The relationship between PLOD1 expression level and glioma prognosis investigated using public databases

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Abstract

Background. Glioma is the most common type of intracranial tumor with high malignancy and poor prognosis despite the use of various aggressive treatments. Targeted therapy and immunotherapy are not effective and new biomarkers need to be explored. Some Procollagen-lysine 2-oxyglutarate 5-dioxygenase (*PLOD*) family members have been found to be involved in the metastasis and progression of tumors. Both *PLOD2* and *PLOD3* had been reported to be highly expressed in gliomas, while the prognostic value of *PLOD1* remains to be further illustrated, so we want to investigate the *PLOD1* expression in glioma and its clinical implication.

Methods. We collected gene expression and corresponding clinical data of glioma from the Chinese Glioma Genome Atlas (CGGA) database, The Cancer Genome Atlas (TCGA) database and Gene Expression Omnibus (GEO) database. First, we analyzed the expression and mutation of *PLOD1* in gliomas and its relationship with clinicopathologic characteristics. Then, we conducted survival analysis, prognostic analysis and nomogram construction of the *PLOD1* gene. Finally, we conducted gene ontology (GO) enrichment analysis and gene set enrichment analysis (GSEA) to explore possible mechanisms and gene co-expression analysis was also be performed.

Results. The results showed that the expression level of *PLOD1* was higher in gliomas than normal tissues, and high expression of *PLOD1* was related to poor survival which can serve as an oncogenic factor and an independent prognostic indicator for glioma patients. Both the GO and GSEA analysis showed high expression of *PLOD1* were enriched in Extracellular matrix (ECM) related pathways, the co-expression analysis revealed that *PLOD1* was positively related to *HSPG2*, *COL6A2*, *COL4A2*, *FN1*, *COL1A1*, *COL4A1*, *CD44*, *COL3A1*, *COL1A2* and *SPP1*, and high expression of these genes were also correlated to poor prognosis of glioma.

Conclusions. The results showed that high expression of *PLOD1* leads to poor prognosis, and *PLOD1* is an independent prognostic factor and a novel biomarker for the treatment of glioma. Furthermore, targeting *PLOD1* is most likely a potential therapeutic strategy for glioma patients.

Introduction

40   Gliomas are the most common type of primary intracranial tumor with high malignancy and poor
41   prognosis, especially in high-grade gliomas (Ostrom et al., 2020). The current standard approach
42   of treatment is maximum surgical resection followed by adjuvant radiotherapy and
43   chemotherapy (van den Bent et al. 2017; Stupp et al., 2009), and newly developed alternating
44   electric field therapy has been recommended for glioblastoma (GBM) in recent years (Sampson,
45   2015). Despite the using of multiple aggressive therapies, the overall survival of gliomas
46   remained poor, so it is urgent to explore more effective treatment methods. In recent years,
47   immunotherapy and targeted therapy have made great progress in many other tumors, but have
48   little effect in glioma (Fecci & Sampson, 2019), and although some of the molecular features
49   showed some prognostic values (Cairncross et al., 2012; Hegi et al., 2005), none of them had
50   became a target for targeting therapy, thus developping novel and targeted therapeutic options is
51   urgent.
52   Extracellular matrix (ECM) is an important constituent of tumor microenvironment, corelating
53   with tumor development and progression. Among the various ECM components, collagens are
54   the most abundant proteins, and its deposition and cross-linking are closely related to tumor
55   proliferation and invasion (Jover et al., 2018). Procollagen-lysine 2-oxyglutarate 5-dioxygenase
56   (PLOD) catalyzed hydroxylysine residue, which is critical for the formation of covalent cross-
57   link (Qi & Xu, 2018). An increasing number of evidences indicate that the *PLOD* family, which
58   consists of *PLOD1*, *PLOD2* and *PLOD3*, plays an important role in the development and
59   progression of tumors. Both *PLOD2* and *PLOD3* had been reported to be highly expressed in
60   gliomas and were associated with tumor progression and prognosis (Song et al., 2017; Tsai et al.,
61   2018). Some previous studies revealed that *PLOD1* promoted tumorigenesis and metastasis in
62   osteosarcoma, bladder cancer and esophageal squamous cell carcinoma (Wu et al., 2020;
63   Yamada et al., 2019; Li et al., 2017), while the expression and prognostic role of *PLOD1* in
64   glioma remain to be further illustrated.
65   Bioinformatics analysis using high-throughput sequencing and clinical data is developing rapidly
66   to identify sensitive biomarkers and prognostic factors for a variety of tumors, including gliomas.
67   The acetylation modification and kinase activity of *PAK1* were considered to be an instrumental
68   role in hypoxia-induced autophagy initiation and maintaining GBM growth, and *PAK1* might
69   represent potential therapeutic targets for GBM treatment (Feng et al., 2020). *HIST1H2BK* was
70   identified as an indicator of poor prognosis and a promising biomarker for the treatment of low-
71   grade glioma (LGG) (Liu et al., 2020). Similarly, *ARL9* had been shown its prognostic value in
72   LGG, and probably played an important role in immune cell infiltration (Tan et al., 2020). All
73   these markers are possible used for advanced decision-making processes in the future, however,
74   many more potential prognostic indicators still need to be explored in gliomas.
75   Therefore, this study attempts to analyze the *PLOD1* gene expression levels in glioma and
76   normal tissue, using public database. We also explored the relationship between *PLOD1*
77   expression and clinical characteristics as well as prognosis. Finally, we identified the *PLOD1*-
78   related signaling pathways and suggested that *PLOD1* acted as a cancer-promoting factor in

79   tumor progression, providing a potential prognostic biomarker and therapeutic target for glioma
80   patients.

Materials & Methods

Data collection and download

The clinical data and gene expression data were obtained from Chinese Glioma Genome Atlas
(CGGA, http://www.cgga.org.cn/) database. Two datasets were downloaded containing 1018
samples (Wang et al., 2015; Liu et al., 2018; Bao et al., 2014; Zhao et al., 2017) up to May 6,
2020, and 20 non-glioma brain tissues were downloaded for analyzed. In addition, The Cancer
Genome Atlas (TCGA, https://portal.gdc.cancer.gov/) database containing 592 glioma samples
was collected for validation. There were 449 LGG samples and 143 GBM samples, respectively.
Then, we searched "glioma" and "GEO" in the Gene Expression Omnibus (GEO,
https://www.ncbi.nlm.nih.gov/geo/) database and selected GSE4290, GSE7696 and GSE50161
as validation sets (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE4290,
https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE7696,
https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE50161). GSE4290 dataset contains
153 glioma samples and 23 normal samples (Sun et al., 2006), GSE7696 dataset contains 80
glioma samples and 4 normal samples (Lambiv et al., 2011), and GSE50161 dataset contains 117
glioma samples and 13 normal samples (Griesinger et al., 2013). Before further analysis, RNA
sequencing data were log2-transformed. All these databases were screened to eliminate samples
missing clinical information.

