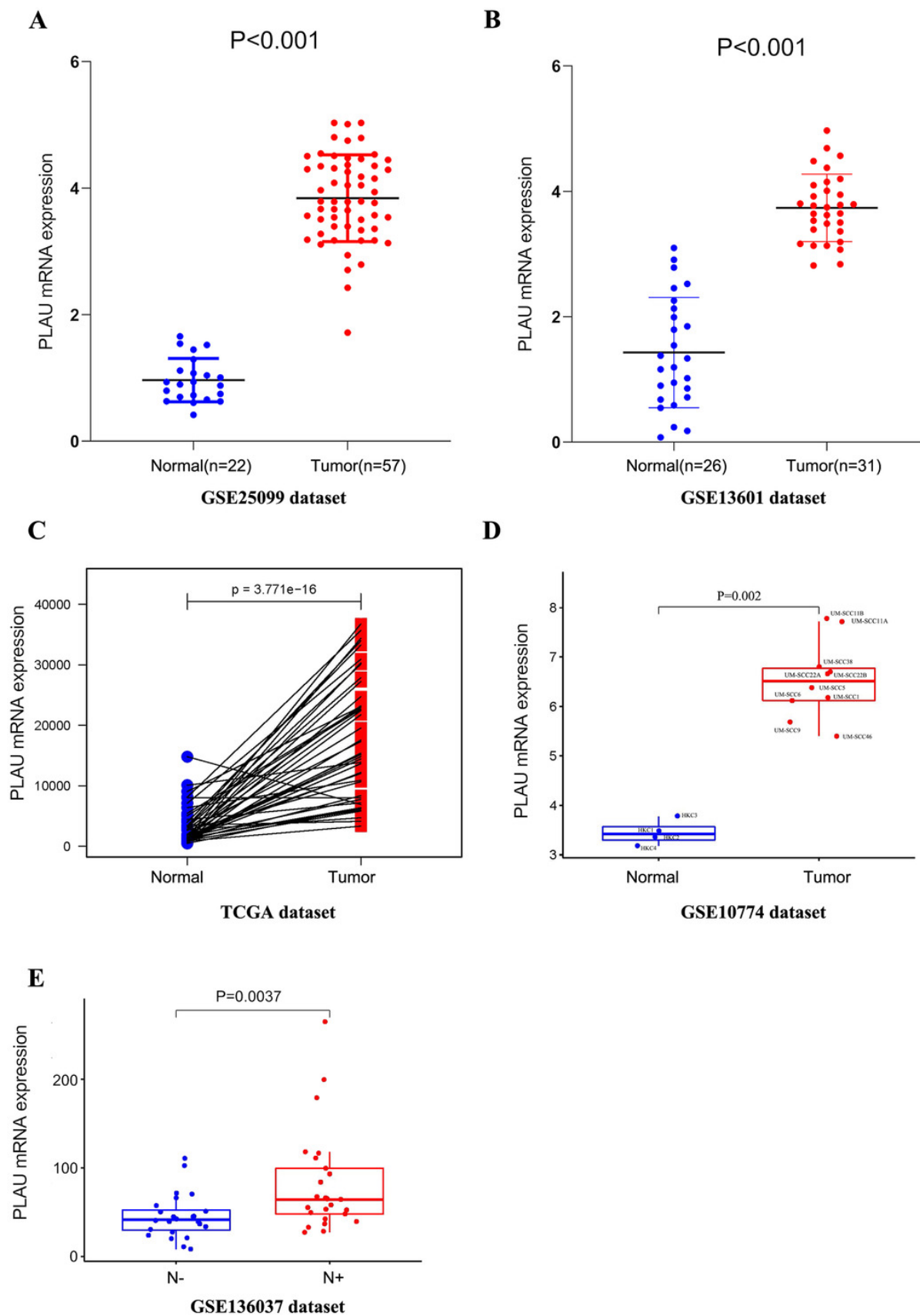



Figure 2

PLAU is an independent predictor of HNSCC prognosis

(A) Multivariate cox analysis related to PLAU. **(B, C)** The TCGA dataset and the GSE65858 database were used to assess the effect of PLAU expression on overall survival (OS). **(D,E)** The TCGA dataset assessed the effect of PLAU expression on progression-free interval (PFI) and relapse free survival (RFS)

