Global Journal of Life Sciences Dec. 30, 2020, Vol 1, No 1

Global *Journal of Life Sciences*
2020; 1(1): 49-62.
ISSN Online: 2766-9130; ISSN Print: 2766-9149
Website: http://naturescholars.com
Email: Glo_J_Ls@126.com
Publisher: Scholars Publishing, LLC

Article

Expression of PLG in Kidney Renal Clear Cell Carcinoma and its Significance

Chao-Qun Xing[1,*], Jiang-Rong Huang [1,*,✉]

[1] Department of Traditional Chinese Medicine, Health Science Center, Yangtze University, Jingzhou 434023, China.

*** These authors contributed equally.**
✉ Correspondence
Jiang-Rong Huang, Department of Traditional Chinese Medicine, Health Science Center, Yangtze University, Jingzhou, Hubei Province, China, 434023, China. Phone: +86-13607216868.; Email: huangjr999@sina.com.

Received: July 31, 2020. Accepted: October 14, 2020. Published online: December 30, 2020.
Cite this paper: Chao-Qun Xing, Jiang-Rong Huang (2020) Expression of PLG in Kidney Renal Clear Cell Carcinoma and its Significance. *Global Journal of Life Sciences*, 1(2): 49-62. http://naturescholars.com/gjls.010107. https://doi.org/10.46633/gjls.010107.
Copyright © 2020 by Scholars Publishing, LLC.

Abstract

We aimed to identify if Plasminogen (PLG) could be used as a new diagnostic and therapeutic biomarker in Kidney Renal Clear Cell Carcinoma (KIRC) patient. Expression of PGL in KIRC was analyzed by using GEPIA (Gene Expression Profiling Interactive Analysis) and UALCAN databases. GEPIA and CBioPortal tools were applied to determine patients' survival and PLG mutations, respectively. PPI (Protein-Protein Interaction) networks were further built by STRING (Search Tool for the Retrieval of Interacting Genes) Web portals. The data demonstrated that the expression of PLG in KIRC was significantly enhanced when compared to normal kidney tissues ($P < 0.001$). A lower PLG expression resulted in a remarkably shorter overall survival. Moreover, the expression of PLG in KIRC was related to KIRC patients' stages, race and grade. PPI networks and GO enrichment analysis suggested that PLG might be associated with negative regulation of cellular protein metabolic process and complement and coagulation cascades signaling pathway etc. Finally, five specific gene mutations L469P, S292C, X317_splice, Y530C and N809S of PLG occurred in KIRC and these unique mutations were not seen in any other tumor tissues. Taken together, our novel findings suggest that PLG is implicated in the poor prognosis of KIRC.

Key words: Plasminogen (PLG), Kidney Renal Clear Cell Carcinoma (KIRC), TCGA, Biomarker, Cancer Therapy.

1 Introduction

Renal cell carcinoma (RCC), a type of kidney cancer, is the leading cause of cancer-related death, with more than 270,000 new cases worldwide each year (1). RCC is the most common type of kidney

cancer in adults, accounting for 90 to 95 percent. Clear cell renal cell carcinoma (ccRCC), also known as clear cell renal cell carcinoma (KIRC), is the most common subtype (about 80%) (2). Progressive accumulation of alterations in cancer drives genes and dysregulation of their associated signaling pathways, causing the occurrence and progression of KIRC. KIRC that is closely associated with loss/inactivation of the von Hippel-Lindau (VHL) tumor suppressor gene (3). KIRC is insensitive to radiotherapy, and there is no uniform standard for the optimal dose of radiotherapy (4). Although kidney immunotherapy combined with chemotherapy is a potential therapy for KIRC, its application is limited by the renal dysfunction (5). For targeted therapy, it can block the effect of key molecules in the formation and progression of kidney cancer. Targeted drugs affect kidney cancer cells more than normal cells. However, only a few drugs are currently available for patients with advanced kidney cancer (6). Therefore, it is significant to investigate the novel key genes and major signal pathways involving in the development of KIRC. To date, the efficacy of immunotherapy and molecular targeted drugs is not ideal (7), and the molecular pathological mechanism of renal carcinoma is still unclear. Surgery is the primary treatment option for localized renal cancer due to radiation and chemotherapy resistance (8). Therefore, screening potential diagnostic biomarkers or therapeutic targets for the treatment of KIRC is urgently needed (9).PLG, is a plasminogen (synthesized in the liver) that is then activated by plasminogen activator to produce PLG, the main enzyme for fibrin clot degradation in the body (10). Plasmin dissolves the fibrin of blood clots and acts as a proteolytic factor in a variety of other processes including embryonic development, tissue remodeling, tumor invasion, and inflammation (11). PLG improves the cellular activity and mitochondrial function of primary neurons. These protective effects are achieved by inhibiting apoptosis and inducing autophagy by enhancing phosphorylation of BCL2 at Ser70 (12). However, PLG is not only a pro-tumor factor, but also an anti-tumor factor, because the proteolysis of PLG can release angiotensin, which will play a role in cancer progression (13, 14). This may explain that in our study, PLG expression level in KIRC samples was lower than that in adjacent normal tissues, suggesting that low expression of PLG is important for the progress of KIRC (15). It has been reported that KIRC is a malignant disease, involving the reprogramming of energy metabolism mechanisms (16), such as aerobic glycolysis, over-utilization of amino acids such as tryptophan, glutamine and arginine, tricarboxylic acid (TCA) cycle, mitochondrial dysfunction and oxidative phosphorylation (17). Some previous studies have shown that inhibiting or upregulating autophagy regulates the metabolic reprogramming of cancer cells (18). Currently, the role of autophagy in KIRC is controversial. Studies have shown that autophagy inhibits cancer occurrence but promotes cancer progression and regulates cancer response to various therapies (19). In summary, different components of the PLG/PLA system have been involved at different stages of tumorigenesis. However, there is increasing evidence that abnormal PLG-R expression and cellular localization may be associated with the development and progression of different types of human cancers (20).Therefore, to verify the value of PLG in the diagnosis and treatment of KIRC, it is very important to analyze the expression and significance of PLG in kidney cancer tissues.

2 Materials and Methods

2.1 UALCAN Analysis

UALCAN (http://ualcan.path.uab.edu/analysis.html) is a web tool to profile gene expressions between tumor and non-tumor tissues and provides interactive data analyses. In this study, we utilized this online

tool to analyze the expression levels of PLG between KIRC specimen and normal tissues.