**Gene expression analysis, GEPIA database analysis, mutation analysis and Cancer Cell
Line Encyclopedia (CCLE) analysis**

The expression data of *PLOD1* in normal brain tissue and glioma in CGGA dataset were
imported into GraphPad Prism 8 software for analysis, and then validated by GSE4290 dataset.
In addition, *PLOD1* expression analyses of GBM and LGG were also performed in GEPIA
(http://gepia.cancer-pku.cn/) (Tang et al., 2017). In addition, we performed mutation analysis to
better comprehend the genomics profile of *PLOD1* based on the cBioPortal online database (Gao
et al., 2013). Furthermore, we used the CCLE database (https://portals.
broadinstitute.org/ccle/home) to assess *PLOD1* expression in different cancers.

**Correlations between *PLOD1* expression and clinical outcomes and clinicopathologic
characteristics**

The data from the CGGA datasets were mainly used to research the prognostic role of *PLOD1* in
gliomas by using R software. According to the median expression level of *PLOD1*, high and low
group were divided. The "survival" and "survminer" package were used in R software to plot
survival curves for different *PLOD1* expression level. And then, the survival data of different
IDH and MGMT promoter methylation status were imported into GraphPad Prism 8 software for
survival analysis, so as to explore the survival of *PLOD1* expression levels in different molecular
types of gliomas. We used the "survival ROC" package to calculate receiver operator
characteristic (ROC) curves for *PLOD1* at 1, 3, and 5 years using the Kaplan-Meier method.
Univariate and multivariate Cox analysis were also performed to assess the

119 predictive value of *PLOD1* at a significance level of P < 0.001. Based on the TCGA datasets,
120 survival curve and ROC curves were performed to validate. To develop an individual prognostic
121 signature for the 1-, 2- and 3-year survival rates, we constructed a nomogram in CGGA cohort
122 using the "survival" and the "rms" package in R software. Following that, calibration curves
123 were plotted to evaluate the concordance between actual and predicted survival. In addition, the
124 correlationship between *PLOD1* expression and clinicopathologic characteristics was performed
125 using the "beeswarm" package in R software.

126 **Differential genes expression analysis and enrichment analysis for significant pathways**
127 The mRNA sequencing datas in glioma from CGGA datasets were normalized and the
128 differentially expressed genes (DEGs) including significantly upregulated and downregulated
129 genes were screened with an adjusted p value < 0.05 and absolute log2 fold change (FC) > 2, and
130 then a volcano plot of DEGs was generated using the "limma" package in R software (Ritchie et
131 al., 2015). Using the screened DEGs, gene ontology (GO) enrichment analysis was performed on
132 the online tool-Metascape (Zhou et al., 2019) (http://metas cape.org/gp/index .html#/main/step1).
133 In addition, gene set enrichment analysis (GSEA) was also performed to indirectly explain the
134 function of *PLOD1* (Subramanian et al., 2005). A gene set was considered as an enriched group
135 when the NES>1 and FDR score < 0.05.

136 **Co-expression analysis**
137 The GO and KEGG analysis both enriched in extracellular matrix (ECM). The common genes of
138 DEGs and the key genes in the ECM pathways enriched by the KEGG analysis were performed
139 for correlation analysis between *PLOD1*. Pearson correlation analysis was used for parametric
140 tests, Spearman correlation analysis was used for non-parametric tests. A circular plot and a
141 pheatmap of the common genes positively associated with *PLOD1* were generated by R
142 software. In addition, every common gene was preformed in GEPIA to examination the
143 expression and survival in gliomas.

144 **Statistical analysis**
145 Statistical analyses were performed with R software v3.6.3 (http://www.r-project.org/) (Hjalt et
146 al., 2001), and Prism 8 (GraphPad Software, Inc). The "survival" package (https://CRAN.R-
147 project.org/package=survival), "survminer" package (https://CRAN.R-
148 project.org/package=survminer), "survivalROC" package (https://CRAN.R-
149 project.org/package=survivalROC), "rms" package, "beeswarm" package (https://CRAN.R-
150 project.org/package=beeswarm), "limma" package, "ggplot2" package, "pheatmap" package
151 (https://CRAN.R-project.org/package=pheatmap), "corrplot" package
152 (https://github.com/taiyun/corrplot), and "circlize" package (Gu et al., 2014) of R software were
153 used successively. Data were considered significant at P<0.05.

154 # Results

155 **Characteristics of the samples**
156 The workflow of our study is shown in Fig. 1. After screening, a total of 636, 592, 153, 80 and
157 117 glioma patient samples were obtained from the CGGA, TCGA, GSE4290, GSE7696 and
158 GSE50161 datasets. Both CGGA and TCGA datasets contained grading data, age, gender, IDH

159 mutation status and 1p19 codeletion status. Additionally, postoperative radiotherapy or
160 chemotherapy follow-up and MGMT methylation data were only included in the CGGA dataset.
161 Detailed clinical information classification and percentages of glioma patients are shown in
162 Table 1.

163 **PLOD1 gene expression and genomic characteristics in glioma**
164 The expression level of *PLOD1* was significantly higher in gliomas than in normal tissues based
165 on the CGGA and GEO datasets (Fig. 2A, B, C, D), and the same results were obtained in
166 GEPIA online analysis in both GBM and LGG (Fig. 2E). The genomic alteration of *PLOD1* was
167 shown in Fig. 2F, and several mutation types were provided using cBioPortal online database
168 (Fig. 2G). In addition, the results from the CCLE database showed that *PLOD1* expression in
169 glioma ranked 4th among the cell lines from different cancer tissues (Fig. 2H).

170 **Survival analysis and prognostic values of *PLOD1* in glioma patients**
171 The Kaplan-Meier survival analysis of the CGGA datasets showed that high level expression of
172 *PLOD1* related to poor survival (p<0.001, Fig. 3A), and the same results were obtained by using
173 the TCGA datasets (p<0.001, Fig. 3B). Furthermore, we investigated the correlation between
174 *PLOD1* expression and IDH and MGMT promoter methylation status on survival. The results
175 showed that patients with IDH mutation had a longer survival regardless of the level of *PLOD1*
176 expression (p<0.001, Fig. 3C). However, patients with low expression of *PLOD1* had a longer
177 survival regardless of the MGMT promoter methylation status (p<0.001, Fig. 3D). In addition,
178 based on the CGGA datasets, ROC curve revealed that *PLOD1* was a predictive marker of 1-year
179 (AUC=0.816), 3-year (AUC=0.793), and 5-year survival (AUC=0.707) (Fig. 3E). Similarly,
180 ROC curve analysis using TCGA datasets also verified this result. The area under curve for OS
181 was 0.786 at 1 years, 0.805 at 3 years, and 0.796 at 5 years, respectively (Fig. 3F).

