PDK2: a novel diagnostic and prognostic biomarker for liver cancer

Zhi-Cheng Liu
The First Hospital of Jilin University
Yan-Qing Li
College of Basic Medical Sciences,Jilin University
Yan Jiao
The First Hospital of Jilin University
Yue-Chen Zhao (drzhaoyc@163.com)
The Second Hospital of Jilin University https://orcid.org/0000-0002-7098-5322

Primary research

Keywords: Pyruvate dehydrogenase kinase2 (PDK2), Liver neoplasms, Prognosis, The Cancer Genome Atlas (TCGA)

Posted Date: February 26th, 2020

DOI: https://doi.org/10.21203/rs.2.24570/v1

PDK2: a novel diagnostic and prognostic biomarker for liver cancer

Zhi-Cheng Liu[1,2] Yan-Qing Li[3] Yan Jiao[1*] Yue-Chen Zhao[4*]

[1] Department of Hepatobiliary and Pancreatic Surgery, The First Hospital of Jilin University, Changchun, Jilin 130021, P.R. China.

[2] Department of Gastrointestinal Surgery, The First Hospital of Jilin University, Changchun, Jilin 130021, P.R. China.

[3] Department of Pathophysiology, College of Basic Medical Sciences, Jilin University, Changchun, Jilin 130021, P.R. China.

[4] Department of Radiation Oncology, The Second Hospital of Jilin University, Changchun, Jilin 130022, P.R. China.

***Corresponding author:**

Yan Jiao and Yue-Chen Zhao contributed equally to this work.

Address: Department of Hepatobiliary and Pancreatic Surgery, The First Hospital of Jilin University, Changchun, Jilin 130021, P.R. China. (YJ); Department of Radiation Oncology, The Second Hospital of Jilin University, Changchun, Jilin 130022, P.R. China. (YCZ)

Phone number: +8613843101157(YJ); +8619904449098(YCZ)

E-mail: lagelangri1@126.com(YJ); drzhaoyc@163.com (YCZ)

Abstract

Background: Liver cancer (LC) is a common malignancy with very high morbidity. Pyruvate dehydrogenase kinases (PDKs) are regulators of mitochondrial pyruvate dehydrogenase complexes (PDCs) and play an important role in regulating cellular energy metabolism. In this

study, The Cancer Genome Atlas (TCGA) database was used to analyze the expression of PDK2 mRNA in LC, and to explore the value of PDK2 in the diagnosis and prognosis of LC.

Methods: The TCGA database, containing the clinical data of 373 LC patients, includes information on PDK2 expression values. The receiver operating characteristic (ROC) curve of PDK2 was drawn to evaluate its diagnostic ability. Patients were divided into PDK2 high- and low-expressing groups by threshold levels. The Chi-square test was used to evaluate the correlation between PDK2 levels and clinicopathological characteristics. The Kaplan-Meier estimator and Cox regression analysis were performed to assess the effect of PDK2 levels on survival outcomes.

Results: PDK2 expression in LC tissue was lower than that in normal liver tissues. According to the area under the curve (AUC) value calculated by ROC, PDK2 has a considerable diagnostic value for LC prognosis. The decreased expression of PDK2 is associated with clinical parameters, such as histologic grade (P=0.0001), radiation therapy (P=0.0490), vital status (P=0.0240), and overall survival (OS) (P=0.0222). Multivariate analysis shows that decreased PDK2 level is an independent risk factor for predicting poor prognosis in LC.

Conclusions: PDK2 has a significant impact on the prognosis of LC and is a potential biomarker for the diagnosis and prognosis of LC.

Keywords: Pyruvate dehydrogenase kinase2 (PDK2). Liver neoplasms. Prognosis. The Cancer Genome Atlas (TCGA).

Background

Liver cancer (LC) is one of the most common malignancies and has attracted worldwide attention. The morbidity of LC is higher than that of any other cancer, irrespective of gender [1]. LC is characterized by occult onset and rapid progression, together with a high degree of

invasiveness, metastasis and recurrence, with the result that the overall prognosis is extremely poor [2]. Studies have shown that the five-year survival rate of LC is more than 70% if it is diagnosed early, but is reduced to 10% if diagnosed at an advanced stage [3, 4]. Since most diagnoses occur in the middle or late stages, patients are only able to choose between interventional therapy, ablation therapy, radiation therapy and chemotherapy. Surgical resection is the choice of less than 20% of LC patients [5]. Therefore, there is a critical need to develop reliable novel biomarkers for the early diagnosis and prognosis of LC in order to choose the appropriate individualized treatment strategy.