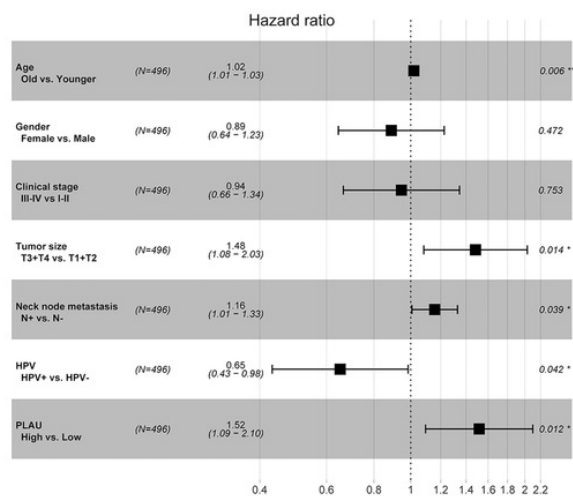
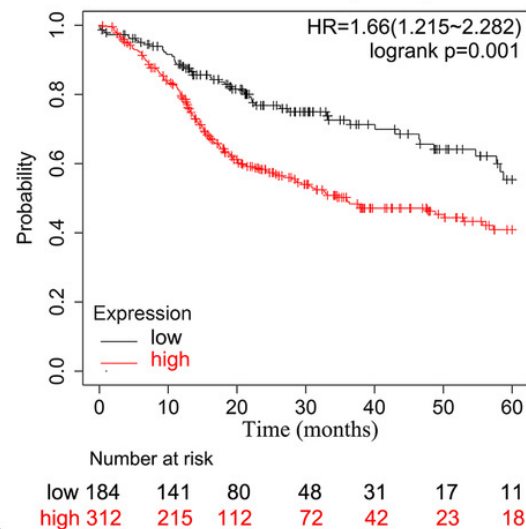
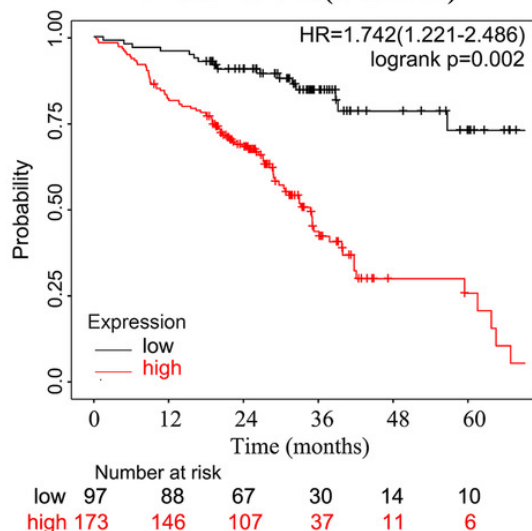
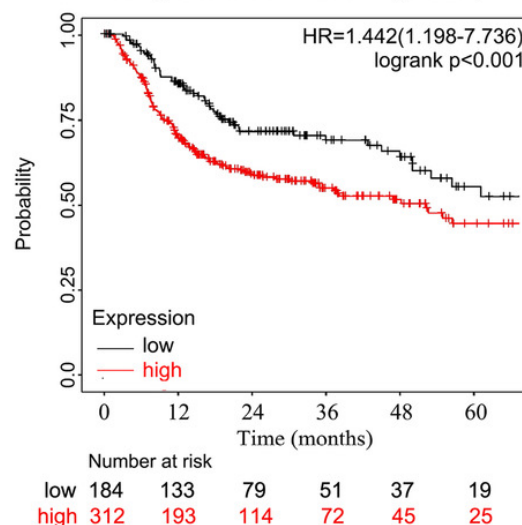
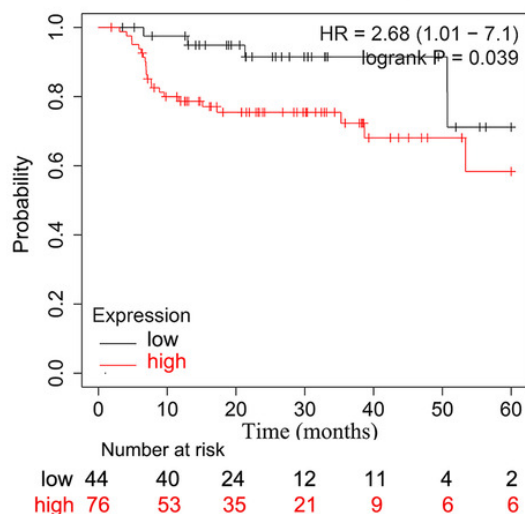