182 **Independent prognostic analysis and development of nomogram of *PLOD1* in glioma**
183 To identify whether *PLOD1* was an independent prognostic index, univariate and multivariate
184 Cox regression analyses were performed in CGGA datasets. Univariate analysis showed that
185 *PLOD1* expression (HR=1.986; 95% CI=1.796–2.198; P<0.001), PRS type, grade, age, IDH
186 mutation, and 1p19q codeletion were significantly associated with OS (Fig. 4A). Furthermore,
187 multivariate Cox regression analysis revealed that *PLOD1* expression (HR=1.283; 95%
188 CI=1.128–1.460; P<0.001), PRS type, grade, chemotherapy after resection, IDH mutation, and
189 1p19q codeletion remained significantly correlated with OS (Fig. 4B). These results indicated
190 that *PLOD1* expression has a strong prognostic value in gliomas. Furthermore, to quantitatively
191 predict the prognosis of glioma patients, we constructed a nomogram using grade, IDH mutation
192 status, MGMT promoter methylation status, 1p19q codeletion status and *PLOD1* expression
193 level. It revealed that the *PLOD1* expression level was the leading factor for predicting
194 nomogram (Fig. 4C). Calibration curves indicated that actual and predicted survival matched
195 very well, especially for 3-year survival (Figure. 4D-F).

196 **Correlations between *PLOD1* expression and clinicopathologic characteristics**
197 Among the analysis, patients older than 42 years had significantly higher levels of *PLOD1*
198 expression (p<0.001, Fig. 5A). The expression of *PLOD1* increased with the increase of glioma

199 grade (p<0.001, Fig. 5B) and was higher in recurrent and secondary tumor than primary tumor
200 (p<0.001, Fig. 5C). For molecular type, *PLOD1* expressed lower in patients with 1p19q
201 codeletion and IDH1 mutants (p<0.001, Fig. 5D, E).

202 **DEGs and enrichment pathways analysis of *PLOD1***
203 A total of 67 DEGs were identified in analysis, which included 61 upregulated and 6
204 downregulated genes (Fig. 6A). Metascape analysis demonstrated that the biological
205 processes of these DEGs were significantly enriched in extracellular structure organization (Fig.
206 6B). In addition, GSEA was used to identify hallmarks of glioma. The results showed that ECM
207 receptor interaction was significantly enriched in high-expression *PLOD1* phenotypes
208 (NES=1.96, normalized P=0.002) (Fig. 6C). As Metascape analysis and GSEA analysis both
209 enriched in ECM related pathways, we screened out the core genes of ECM-related pathways
210 enriched in GSEA analysis and intersected the core genes with DEGs to obtain 10 common
211 genes (Fig. 6D).

212 **Co-expression analysis of *PLOD1***
213 Through gene co-expression analysis, we found that *PLOD1* was closely related to all of the
214 screened common genes. The *PLOD1* was positively associated with *HSPG2, COL6A2,*
215 *COL4A2, FN1, COL1A1, COL4A1, CD44, COL3A1, COL1A2, SPP1* (Fig. 7A-J). A heatmap of
216 the intersection genes associated with *PLOD1* was plotted (Fig. 7K), and a circular plot of these
217 genes was also generated (Fig. 7L). Furthermore, we performed gene expression and survival
218 analysis of the the common genes in GEPIA, the results showed that all the genes were highly
219 expressed in glioma and high expression level related to poor prognosis (Fig. 8A-T).

Discussion

221 Glioma, especially high-grade glioma, has a poor prognosis due to its aggressive growth and
222 high recurrence rate. The standard treatment for glioma is a combination of traditional surgery,
223 radiotherapy and chemotherapy (Buckner et al., 2016; Jaeckle et al., 2020; Herrlinger et al.,
224 2019). Emerging alternating electric field therapy has been included in the NCCN guidelines in
225 recent years due to its significant survival benefits in glioblastoma (Stupp et al., 2017). However,
226 the prognosis is still poor, and the choice of multiple treatment options is a clinical challenge.
227 Therefore, it is important to explore potential biomarkers and therapeutic targets of glioma.
228 In this study, we found that *PLOD1* expression in glioma was higher than that in normal tissues
229 using the CGGA datasets, which was verified by the GEO datasets and GEPIA. In addition, the
230 expression of *PLOD1* in glioma was higher than in most cancers based on the CCLE database.
231 Previous studies have revealed that *PLOD2* and *PLOD3* have predictive effects on the prognosis
232 of glioma (Song et al., 2017; Tsai et al., 2018), we speculated that *PLOD1* also influence the
233 prognosis of glioma, although it has not been explored. The sequential Kaplan-Meier survival
234 analysis showed high expression level of *PLOD1* related to poor prognosis based on both the
235 CGGA and TCGA datasets. We also found that patients with different IDH mutation and MGMT
236 promoter methylation status had different survival at different *PLOD1* expression levels.
237 Through comprehensive univariate and multivariate Cox analysis, we found that *PLOD1* was an
238 independent prognostic factor in glioma patients. Moreover, the AUC values of ROC curves for

239 *PLOD1* at 1, 3, and 5 years were all >0.7, which also suggested that *PLOD1* was a predictor of
240 survival. All of the above results confirmed our previous hypothesis. According to these
241 prognostic signatures, we constructed a nomogram to quantitatively predict the survival of
242 glioma patients, and the results showed that the *PLOD1* expression level was the leading factor.
243 We can use this model to predict the survival of glioma patients. Therefore, it can help to make
244 clinical decisions for patients, which can avoid overtreatment or undertreatment, so as to
245 individually select the best treatment strategies for glioma patients.
246 ECM is an important component of tumor microenvironment and plays an important role in
247 cancer development and progression (Mohan, Das & Sagi, 2020; Lu, Weaver & Werb, 2012).
248 Collagen is a major component of ECM, and its elevated deposition and cross-linking can
249 worsen tumor progression depending on the hydroxylation of lysine residues, which is mainly
250 catalyzed by *PLODs* (Gjaltema & Bank, 2017; Qi & Xu, 2018). In this study, both GO and
251 GSEA analysis were performed to suggest that *PLOD1* was enriched in ECM-related pathways,
252 which was consistent with its pathophysiological mechanism. Using cBioPortal online analysis,
253 we found that the mutation frequency of *PLOD1* is not high (1%), which suggests that the
254 aberrant expression of *PLOD1* may be a result of post-transcriptional regulations or translation
255 modifications. Previous studies reported that the *PLOD* expression was mainly regulated at the
256 transcription level (Gjaltema et al., 2015). Compared with *PLOD2*, the regulation of *PLOD1*
257 expression has not been well investigated. Some preliminary studies have shown that *PITX2* can
258 directly regulate *PLOD1* expression by binding to the promoter region, using chromatin
259 immunoprecipitation and luciferase reporting experiments (Hjalt, Amendt & Murray, 2001), and
260 both *BMP-2* and *TGF-β1* can induce *PLOD1* expression in adipose tissue-derived mesenchymal
261 stem cells (Knippenberg et al., 2009). However, the mechanism of its expression regulation still
262 needs more exploration to identify.
263 As we found in glioma, increased expression of *PLOD1* is present in many types of cancer, and
264 the high expression leads to short disease-related survival (Wu et al., 2020; Yamada et al., 2019;
265 Li et al., 2017). Therefore, targeting *PLOD1* is a potential therapeutic strategy, while there is no
266 potent *PLOD1* inhibitor available. So, it is of great significance to explore specific inhibitors of
267 *PLOD1* for preventing tumor progression. In addition, another potential strategy is to reduce
268 *PLOD1* expression, this means that further understanding of the regulatory mechanism of
269 *PLOD1* in the development of cancer may lead to the exploration of novel signaling pathways to
270 target *PLOD1*.
271 Finally, we screened out the common genes between the core genes of ECM-related pathways
272 and DEGs, and then performed co-expression analysis with *PLOD1*. We found that *PLOD1* was
273 positively related to *HSPG2, COL6A2, COL4A2, FN1, COL1A1, COL4A1, CD44, COL3A1,*
274 *COL1A2, SPP1*, suggesting that they jointly contribute to the occurrence and development of
275 gliomas. Moreover, based on GEPIA online analysis, all of them were highly expressed in
276 glioma and its expression levels were closely related with patients' survival, therefore, it is of
277 great significance to carry out more studies on these genes in glioma in the future.
278 **Conclusions**