2.2 Survival Analysis

GEPIA (http://gepia.cancer-pku.cn/) is an interactive web resource and database for analyzing cancer transcriptome and patients' survival. In this study, we utilized this online tool to analyze patients' survival. Using GEPIA, disease free survival (DFS) and overall survival (OS) were presented, and the hazards ratio was calculate based on Cox PH Model, 95% confidence interval was added as dotted line. The thresholds for high and low expression level cohorts are 50%, respectively.

2.3 Histopathological Analysis

Human Protein Atlas is a Swedish-based program initiated in 2003 with the aim to map all the human proteins in cells, tissues and organs using integration of various omics technologies, including antibody-based imaging, mass spectrometry-based proteomics, transcriptomics and systems biology. (http://www.proteinatlas.org/). Compare normal tissue with KIRC tissue and find out the difference of PLG expression between the two.

2.4 Construction of the PPI Networks

The STRING database (http://string-db.org/) and Metascape (http://metascape.org/) tool was used to analyze the PPI networks. In this study, the PPI networks of the PLG gene was constructed using STRING and Metascape database. The non-interacting genes were excluded to simplify the PPI network. The top 11 genes with the highest degree of connection to the others were presented.

2.5 GO and KEGG Analysis

The STRING database (http://string-db.org/) is an online tool for high-throughput functional analysis of genes. In this study, the potential associations between the 11 core genes and PLG were assessed through the GO annotation analysis and KEGG pathway enrichment analysis. P-values < 0.05 were considered as statistically significance.

2.6 Analysis of Genetic Alterations

cBioPortal (https://www.cbioportal.org/) is a database with integrated genetic data, including DNA mutations, gene amplifications and protein alterations. In our study, the cBioPortal database was used to analyze the association between genetic mutations and the development of KIRC. The top 11 genes which are related to PLG were analyzed by using cBioPortal database. And we performed PLG gene mutations analysis across all tumor samples from the TCGA-KIRC database.

2.7 Correlation analysis of upstream and downstream genes

The linkedomics database (http: http://www.linkedomics.org/) contains multigroup data on 32 types of cancer and clinical data on the genome atlas (TCGA) project of 11 158 cancer patients. PLG was analyzed using LinkedOmics database according to Pearson Correlation test.

3 Results

3.1 The Expression Levels of PLG in KIRC Patients

To verify PLG expression levels in KIRC tissues and the value to the diagnosis and surveillance of KIRC, GEPIA database was applied. As shown in Figure 1a, the data showed that the expression level of PLG in the KIRC group is significantly lower than normal kidney group ($P < 0.001$). Moreover, the relationship between PLG expression levels and KIRC patients' clinicopathological parameters were further analyzed by UALCAN databases. As shown in Figure 1b. we also found there are gradually decreased expression

of PLG from stage 1 to stage 2 but obviously declined in stage 4 (stage 1 vs stage 2, P < 0.01 and stage 1 vs stage 4, P < 0.001). The result demonstrated that expression of PLG was lower in Asian KIRC patients than Caucasian patients (P < 0.01, Figure 1c). The expression of PLG decreased from grade 1 to grade4 of KIRC, suggesting PLG was remarkably correlated with KIRC patients' grade (Figure 1d) (grade 1 vs grade 3, P < 0.01; grade 1 vs grade 4, P < 0.01; grade 2vs grade 3, P < 0.05).