Oxidative phosphorylation and glycolysis are two main ways for mammalian cells to obtain energy. Under aerobic conditions, ATP is produced mainly by oxidative phosphorylation, while under hypoxia, ATP is produced mainly by glycolysis [6]. Pyruvate dehydrogenase complexes (PDCs) are the key enzymes of the aerobic oxidation of glucose, catalyzing the conversion of pyruvate to acetyl-CoA which then enters the citric acid cycle, an important control point of glucose metabolism [7]. Pyruvate dehydrogenase kinases (PDKs) are the regulatory enzymes of PDCs and can negatively regulate PDC activity by phosphorylation. Therefore, an increase or decrease in PDKs expression will have a significant impact on glucose metabolism and cell bioenergetics [8]. Current research has found that PDKs are closely related to cancer cell metabolism, cancer drug resistance and cell steatosis [9-11].

PDKs include four subtypes, PDK1, PDK2, PDK3, and PDK4. Among them, PDK2 is the most widely distributed and is expressed in almost all tissues [12]. At present, it is generally believed that PDK2 is closely related to proto-oncogenes, transcription factors and growth factors in tumorigenesis and development [13]. However, there are few reports on the prognostic value of PDK2 levels in cancer cells. In this study, we performed a retrospective analysis of The Cancer Genome Atlas Liver Hepatocellular Carcinoma (TCGA-LIHC) level 3 data to evaluate the relationship between PDK2 levels and prognosis of LC. The value of PDK2

as a biomarker for diagnosis and prognosis of LC was explored.

Materials and methods

Data Mining

RNA-seq and clinical data of PDK2 were obtained from The Cancer Genome Atlas (TCGA) database (https://cancergenome.nih.gov). There is no need for ethical review as all information is in the public domain.

Statistical analysis

We used the ggplot2 software package in R (version 3.6.1) to draw boxplots of clinical features to visualize the differences between discrete variables[14, 15]. The SPSS software (version 20.0) was used to test the relationship between PDK2 expression and clinicopathological characteristics using the Chi-square test and Fisher's exact test. The receiver operating characteristic (ROC) curve was drawn using the PROC software package[16]. According to the threshold PDK2 value calculated in ROC, patients were divided into PDK2 high- and low-expressing groups. Area under the curve (AUC) was calculated to measure the diagnostic value of parameters [16]. The Kaplan-Meier curve was generated to compare the survival times of the different PDK2 expression groups, using the R-Survival package[17, 18]. The statistical significance of the above differences was calculated by log-rank test. The univariate and multivariate Cox proportional hazard models of overall survival (OS) and relapse-free survival (RFS) were established to explore the impact of PDK2 expression on prognosis and other clinical parameters.

Results

Data Overview

This study used the TCGA database of 373 LC patients with complete clinical data and PDK2 expression values. The clinical data include age, gender, histological type, histologic grade, stage, radiation therapy and residual tumor (Table 1).

Comparison of hepatic PDK2 expression in cancerous and normal tissues

Boxplots were prepared to examine the correlation between PDK2 expression and different histological features (Figure 1). The expression of PDK2 mRNA in LC tissue was lower than that in normal liver tissue (P=1.1e^{-10}). In addition, PDK2 expression was clearly related to histological type (P=0.0019), histologic grade (P=0.00043) and radiation therapy (P=0.0089).

The diagnostic value of PDK2 in LC

The ROC curve shows that the AUC value of all LC patients is 0.781, suggesting that PDK2 has considerable diagnostic ability (Figure 2). The subsequent subgroup analysis showed that the AUC of stages I, II, III, and IV were 0.774, 0.760, 0.816, and 0.844, respectively, suggesting that the diagnostic sensitivity and specificity were acceptable. These results indicate that PDK2 has a high degree of accuracy in predicting the prognosis of LC.

Correlation between PDK2 expression and clinical parameters in LC

Based on the threshold expression value, all patients were divided into high- and low-expressing groups and the correlations between PDK2 expression and clinical parameters were examined (Table 1). The Chi-square test indicated that PDK2 expression was clearly correlated with histologic grade (P = 0.0001), radiation therapy (P=0.0490), vital status (P=0.0240) and

OS (P =0.0222).

Hepatic PDK2 expression correlates with OS and RFS in all patients

The Kaplan–Meier curve was drawn to determine the correlation between PDK2 levels and patient survival (Figure 3). Patients were grouped as above. The statistical significance of the differences was calculated by log-rank test. The results showed that PDK2 levels were significantly associated with OS (P <0.0001) and RFS (P=0.0032). It is not difficult to conclude that decreased PDK2 levels can predict unsatisfactory OS and RFS in all patients. Next, the same method was used to explore the effect of PDK2 expression on patient prognosis in subgroup analysis.