279   To analyze the relationship between the expression level of *PLOD1* and the prognosis of glioma,
280   we use the CGGA, TCGA and GEO datasets performing bioinformatics analysis. The results
281   showed that the expression level of *PLOD1* was higher in glioma than normal tissues and high
282   expression of *PLOD1* was related to poor survival which can serve as an independent prognostic
283   indicator for glioma patients. Additionally, the GO and GSEA analysis verified that the
284   mechanism of *PLOD1*'s oncogenic effect was related to ECM, the co-expression analysis
285   revealed that *PLOD1* was positively correlated with *HSPG2, COL6A2, COL4A2, FN1, COL1A1,*
286   *COL4A1, CD44, COL3A1, COL1A2* and *SPP1*. All of them were ECM related genes and the
287   expression of these genes was also correlated with the prognosis of glioma. In conclusion, this
288   study indicated that targeting *PLOD1* is a potential therapeutic strategy for glioma patients, and
289   the expression level of *PLOD1* may provide a reference for the selection of treatment regimens
290   for glioma patients, which suggests that the biological functions and mechanisms of *PLOD1*
291   need to be explored in the future. However, this study mainly relies on bioinformatics
292   analysiswith certain limitations and more in vitro or in vivo validation experiments can make it
293   more reliable.

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The characteristics of the public database samples

Notes: *Abbreviations: CGGA, Chinese Glioma Genome Atlas; TCGA, The Cancer Genome Atlas; IDH, Isocitrate Dehydrogenase; MGMT, O6-methylguanine-DNA methyltransferase.*

Characteristics	CGGA(n=636)		TCGA(n=592)	
	case	proportion	case	proportion
WHO Grade				
II	164	25.8%	211	35.6%
III	207	32.5%	238	40.2%
IV	265	41.7%	143	24.2%
Age(years)				
≥42	342	53.8%	349	59.0%
<42	294	46.2%	243	41.0%
Gender				
Male	371	58.3%	344	58.1%
Female	265	41.7%	248	41.9%
IDH mutation				
Yes	336	52.8%	372	62.8%
No	300	47.2%	220	37.2%
1p19q codeletion				
Yes	126	19.8%	149	25.2%
No	510	80.2%	443	74.8%
MGMTp methylation				
Yes	347	54.6%		
No	289	43.4%		
Radio				
Yes	501	78.8%		
No	135	21.2%		
Chemo				
Yes	465	73.1%		
No	171	26.9%		

2 **Notes: Abbreviations: CGGA, Chinese Glioma Genome Atlas; TCGA, The Cancer Genome Atlas;**

3 **IDH, Isocitrate Dehydrogenase; MGMT, O^6-methylguanine-DNA methyltransferase.**

Figure 1

Workflow of the whole study

Notes: *Abbreviations: CGGA, Chinese Glioma Genome Atlas; TCGA, The Cancer Genome Atlas; GSE, GEO Series; GEO, Gene Expression Omnibus; HPA, The Human Protein Atlas; ROC, Receiver Operator Characteristic; DEGs, Differentially Expressed Genes; GSEA, Gene Set Enrichment Analysis; ECM, Extracellular Matrix.*
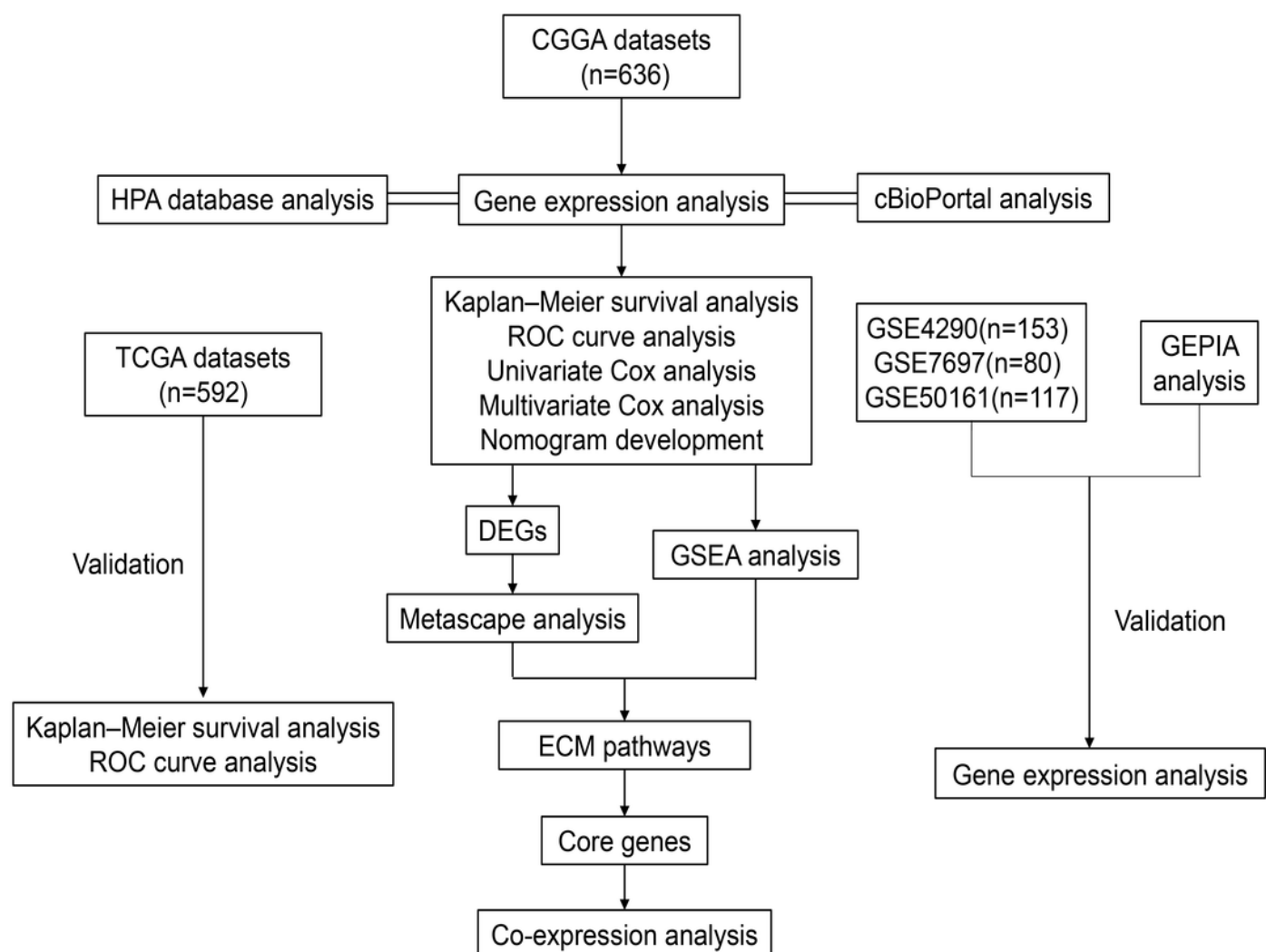
Figure 2