Figure 3

ROC analysis of the PLAU expression with the clinical parameters of HNSCC in TCGA datasets.

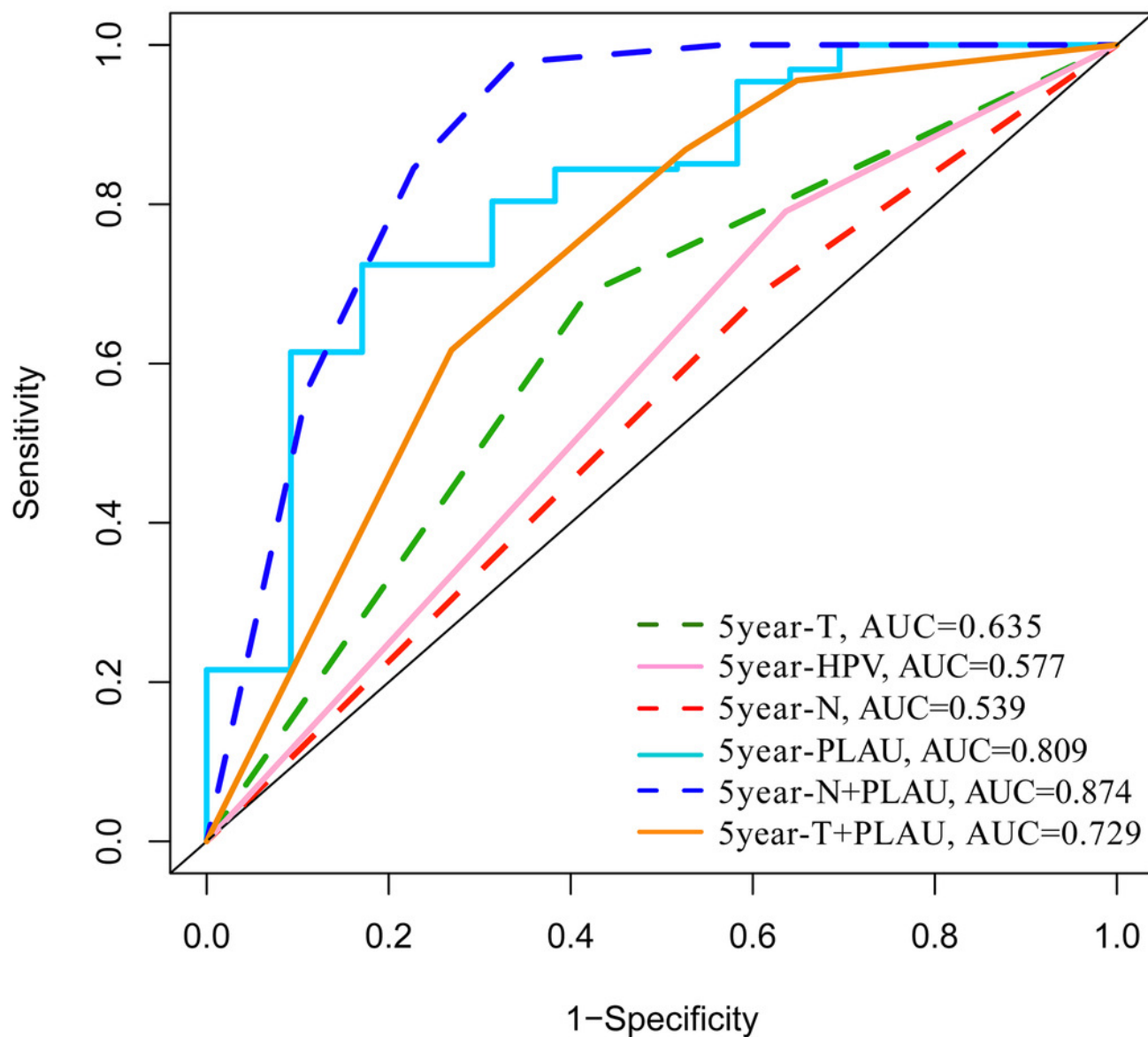



Figure 4

Relationship between PLAU methylation and HNSCC patients

(A) S eparating the groups by HPV status, PLAU methylation compared with the normal samples were evaluated in HNSCC samples of TCGA databases. **(B)** The correlation between the expression of PLAU and the beta value of methylation were evaluated by TCGA datasets. **(C)** The methylation of PLAU on OS by TCGA datasets.

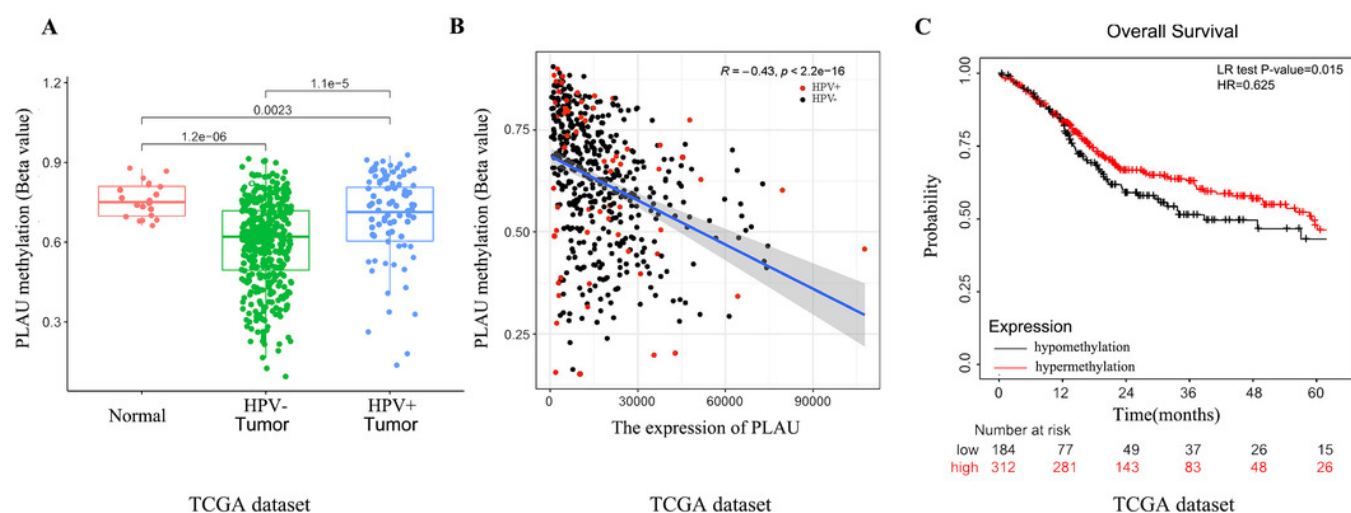



Figure 5

Network establishment for PLAU correlated genes in HNSCC

(A) The top 20 genes of positively or negatively correlated with PLAU were showed in Heatmap. **(B)** The PPI networks of PLAU interaction partners generated by STRING and Cytoscape. The color represents the degree score (represent the intensity of the hub interacting with its neighbors). Degree score <0.5 represented low value (colored yellow), degree score ≥0.5 represented high value (colored orange or red). **(C)** Major biological process, cellular component and molecular functions of PLAU biology by GO enrichment analysis. **(D)** KEGG pathway analyses further illuminate enriched function pathway related to PLAU.