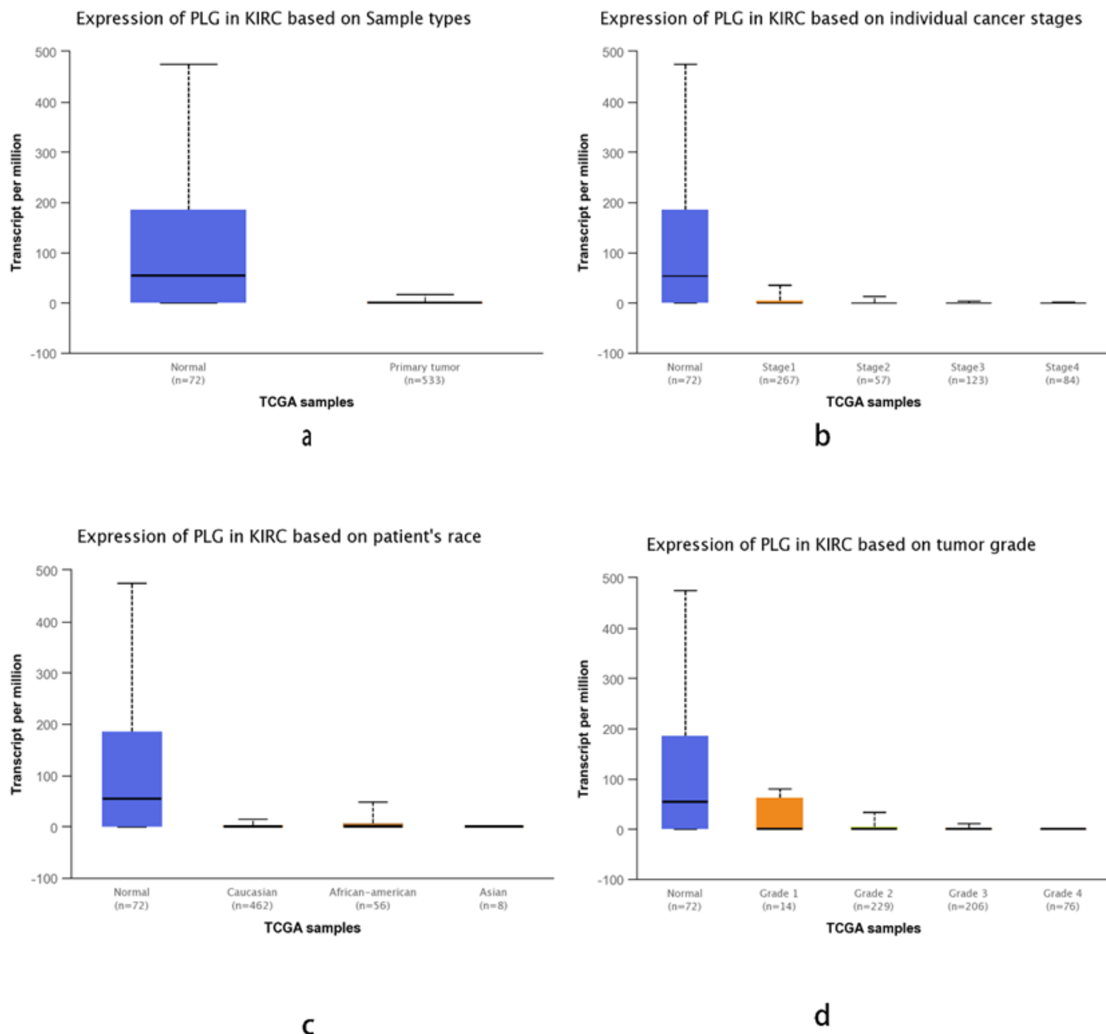

Figure 1. Low-expression of PLG is associated with malignancy of KIRC. (a) PLG expression in normal and KIRC tissues from TCGA data-sets; (b) The expressions of PLG were partially related to cancer stages. (c) The expressions of PLG were partially related to patient of race; (d) The expression of PLG was significantly related to cancer grade.

3.2 Survival Analysis of KIRC Patients Based on PLG Expression

PLG expression of KIRC patients was divided into low-expression group and high-expression group (cutoff-high is 50%, cutoff-low is 50%). As shown in Figure 2, survival was higher in the high expression group (Figure 2a), There were significant differences in gender (Figure 2b), race (Figure 2c) and tumor grade (Figure 2d) between the two groups. The overall survival (Figure 2f) was significant better in high PLG expression group than low PLG expression group (P < 0.001).

©Scholars Publishing, LLC

Survival curves analysis showed that PLG was suitable for predicting kidney cancer patients' prognosis.

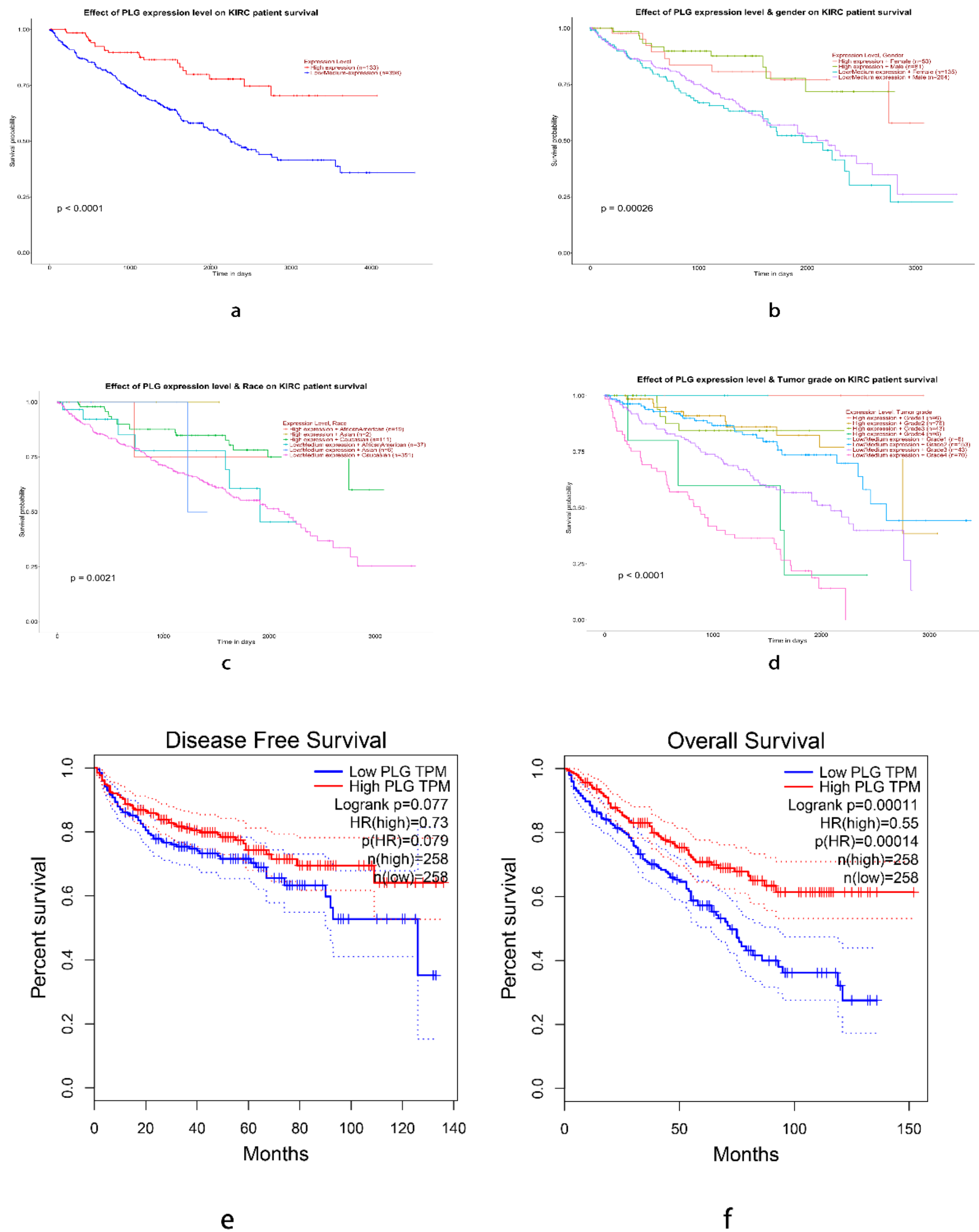

Figure 2. PLG expression of KIRC patients and survival. (a) PLG high-expression group and low-expression group (p<0.0001); (b) The expressions of PLG in gender (p=0.00026). (c) The expressions of PLG in race (p=0.0021); (d) The expression of PLG in cancer grade (p<0.0001).
Prognostic value of PLG in kidney cancer patients. lower expressions of PLG were associated with poorer DFS (f) in KIRC patients. DFS no significant difference (e).