PLOD1 gene expression in glioma and normal tissue.

Figure 2: PLOD1 gene expression in glioma and normal tissue. (A) Comparison of PLOD1 expression levels between glioma and normal tissue in CGGA datasets. (B) GSE4290 dataset. (C) GSE7696 dataset. (D) GSE50161 dataset. (E) PLOD1 expression levels of GBM and LGG in GEPIA analysis, comparing to normal tissue respectively. (F) The genomic alteration of PLOD1 using cBioPortal online analysis. (G) Mutation types of PLOD1. (H) PLOD1 expression level across various cancer cell lines, including glioma cell lines (rank 4th, indicated by red boxes) from the CCLE database (Y-axis represents the expression level of PLOD1 in different cancer cell lines).
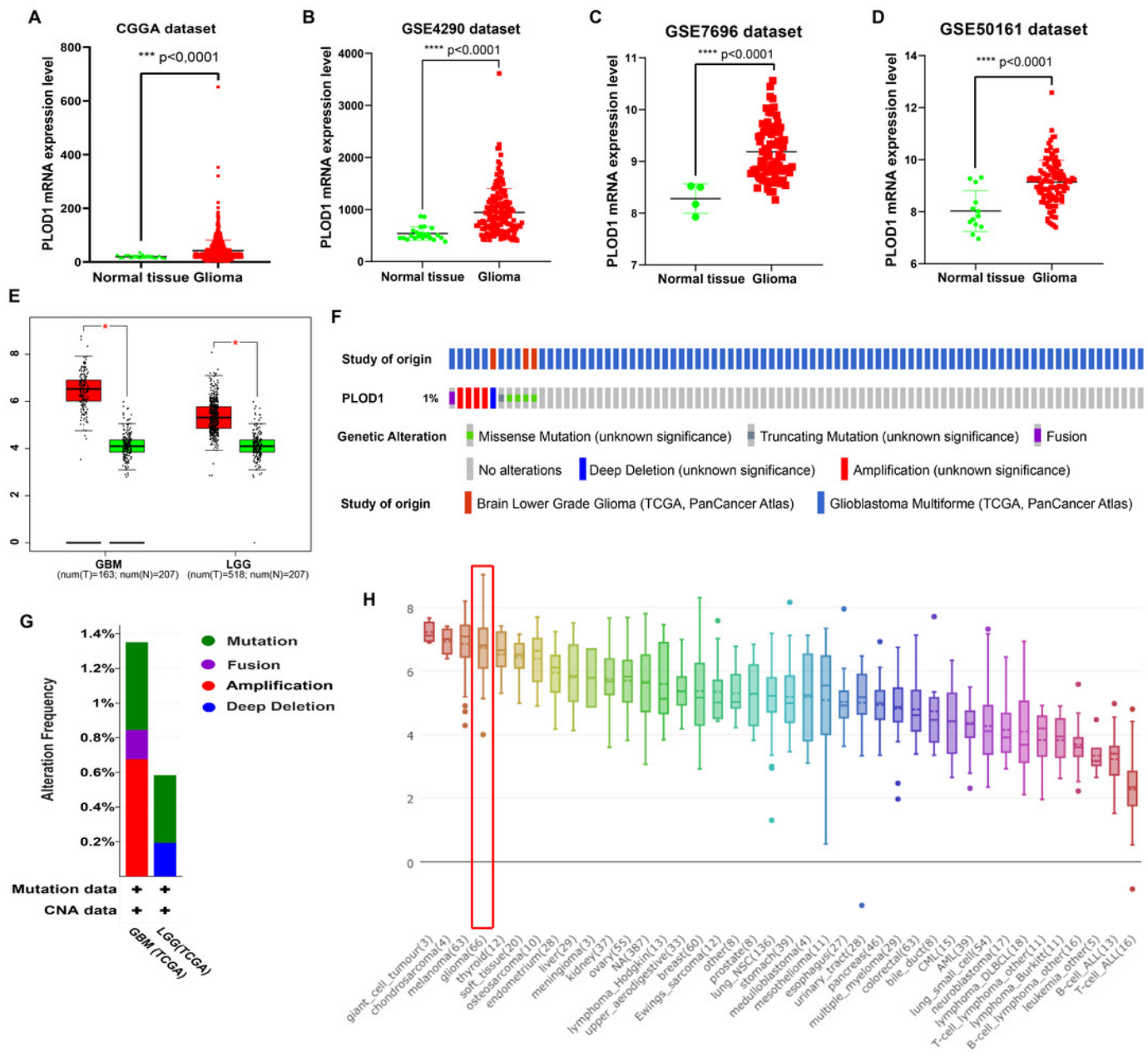
Figure 3

Survival analysis and prognostic values of PLOD1.