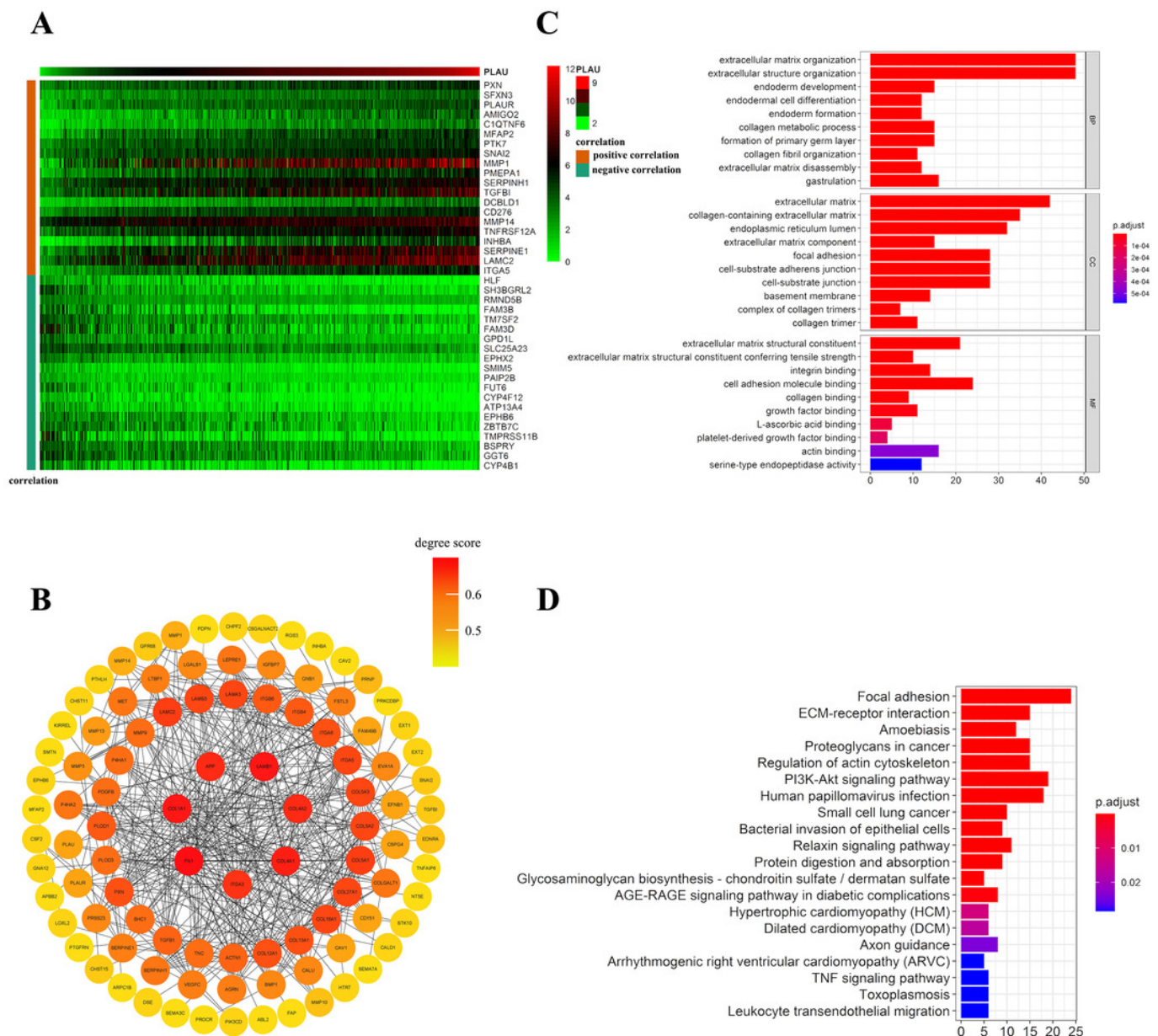
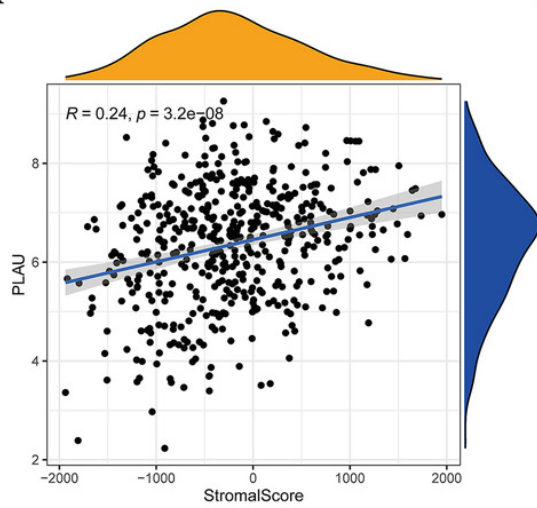
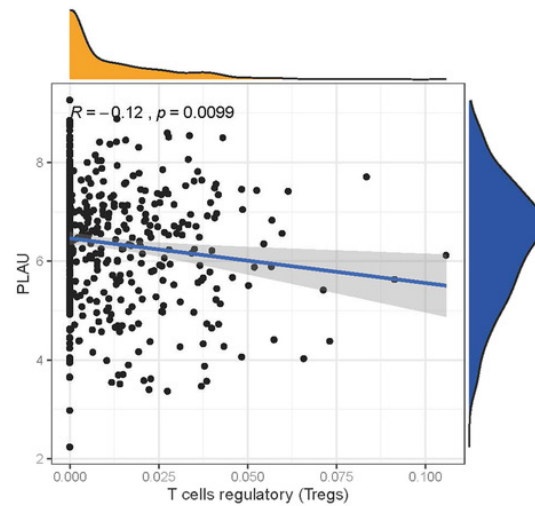