Relationship of hepatic PDK2 expression with OS in subgroup analysis

Subgroup analysis showed that PDK2 levels have remarkable predictive value for OS (Figure 4). This was seen for PDK2 levels in relation to early clinical stage (I/II) (P <0.0001), early and late histologic stage (G1/G2 and G3/G4, P=0.00032 and P =0.0027, respectively), gender (P=2e^{-04} for males and P=0.0025 for females) and older and younger age (P=0.00024 and P=0.0013, respectively). In these subgroups, decreased hepatic PDK2 levels predict unsatisfactory OS.

Relationship of hepatic PDK2 expression with RFS in subgroup analysis

Subgroup analysis showed that PDK2 levels in early clinical stages (I/II) (P=0.00097), early histologic stage (G1/G2) (P<0.0001), male gender (P=0.0049) and older age (P=0.015) have remarkable predictive value for RFS (Figure 5). In the above subgroups, decreased hepatic PDK2 levels predict unsatisfactory RFS.

Univariate and multivariate analysis of OS of LC

Cox proportional hazard models were established for single and multiple factor analysis of OS (Figure 6). In univariate analysis, stage ($P<0.001$), T classification ($P<0.001$), residual tumor ($P=0.003$) and PDK2 expression ($P<0.001$) were clearly related to OS. Multivariate analysis showed that low expression of PDK2 was an independent risk factor for OS (risk ratio: 0.372, 95% CI: 0.226-0.613, $P<0.001$).

Univariate and multivariate analysis of RFS of LC

Cox proportional hazard models were established for single and multiple factor analysis of RFS (Figure 7). In univariate analysis, stage ($P<0.001$), T classification ($P<0.001$), residual tumor (P=0.042) and PDK2 expression ($P=0.004$) were clearly related to RFS. Multivariate analysis showed that T classification and residual tumor were independent risk factors for RFS (risk ratio: 1.659, 95% CI: 1.279-2.153, $P<0.001$ and risk ratio: 1.282, 95% CI: 1.004-1.637, $P=0.046$).

Discussion

Our group has, after a long period of research, identified a variety of novel LC biomarkers [19-38]. This study confirmed that hepatic PDK2 levels were lower in cancerous tissue than in normal tissue. PDK2 mRNA expression was found to be related to histologic grade, radiation therapy, vital status, and OS of LC patients. We also found that PDK2 plays an important role as a diagnostic indicator of LC. Patients with low PDK2 expression in LC have unsatisfactory OS and RFS, especially those in stage I/II. The Cox proportional hazard model confirmed that low expression of PDK2 is an independent risk factor for poor prognosis of LC. PDK2 may be a novel and reliable biomarker for clinical evaluation of LC prognosis.

Both the occurrence and development of tumors are closely related to energy metabolism. Even with sufficient oxygen content, malignant cancer cells still use glycolysis instead of aerobic oxidation as the main source of energy metabolism. This metabolic feature is called "Warburg metabolism". The traditional view is that Warburg metabolism is a prominent feature of malignant cells and that this metabolism is conducive to the rapid proliferation of cancer cells [39]. Previous studies have also confirmed that PDKs, as the regulatory enzyme of PDCs, can inhibit the activity of PDCs through phosphorylation, thus inhibiting the aerobic oxidation promoted by PDCs. This will shift the metabolism of cancer cells to glycolysis, which will promote the growth of cancer cells and inhibit their death [40]. It has been found that PDK2 expression is increased in tumor tissues such as glioblastoma, lung cancer, and gastric cancer [41-43]. Cui et al. found, by mining TCGA database in a similar method to ours, that PDK2 overexpression can lead to poor prognosis of acute myeloid leukemia [10].

However, in this study, we found the opposite phenomenon. PDK2 expression in LC tissue was observed to be lower than in normal tissue and low expression of PDK2 can lead to poor prognosis of LC. This difference may be due to the different type of cell: it is possible that PDK2 may have a unique role and mechanism in LC. Although the presence of Warburg metabolism has been confirmed in most cancer cells, not all cancer cells appear to utilize it to the same extent. In addition, different types of tumors have different bioenergetic demands [44, 45]. Approximately 50-70% of ATP in cancer cells is generated by glycolysis [46, 47]. Besides, cancer cells also use aerobic oxidative metabolism and thus have a dynamic balance between oxidative metabolism and glycolysis [48, 49]. The alteration in metabolic functioning in hepatocellular carcinoma cells is one of the most important features that distinguish it from other malignant cells [50]. We speculate that the potential mechanism of PDK2 in regulating LC metabolism may be inconsistent with other types of tumors. The low expression of PDK2 may provide favorable conditions for the proliferation and progression of hepatocellular

carcinoma cells by regulating the cell cycle and other pathways.

To our knowledge, this is the first study to investigate the prognostic significance of PDK2 in LC. We report that low PDK2 expression may be an independent risk factor for poor prognosis of LC. However, there are few in vivo and in vitro experiments on PDK2 in LC, and the specific molecular mechanism of PDK2 in LC is still not clear. The data of this study are only from a single public database and the sample size is limited. In future research, we intend to explore the potential prognostic value of PDK2 for LC in different populations and test its molecular function through in vivo and in vitro experiments.