Survival analysis of glioma patients in the high PLOD1 and low PLOD1 groups. Red lines represent high expression and blue lines represent low expression. (A) Based on CGGA datasets. (B) Based on TCGA datasets. (C) Relationship between PLOD1 expression and IDH status on glioma survival. Green line represents low expression and IDH wildtype, red line represents high expression and IDH wildtype, blue line represents low expression and IDH mutation, purple line represents high expression and IDH mutation. (D) Relationship between PLOD1 expression and MGMT promoter methylation status on glioma survival. Blue line represents low expression and MGMT promoter methylation, green line represents high expression and MGMT promoter methylation, purple line represents low expression and MGMT promoter unmethylation, red line represents high expression and MGMT promoter unmethylation. Receiver operator characteristic curve analysis of PLOD1. Red lines represent 5 year survival, green lines represent 3 year survival and blue lines represent one 1 survival. (E) Based on CGGA datasets. (F) Based on TCGA datasets.
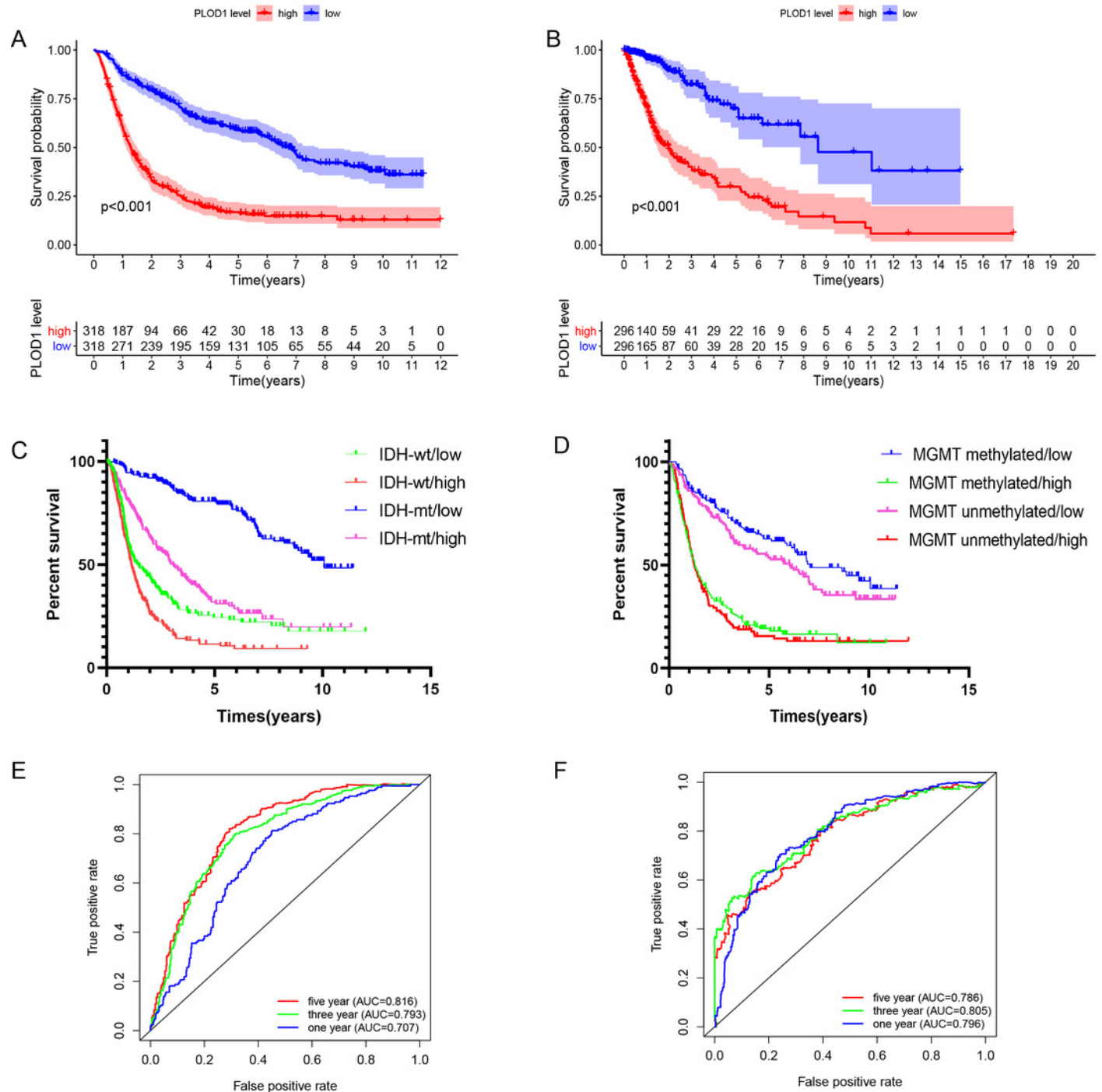
Figure 4

Independent prognostic analysis and development of nomogram of PLOD1 in glioma.

(A) Univariate analysis of PLOD1 based on CGGA datasets. (B) Multivariate analysis of PLOD1 based on CGGA datasets. (C) Prognostic nomogram to predict the survival of glioma patients based on the CGGA datasets. The values of grade, IDH, MGMT, 1p19q and PLOD1 are acquired from each variable axis. The total points on the axis are the sum values of these factors, which can predict the 1-, 2-, and 3-year survival. Calibration curves of the nomogram for predicting survival at 1, 2, and 3 years in the CGGA training cohort (D-F).
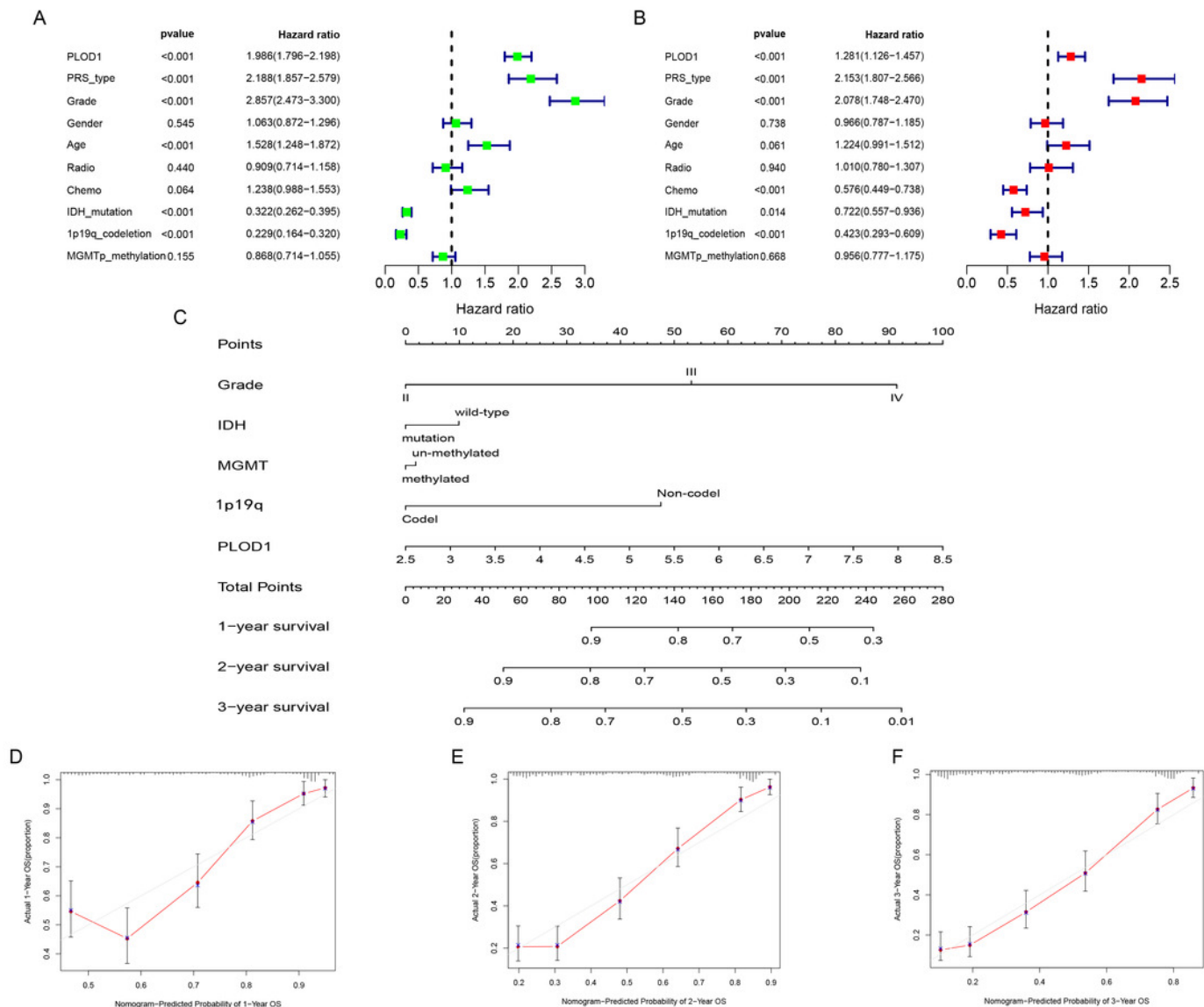
Figure 5