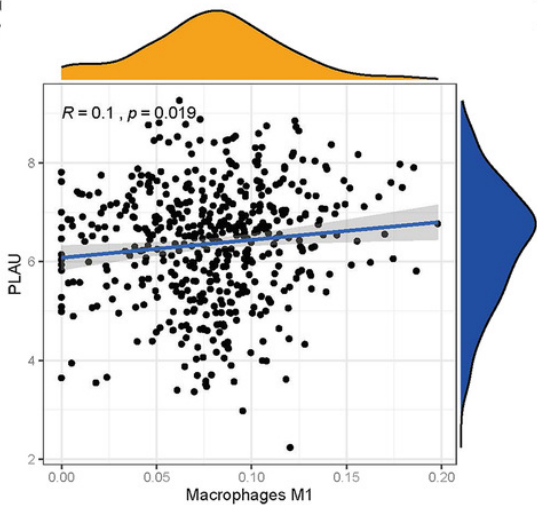
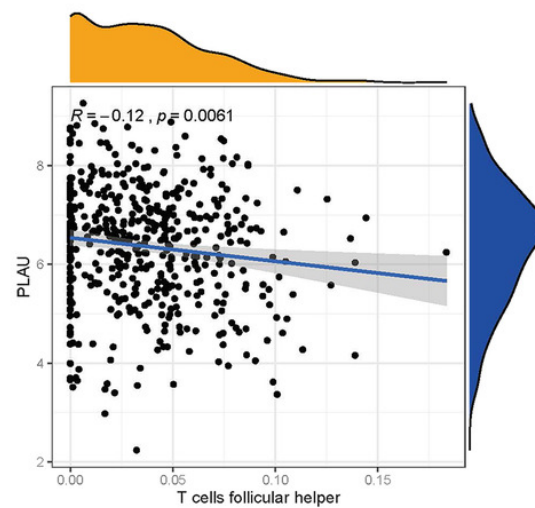
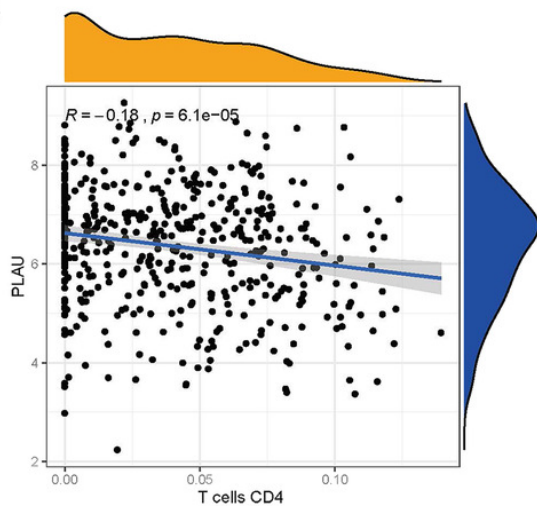