3.3 Histopathological Analysis of KIRC Patients

Histologically, renal parenchyma consists of four parts: glomeruli, tubules, stroma, and blood vessels. The glomerulus is a complex vascular structure composed of a cluster of capillaries composed of specialized endothelial cells, epithelial cells, and mesangial cells arranged around a relatively thick basement membrane Cells in tubules. As shown in (Figure 3a). Microscopically, KIRC is typically characterized by a homogeneous network of small vessels and abundant clear tumor cytoplasm. The clarity of the cytoplasm is due to the abundance of lipids and glycogen. Large and small cysts, areas of hemorrhage, and necrosis are often seen within the tumor. As shown in (Figure 3b). Furthermore, Immunohistochemical results: Antibody HPA048823 is moderately expressed in cells in tubules as shown in (Figure 4a). Antibody HPA048823 is lowly expressed in tumor cells as shown in (Figure 4b).

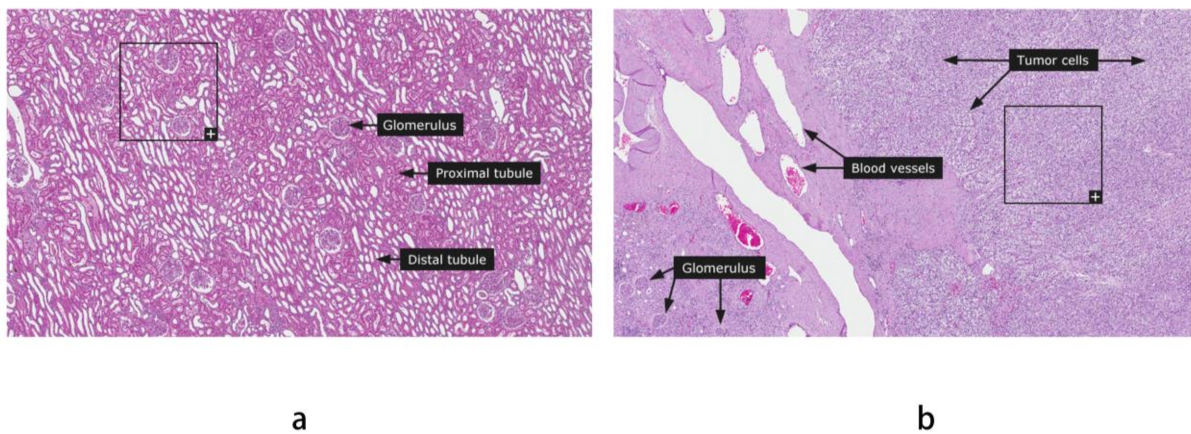

Figure 3. Histologically, normal renal parenchyma. (a) glomeruli, tubules, stroma, and blood vessels; (b) KIRC tumor tissue

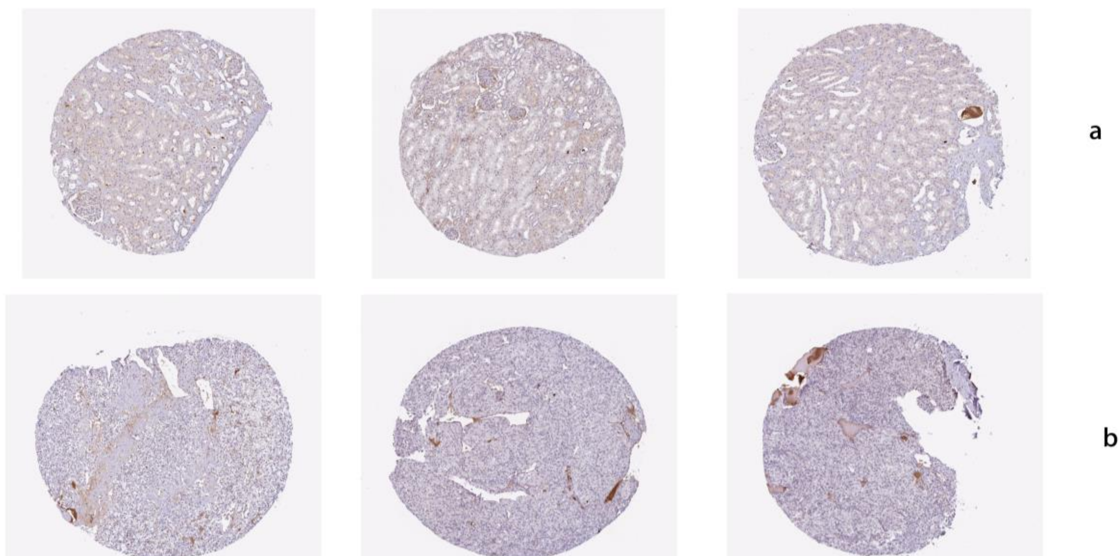

Figure 4. Cells in tubules. Antibody HPA048823 ; Staining: Medium, Intensity: Moderate, Quantity: >75%, Location: Nuclear (a). KIRC Tumor cells: Staining: low, Intensity: Moderate, Quantity:

<25%, Location: Nuclear(b).

3.4 PPI Networks and GO Enrichment Analysis of PLG

The functional interactions between proteins can provide us some information in molecular mechanism. In this study, PPI network was constructed by the Metascape database. PPI network analysis indicated that PLG has more interactions with other 11proteins, including SERPINC1, SERPINF2, VWF, FN1, HRG, SERPINE1, SERPINB2, MMP9, PLAUR and ANXA2 (Figure 5). To predict the biological functions and signaling pathways in which PLG were involved in KIRC, GO enrichment and KEGG pathway analyze were further performed (Table 1). The results showed that those proteins were biologically closely associated with fibrinolysis, secretion, negative regulation of cellular protein metabolic process and complement and coagulation cascades signaling pathway etc.

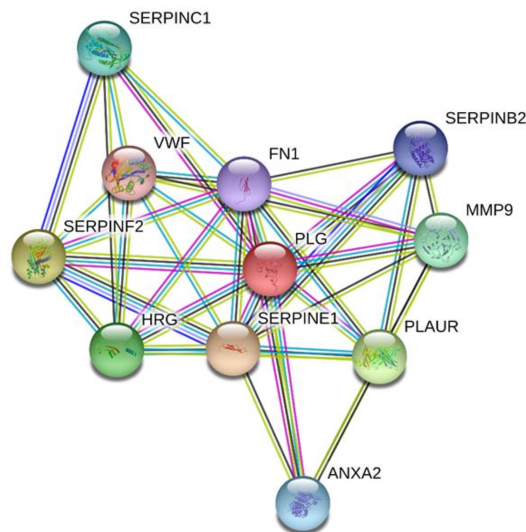

Figure 5. PPI network and GO enrichment analysis of 11 hub genes related to PLG. PPI network and MCODE components identified in the gene lists.