Correlation analysis with clinicopathologic characteristics of PLOD1 in glioma.

(A) age (<42, n=294; ≥42, n=342), (B) grade (WHO II, n=164; WHO III, n=207; WHO IV, n=265), (C) PRS type (Primary, n=433; Recurrent, n=179; Secondary, n=24), (D) 1p19q codeletion status (Codel, n=126; Non-codel, n=510), and (E) IDH mutation status (Mutant, n=347; Wildtype, n=289).
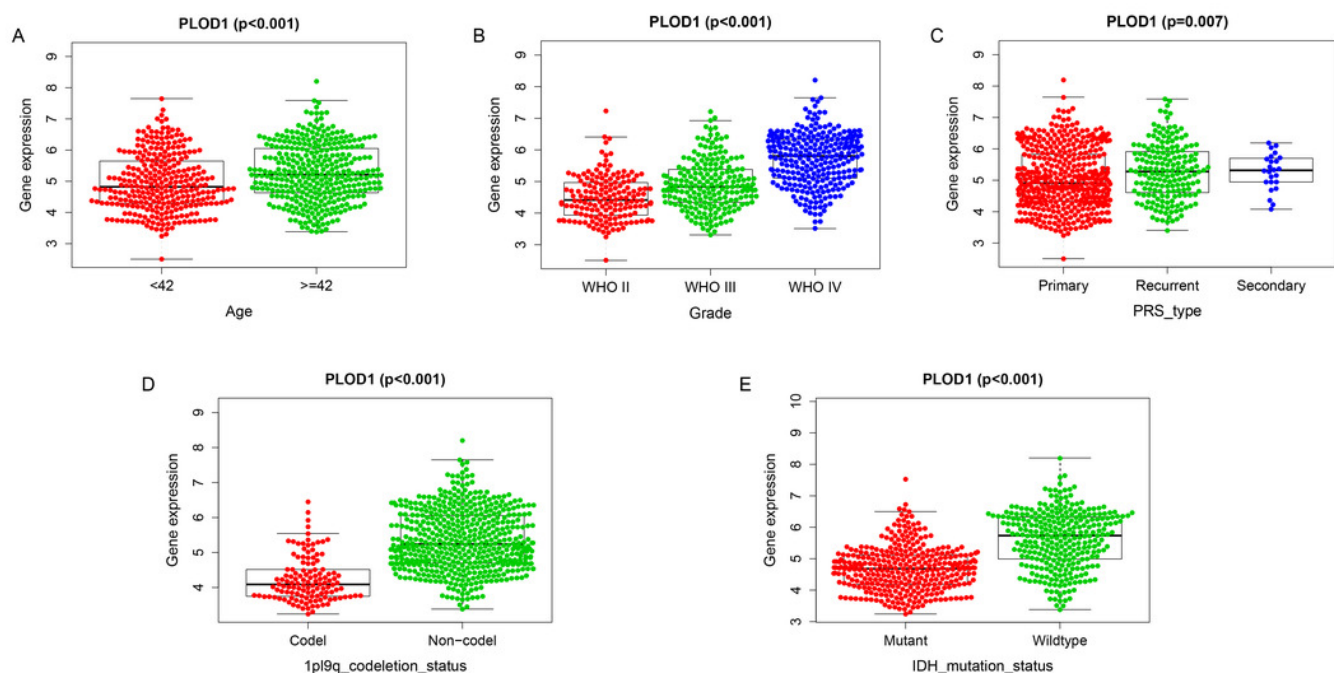
Figure 6

DEGs and enrichment pathways analysis of PLOD1.