Figure 6

Relationship between PLAU and tumor immune microenvironment of HNSCC

(A) The expression of PLAU is positively correlated with stromal score. **(B-E)** The expression of PLAU was correlated with Tregs cell, M1 type macrophages, follicular helper T cell and CD4 + T cell.

Cutoff identification for survival time by clinical parameters

1 **Table 1. Cutoff identification for survival time by clinical parameters**

Index	Cutoff	AUC	95%CI	Sensitivity	Specificity	PPV	NPV
T	/	0.532	0.451~0.616	0.711	0.474	0.523	0.669
N	/	0.58	0.495~0.668	0.768	0.437	0.549	0.679
HPV	/	0.531	0.437~0.624	0.702	0.458	0.569	0.602
PLAU	9532	0.795	0.724~0.863	0.901	0.679	0.749	0.798
N+PLAU	/	0.862	0.663~0.887	0.885	0.639	0.704	0.801
T+PLAU	/	0.778	0.821~0.906	0.995	0.619	0.748	0.812

PPV: Positive Predictive Value. NPV: Negative Predictive Value

The PLAU expression in HNSCC patients with different clinical parameters

1 **Table 2. The PLAU expression in HNSCC patients with different clinical parameters**

Clinical parameters	PLAU mRNA expression		P-value
	Low (n=184)	High (n=312)	
Age(years)			0.949
Mean (SD)	61.6(11.8)	60.7 (12.0)	
Median [Min, Max]	61.0 [26.0, 87.0]	60.5[19, 90]	
Gender			0.943
Female	49(36.8%)	84(37.2%)	
Male	135(63.2%)	228(62.8%)	
Clinical stage			0.913
I-II	136(37%)	232(37.5%)	
III-IV	48(63%)	80(62.5%)	
Tumor stage			0.23
T1-2	88(47.8%)	126(40.4%)	
T3-4	93(50.6%)	182(58.3%)	
Missing	3(1.6%)	4(1.3%)	
Neck nodal metastasis			0.033*
N-	82(44.6%)	105(33.7%)	
N+	101(54.9%)	202(64.7%)	
Missing	1(0.5%)	5(1.6%)	
HPV			0.001***
HPV-	134(72.8%)	270(86.5%)	
HPV+	49(26.7%)	38(12.2%)	
Missing	1(0.5%)	4(1.3%)	

2 **P<0.05*, P<0.01**, P<0.005*****

The hazard ratio of PLAU expression and clinical parameters in 496 HNSCC patients

1   **Table 3. The hazard ratio of PLAU expression and clinical parameters in 496 HNSCC**
2   **patients**

Parameter	Univariate analysis		
	HR	95%CI	P-value
Age			0.018*
Older vs. Younger	1.021	1.003~1.034	
Gender			0.087
Female vs. Male	0.728	0.507~1.047	
Clinical stage			<0.001***
III-IV vs I-II	2.902	1.605~5.25	
Tumor size			0.003**
T3+T4 vs. T1+T2	1.562	1.158~2.109	
Neck node metastasis			<0.001***
N+ vs. N-	1.725173	1.258~2.365	
HPV			<0.001***
HPV+ vs. HPV-	0.468349	0.312~0.703	
PLAU			0.001***
High vs. Low	1.667282	1.218-2.282	

P<0.05*, P<0.01**, P<0.001***