3.5 Specific Mutations of PLG Genes in KIRC Patients

To analyze the mutations of PLG gene in KIRC patient's tissues, the cBioPortal database analysis was employed. As shown in Figure 4, we performed PLG gene mutations analysis across all tumor samples from the TCGA-KIRC database (https://www.cbioportal.org/). Intriguingly, there were three specific mutations L469P, S292C, X317_splice, Y530C and N809S (Figure 6a and Table 2) in the KIRC samples that were not present in any other tumor samples. These particular mutations of PLG in KIRC patients might contribute greatly to KIRC clinical diagnosis and monitoring. Moreover, the mutations between PLG and its interacted genes (SERPINC1, SERPINF2, VWF, FN1, HRG, SERPINE1, SERPINB2, MMP9, PLAUR and ANXA2) were analyzed through the cBioPortal dataset. The alteration statuses of 11 key genes were analyzed using TCGA HCC patients' data of cBioPortal database. The genetic alteration of PLG genes was altered in 1.2% of KIRC patients (Figure 6b-c).

3.6 Correlation analysis of genes expression with PLG in KIRC Patients

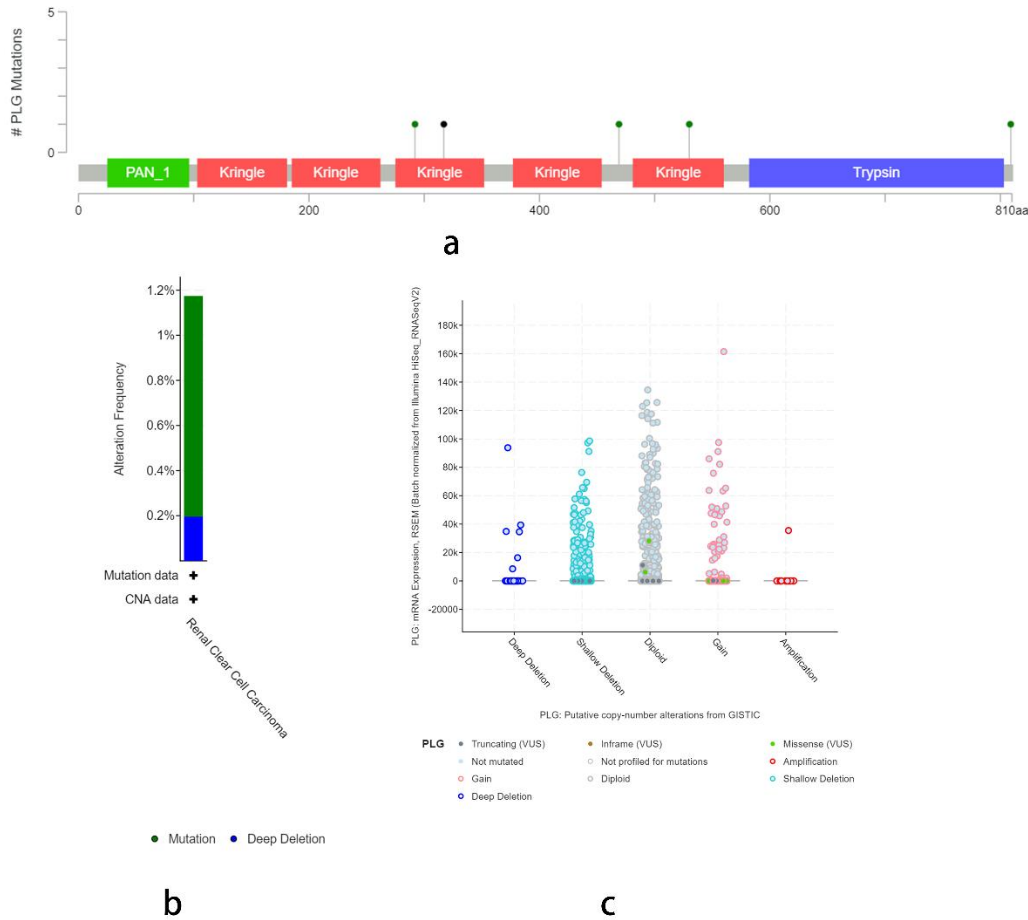

Figure 6. (a) and (c) The particular mutations (L469P, S292C, X317_splice, Y530C and N809S) of PLG in KIRC patients. (b) A visual summary of genetic alterations (data from KIRC in TCGA) shows the genetic alteration of PLG genes which were altered in 1.2% of KIRC patients.

Table2 Specific Mutations of PLG Genes

Cancer Type	Sample ID	Protein Change
Renal Clear Cell Carcinoma	TCGA-A3-3378-01	L469P
Renal Clear Cell Carcinoma	TCGA-B8-5553-01	S292C
Renal Clear Cell Carcinoma	TCGA-DV-5566-01	X317_splice
Renal Clear Cell Carcinoma	TCGA-B0-4698-01	Y530C
Renal Clear Cell Carcinoma	TCGA-BP-4974-01	N809S

We speculated that the role of PLG in KIRC might be closely related to the function of its neighbor genes in KIRC. We used the LinkedOmics database to analyze the co-expressed genes of PLG in 533 KIRC cases. As shown in Fig. 7a, there were 3218 genes represented by dark red dots, having an obviously positive connection with PLG. Conversely, there were 2931 genes, represented by dark green dots, having a notably negative correlation with PLG (false discovery rate [FDR].