(A) Volcano plot of differentially expressed genes. Red nodes represent the significantly up-regulated genes with logFC > 2 and p < 0.05. Green nodes represent the significantly down-regulated genes with logFC < -2 and p < 0.05. (B) Heatmap of enriched terms across DEGs in Metascape analysis. (C) Enrichment plot of ECM receptor interaction pathway from GSEA. (D) The Venn diagram of the common genes, which is obtained by intersecting the core genes of the ECM pathway with DEGs.
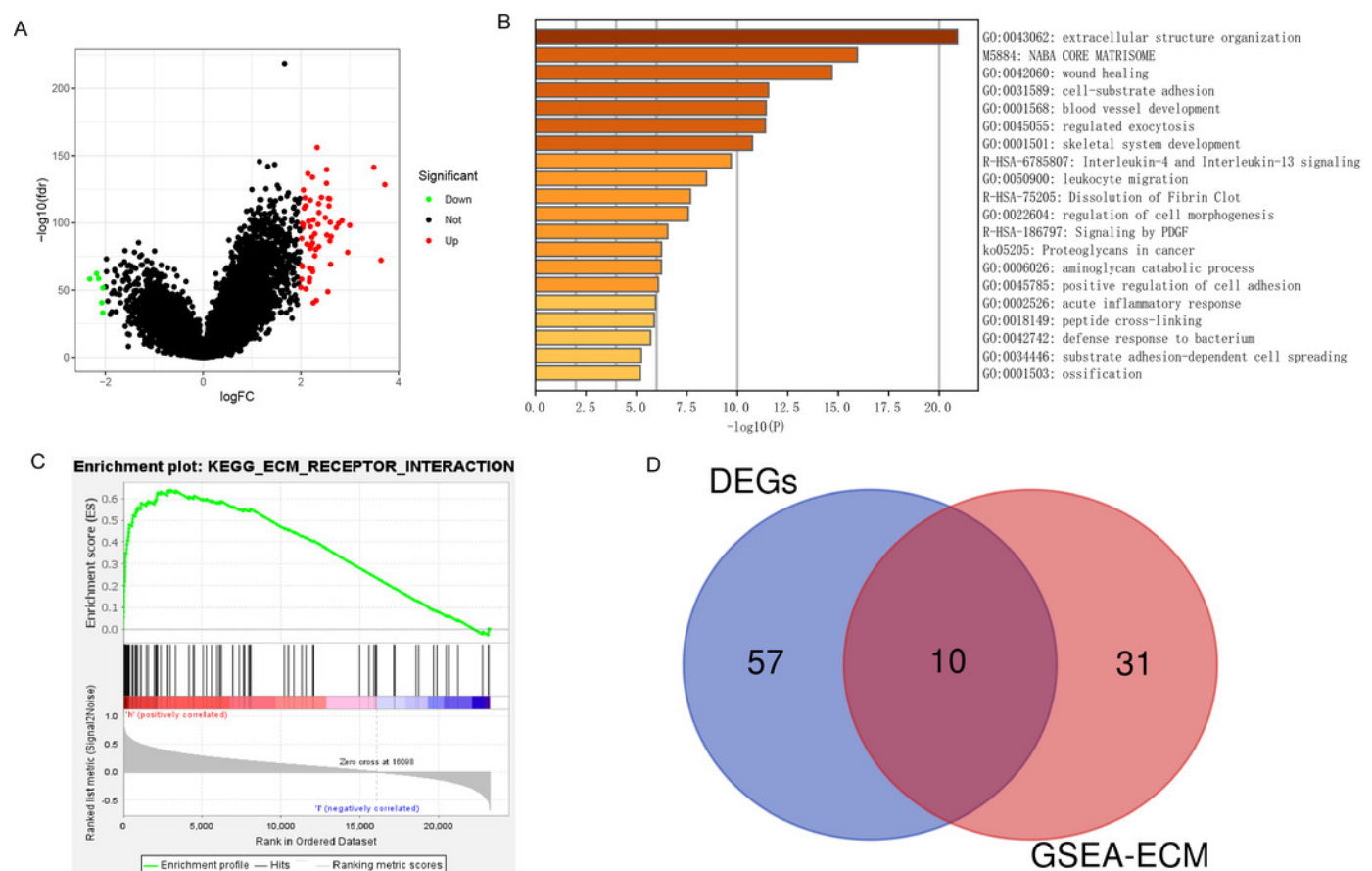
Figure 7

Co-expression analysis of PLOD1 with common genes.

(A) CD44; (B) COL1A1; (C) COL1A2; (D) COL3A1; (E) COL4A1; (F) COL4A2; (G) COL6A2; (H) FN1; (I) HSPG2; (J) SPP1. (K) Circular plot of common genes with PLOD1. Red represents positive association. (L) Heatmap of the common genes with PLOD1.
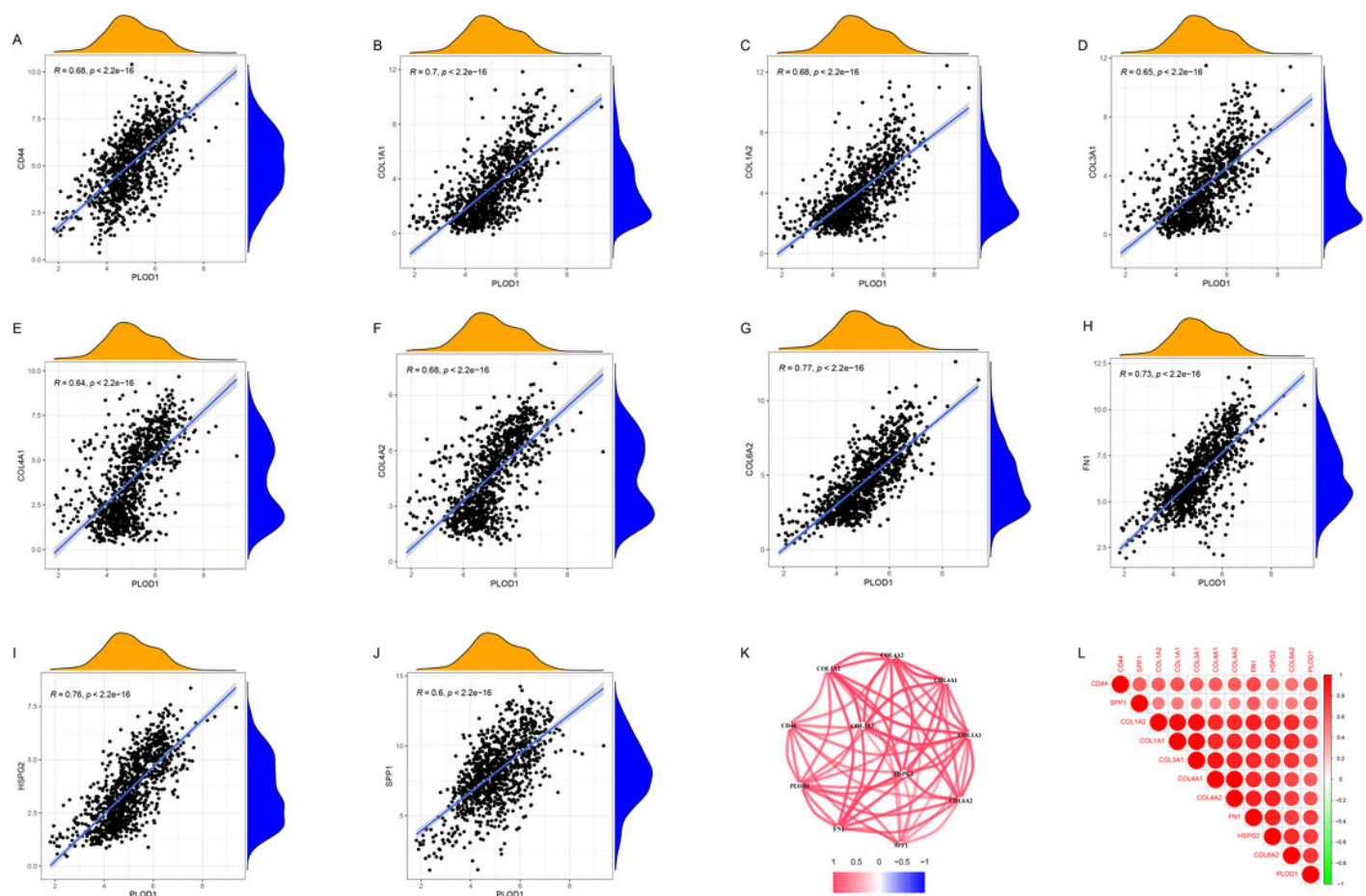
Figure 8

Common genes expression levels in glioma and correlations with survival in GEPIA analysis.

(A, B) CD44; (C, D) COL1A1; (E, F) COL1A2; (G,H) COL3A1; (I, J) COL4A1; (K, L) COL4A2; (M, N) COL6A2; (O, P) FN1; (Q, R) HSPG2; (S, T) SPP1.