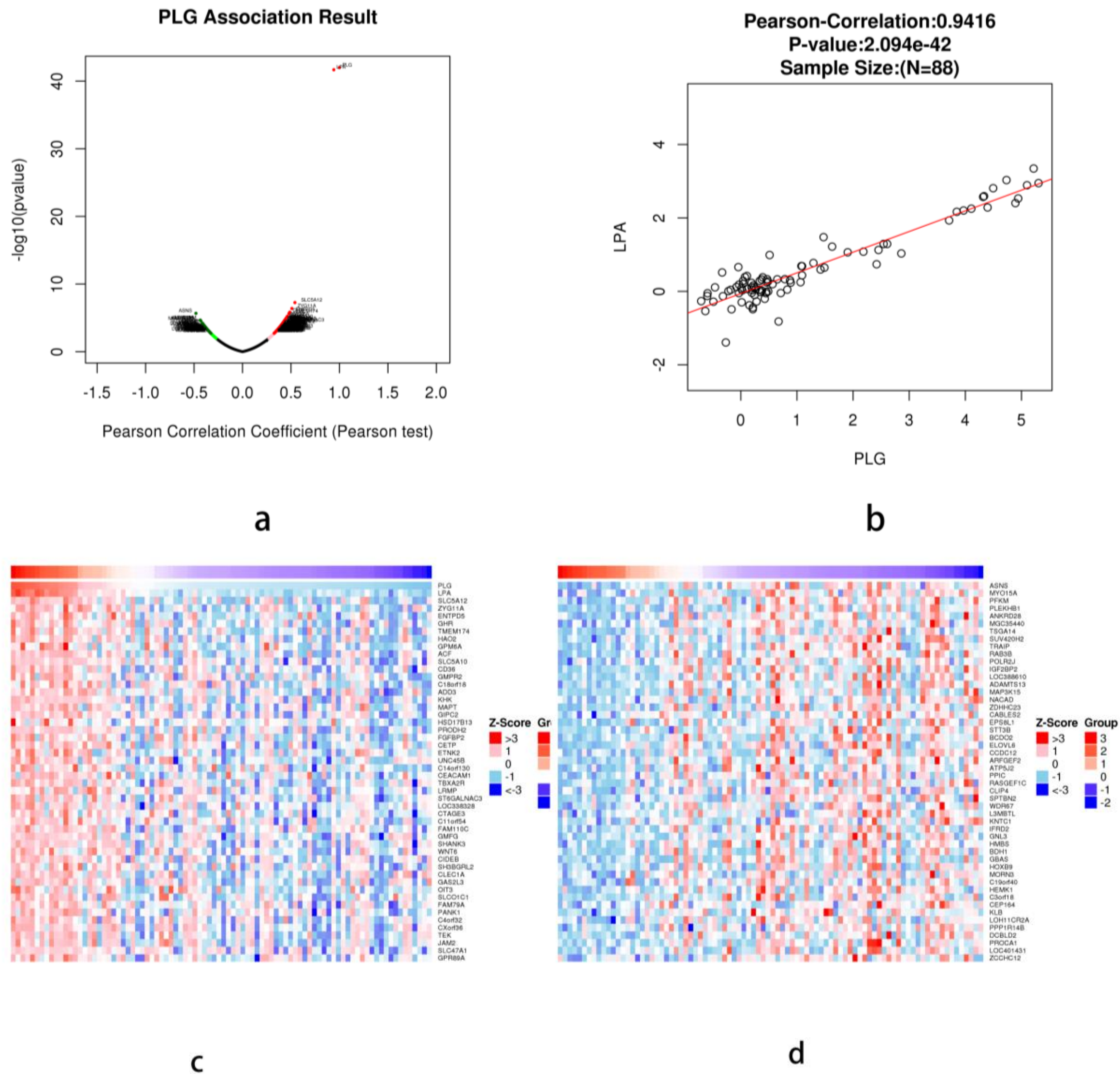

Figure 7. Co-expression genes of PLG in KIRC (LinkedOmics). (a) A pearson correlation test was used to analyze correlations between PLG and genes differentially expressed in KIRC, red indicates positively correlated genes and green indicates negatively correlated genes. (b) PLG and LPA Correlation. (c) Positively Correlated Significant Genes. (d) Negatively Correlated Significant Genes. Heat maps show the top 50 significant genes positively and negatively correlated with PLGin KIRC.

4 Discussion

Bioinformatics methods can provide us with gene expression levels and predict potential therapeutic targets. One of the best ways to reduce mortality is to detect accurately and treat successfully. Identifying key genes associated with the development and the progression of KIRC is crucial for its diagnosis and treatment. In this study, our data showed that PLG expression was lower in KIRC patients than that in normal tissues. PLG expression was remarkably correlated with KIRC patients' grade. A lower PLG expression resulted in a significant shorter OS in KIRC patients, suggesting

that PLG may play an important role in the prognosis of KIRC. By mutation analysis, CBioPortal tool unveiled three specific mutations (L469P, S292C, X317_splice, Y530C and N809S) unique presented in the KIRC samples that were not occurred in any other tumor types. These characteristic mutations of PLG in KIRC patients might facilitate to KIRC clinical diagnosis and monitoring. To determine the probably pathogenic mechanism of PLG in KIRC, PPI networks were further applied. The11 interacted proteins were identified by using PPI network analysis. Go enrichment analysis suggested these genes are enriched in fibrinolysis, secretion, negative regulation of cellular protein metabolic process and complement and coagulation cascades signaling pathway etc. PLG, a protein found in the host's plasma, which is activated into plasmin (PLM), a serine protease that degrades fibrin networks and promotes degradation of extracellular matrix (ECM), aiding maintenance of homeostasis(21). In addition to interacting with fibrin, PlG/PlM can also act on other proteins, such as cell surface receptors, coagulation components (factor V, factor VIII and factor X), metalloproteinases and structural components of ECM(22), including laminin, fibronectin, complement factors (C3 and C5), vitronectin, etc. (23). Therefore, PlG/PlM is associated with multiple physiological and pathological functions such as fibrinolysis and hemostasis, ECM degradation, tumor growth, invasion, migration, tissue remodeling, wound healing, angiogenesis and avoidance of immune response (24, 25). Recent study indicated that PLG is a complement inhibitor (24). In our setting, both C3a and C5a receptors were dramatically up-regulated in the renal cancer tissue, supporting the hypothesis that the two soluble modulators available in situ after the activation of the complement cascade may play a direct or indirect effect on resident cells to sustain carcinogenesis(26). Therefore, PLG may be antitumor by inhibiting complement activation. Furthermore, This may explain the result that the expression level of PLG in KIRC samples was lower than that in adjacent normal tissue in our study, which meant the low expression of PLG was important for KIRC progression (15). On the other hand, there is growing evidence that metabolic changes are linked to cancer (27). In addition to the well-known transition to aerobic glycolysis in cancer cells, mutations or changes in the expression of metabolic enzymes have been identified as potential cancer drivers (28). Changes in metabolite levels can influence the expression profile, epigenetic markers, and chromatin tissue of cancer, leading to changes in cell phenotypes, metastasis potential, and tumor microenvironment (29). In KIRC, in terms of biological process, positive regulation of nitrogen compound metabolic process, cellular biosynthetic process, biosynthetic process, cell cycle, transcription and gene expression etc. are significantly enriched in GO functional enrichment(30). Different evidence suggests that cancer cells reprogram their metabolism to meet the needs of cells to grow and divide. This provides opportunities for cancer diagnosis and treatment based on metabolic biomarkers and targets, respectively (31). In this work, The high expression of PLG in KIRC may play an anti-cancer role in two aspects, one is to regulate cell metabolism, the other is to inhibit complement response pathway.

5 Conclusion

In summary, this study recently found that PLG expression was decreased in KIRC patients compared with normal tissue and was positively correlated with patient survival time. The results of this study may further advance the mechanism of PLG in KIRC and provide high prognostic value of KIRC. However, the molecular mechanism and significance of PLG in KIRC tumorigenesis and treatment require further study.

Table1 GO enrichment and KEGG pathway analyze

Category	Term description	Count	P-Value
Biological Process (GO)			
GO:0042730	fibrinolysis	7	1.36E-15
GO:0030193	regulation of blood coagulation	8	5.61E-15
GO:0050878	regulation of body fluid levels	9	1.24E-11
GO:0046903	secretion	10	1.54E-10
GO:0045055	regulated exocytosis	9	2.55E-10
GO:0052547	regulation of peptidase activity	8	3.88E-10
GO:0010951	negative regulation of endopeptidase activity	7	7.02E-10
GO:0002576	platelet degranulation	6	1.73E-09
GO:0051918	negative regulation of fibrinolysis	4	1.96E-09
GO:0030198	extracellular matrix organization	7	2.16E-09
Molecular Function (GO）			
GO:0002020	protease binding	6	4.67E-09
GO:0061134	peptidase regulator activity	6	3.17E-08
GO:0004867	serine-type endopeptidase inhibitor activity	5	3.73E-08
GO:0004857	enzyme inhibitor activity	6	4.81E-07
GO:0030234	enzyme regulator activity	7	2.97E-06
GO:0019899	enzyme binding	8	2.84E-05
GO:0005518	collagen binding	3	4.48E-05
GO:0051087	chaperone binding	3	0.00016
GO:0005102	signaling receptor binding	6	0.00045
GO:0019865	immunoglobulin binding	2	0.00045
Cellular Component (GO)			
GO:0031093	platelet alpha granule lumen	6	1.46E-10
GO:0030141	secretory granule	9	1.05E-09
GO:0034774	secretory granule lumen	7	3.05E-09
GO:0005576	extracellular region	11	3.49E-09
GO:0044421	extracellular region part	9	3.12E-08
GO:0044433	cytoplasmic vesicle part	9	4.40E-08
GO:0005615	extracellular space	8	2.26E-07
GO:0031012	extracellular matrix	5	2.57E-06
GO:0012505	endomembrane system	10	2.19E-05
GO:0070062	extracellular exosome	3	9.40E-05
KEGG Pathways			
hsa04610	Complement and coagulation cascades	7	2.21E-13
Reactome Pathways			
HSA-75205	Dissolution of Fibrin Clot	7	8.06E-18
HSA-109582	Hemostasis	9	4.69E-11
HSA-114608	Platelet degranulation	5	1.23E-07

©Scholars Publishing, LLC

HSA-76005	Response to elevated platelet cytosolic Ca2+	5	1.23E-07
HSA-76002	Platelet activation, signaling and aggregation	5	2.42E-06
HSA-1474244	Extracellular matrix organization	4	0.00019
HSA-140837	Intrinsic Pathway of Fibrin Clot Formation	2	0.00078
HSA-1592389	Activation of Matrix Metalloproteinases	2	0.0015
HSA-140877	Formation of Fibrin Clot (Clotting Cascade)	2	0.0017
HSA-8950505	Gene and protein expression by JAK-STAT signaling after Interleukin-12 stimulation	2	0.0017

Declarations

1) ***Consent to publication***
   We declare that all authors agreed to publish the manuscript at this journal based on the signed Copyright Transfer Agreement and followed publication ethics.
2) ***Ethical approval and consent to participants***
   Not applicable.
3) ***Disclosure of conflict of interests***
   We declare that no conflict of interest exists.
4) ***Funding***
   None
5) ***Availability of data and material***
   We declare that the data supporting the results reported in the article are available in the published article.
6) ***Authors' Contributions***
   Authors contributed to this paper with the design (Chaoqun Xing), literature search (Chaoqun Xing), drafting (Chaoqun Xing), revision (Chaoqun Xing and Jiangrong Huang), editing (Chaoqun Xing) and final approval (Chaoqun Xing).
7) ***Acknowledgement***
   None
8) ***Authors' biography***
   None

References

1. Zhao E, Li L, Zhang W, Wang W, Chan Y, You B, et al. Comprehensive characterization of immune- and inflammation-associated biomarkers based on multi-omics integration in kidney renal clear cell carcinoma. J Transl Med. 2019;17(1):177.
2. Sinha R, Winer AG, Chevinsky M, Jakubowski C, Chen YB, Dong Y, et al. Analysis of renal cancer cell lines from two major resources enables genomics-guided cell line selection. Nat Commun. 2017;8:15165.
3. Ricketts CJ, Hill VK, Linehan WM. Tumor-specific hypermethylation of epigenetic biomarkers, including SFRP1, predicts for poorer survival in patients from the TCGA Kidney Renal Clear Cell Carcinoma (KIRC) project. PLoS One. 2014;9(1):e85621.
4. Cui H, Shan H, Miao MZ, Jiang Z, Meng Y, Chen R, et al. Identification of the key genes and pathways involved in the tumorigenesis and prognosis of kidney renal clear cell carcinoma. Sci Rep. 2020;10(1):4271.
5. Hsieh JJ, Le V, Cao D, Cheng EH, Creighton CJ. Genomic classifications of renal cell carcinoma: a critical step towards the future application of personalized kidney cancer care with pan-omics precision. J Pathol. 2018;244(5):525-37.
6. Zhang Y, Li Y, Deng J, Ji Z, Yu H, Li H. Sorafenib neoadjuvant therapy in the treatment of high risk renal cell carcinoma. PLoS One. 2015;10(2):e0115896.
7. Klumper N, Ralser DJ, Bawden EG, Landsberg J, Zarbl R, Kristiansen G, et al. LAG3 (LAG-3, CD223) DNA methylation correlates with LAG3 expression by tumor and immune cells, immune cell infiltration,

and overall survival in clear cell renal cell carcinoma. J Immunother Cancer. 2020;8(1).
8. Hao H, Wang Z, Ren S, Shen H, Xian H, Ge W, et al. Reduced GRAMD1C expression correlates to poor prognosis and immune infiltrates in kidney renal clear cell carcinoma. PeerJ. 2019;7:e8205.
9. Song J, Liu YD, Su J, Yuan D, Sun F, Zhu J. Systematic analysis of alternative splicing signature unveils prognostic predictor for kidney renal clear cell carcinoma. Journal of Cellular Physiology. 2019;234(12):22753-64.
10. Vago JP, Sugimoto MA, Lima KM, Negreiros-Lima GL, Baik N, Teixeira MM, et al. Plasminogen and the Plasminogen Receptor, Plg-RKT, Regulate Macrophage Phenotypic, and Functional Changes. Front Immunol. 2019;10:1458.
11. Urano T, Castellino FJ, Suzuki Y. Regulation of plasminogen activation on cell surfaces and fibrin. Journal of Thrombosis and Haemostasis. 2018;16(8):1487-97.
12. Liu J, Liu W, Lu Y, Tian H, Duan C, Lu L, et al. Piperlongumine restores the balance of autophagy and apoptosis by increasing BCL2 phosphorylation in rotenone-induced Parkinson disease models. Autophagy. 2018;14(5):845-61.
13. Zhang HJ, Sun ZQ, Qian WQ, Sheng L. Abnormal gene expression profile reveals the common key signatures associated with clear cell renal cell carcinoma: a meta-analysis. Genetics and Molecular Research. 2015;14(1):2216-24.
14. Wang S, Yu ZH, Chai KQ. Identification of EGFR as a Novel Key Gene in Clear Cell Renal Cell Carcinoma (ccRCC) through Bioinformatics Analysis and Meta-Analysis. Biomed Res Int. 2019;2019:6480865.
15. Zhang Z, Lin E, Zhuang H, Xie L, Feng X, Liu J, et al. Construction of a novel gene-based model for prognosis prediction of clear cell renal cell carcinoma. Cancer Cell Int. 2020;20:27.
16. Peng X, Chen Z, Farshidfar F, Xu X, Lorenzi PL, Wang Y, et al. Molecular Characterization and Clinical Relevance of Metabolic Expression Subtypes in Human Cancers. Cell Rep. 2018;23(1):255-69 e4.
17. Evelonn EA, Landfors M, Haider Z, Kohn L, Ljungberg B, Roos G, et al. DNA methylation associates with survival in non-metastatic clear cell renal cell carcinoma. BMC Cancer. 2019;19(1):65.
18. Lebovitz CB, Robertson AG, Goya R, Jones SJ, Morin RD, Marra MA, et al. Cross-cancer profiling of molecular alterations within the human autophagy interaction network. Autophagy. 2015;11(9):1668-87.
19. Chen VJ, Hernandez-Meza G, Agrawal P, Zhang CA, Xie L, Gong CL, et al. Time on Therapy for at Least Three Months Correlates with Overall Survival in Metastatic Renal Cell Carcinoma. Cancers (Basel). 2019;11(7).
20. Didiasova M, Wujak L, Wygrecka M, Zakrzewicz D. From plasminogen to plasmin: role of plasminogen receptors in human cancer. Int J Mol Sci. 2014;15(11):21229-52.
21. Ayon-Nunez DA, Fragoso G, Bobes RJ, Laclette JP. Plasminogen-binding proteins as an evasion mechanism of the host's innate immunity in infectious diseases. Biosci Rep. 2018;38(5).
22. Hsiao KC, Shih NY, Fang HL, Huang TS, Kuo CC, Chu PY, et al. Surface alpha-enolase promotes extracellular matrix degradation and tumor metastasis and represents a new therapeutic target. PLoS One. 2013;8(7):e69354.
23. Nguyen NTT, Rottgerding F, Devraj G, Lin YP, Koenigs A, Kraiczy P. The Complement Binding and Inhibitory Protein CbiA of Borrelia miyamotoi Degrades Extracellular Matrix Components by Interacting with Plasmin(ogen). Front Cell Infect Microbiol. 2018;8:23.
24. Barthel D, Schindler S, Zipfel PF. Plasminogen is a complement inhibitor. J Biol Chem. 2012;287(22):18831-42.
25. Principe M, Borgoni S, Cascione M, Chattaragada MS, Ferri-Borgogno S, Capello M, et

al. Alpha-enolase (ENO1) controls alpha v/beta 3 integrin expression and regulates pancreatic cancer adhesion, invasion, and metastasis. J Hematol Oncol. 2017;10(1):16.
26. Roumenina LT, Daugan MV, Noe R, Petitprez F, Vano YA, Sanchez-Salas R, et al. Tumor Cells Hijack Macrophage-Produced Complement C1q to Promote Tumor Growth. Cancer Immunol Res. 2019;7(7):1091-105.
27. Andrejeva D, Kugler JM, Nguyen HT, Malmendal A, Holm ML, Toft BG, et al. Metabolic control of PPAR activity by aldehyde dehydrogenase regulates invasive cell behavior and predicts survival in hepatocellular and renal clear cell carcinoma. BMC Cancer. 2018;18(1):1180.
28. Pavlova NN, Thompson CB. The Emerging Hallmarks of Cancer Metabolism. Cell Metab. 2016;23(1):27-47.
29. Noch E, Khalili K. Oncogenic viruses and tumor glucose metabolism: like kids in a candy store. Mol Cancer Ther. 2012;11(1):14-23.
30. Wei PJ, Zhang D, Xia J, Zheng CH. LNDriver: identifying driver genes by integrating mutation and expression data based on gene-gene interaction network. BMC Bioinformatics. 2016;17(Suppl 17):467.
31. Pandey N, Lanke V, Vinod PK. Network-based metabolic characterization of renal cell carcinoma. Sci Rep. 2020;10(1):5955.