Conclusions

In summary, we found that PDK2 levels were down-regulated in LC and were related to several clinicopathological features of patients. Patients with low PDK2 expression in LC have unsatisfactory OS and RFS. Low expression of PDK2 was also found to be an independent risk factor for poor LC prognosis. We conclude that PDK2 has a significant impact on the prognosis of LC and is a potential biomarker for the diagnosis and prognosis of LC. In future study, we plan to conduct complex experiments to further explore the mechanisms behind these findings and the application of PDK2 levels in predicting the prognosis of LC patients in the clinic.

Abbreviations

LC: Liver cancer; PDKs: Pyruvate dehydrogenase kinases; PDCs: Pyruvate dehydrogenase complexes; TCGA: The Cancer Genome Atlas; TCGA-LIHC: The Cancer Genome Atlas Liver Hepatocellular Carcinoma; ROC: Receiver operator characteristic; AUC: Area under the curve; OS: Overall survival; RFS: Relapse-free survival.

Declarations

Ethics approval and consent to participate

There is no need for ethical review as all information is in the public domain.

Consent for publication

All data published here are under the consent for publication.

Availability of data and materials

All data were obtained from The Cancer Genome Atlas (TCGA) database (https://cancergenome.nih.gov).

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable.

Authors' contributions

YJ, YCZ conceived and designed research; ZCL, YQL analyzed and interpreted the data and wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin. 2019; 69(1):7-34. doi:10.3322/caac.21551.
2. Forner A, Reig M, Bruix J. Hepatocellular carcinoma. Lancet. 2018; 391(10127):1301-14. doi:10.1016/S0140-6736(18)30010-2.
3. Altekruse SF, McGlynn KA, Reichman ME. Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005. J Clin Oncol. 2009; 27(9):1485-91. doi:10.1200/JCO.2008.20.7753.
4. Wang JH, Wang CC, Hung CH, Chen CL, Lu SN. Survival comparison between surgical resection and radiofrequency ablation for patients in BCLC very early/early stage hepatocellular carcinoma. J Hepatol. 2012; 56(2):412-8. doi:10.1016/j.jhep.2011.05.020.
5. Hung H. Treatment modalities for hepatocellular carcinoma. Curr Cancer Drug Targets. 2005; 5(2):131-8. doi:10.2174/1568009053202063.
6. Chae YC, Kim JH. Cancer stem cell metabolism: target for cancer therapy. BMB Rep. 2018; 51(7):319-26
7. Patel MS, Nemeria NS, Furey W, Jordan F. The pyruvate dehydrogenase complexes: structure-based function and regulation. J Biol Chem. 2014; 289(24):16615-23. doi:10.1074/jbc.R114.563148.
8. Korotchkina LG, Patel MS. Site specificity of four pyruvate dehydrogenase kinase isoenzymes toward the three phosphorylation sites of human pyruvate dehydrogenase. J Biol Chem. 2001; 276(40):37223-9. doi:10.1074/jbc.M103069200.
9. Go Y, Jeong JY, Jeoung NH, Jeon JH, Park BY, Kang HJ, et al. Inhibition of Pyruvate Dehydrogenase Kinase 2 Protects Against Hepatic Steatosis Through Modulation of Tricarboxylic Acid Cycle Anaplerosis and Ketogenesis. Diabetes. 2016; 65(10):2876-87. doi:10.2337/db16-0223.

10. Cui L, Cheng Z, Liu Y, Dai Y, Pang Y, Jiao Y, et al. Overexpression of PDK2 and PDK3 reflects poor prognosis in acute myeloid leukemia. Cancer Gene Ther. 2018. doi:10.1038/s41417-018-0071-9.
11. Liang Y, Hou L, Li L, Li L, Zhu L, Wang Y, et al. Dichloroacetate restores colorectal cancer chemosensitivity through the p53/miR-149-3p/PDK2-mediated glucose metabolic pathway. Oncogene. 2020; 39(2):469-85. doi:10.1038/s41388-019-1035-8.
12. Rardin MJ, Wiley SE, Naviaux RK, Murphy AN, Dixon JE. Monitoring phosphorylation of the pyruvate dehydrogenase complex. Anal Biochem. 2009; 389(2):157-64. doi:10.1016/j.ab.2009.03.040.
13. Contractor T, Harris CR. p53 negatively regulates transcription of the pyruvate dehydrogenase kinase Pdk2. Cancer Res. 2012; 72(2):560-7. doi:10.1158/0008-5472.CAN-11-1215.
14. Team RDCJC. R : A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2009; 14:12-21
15. Wickham H. Ggplot2 :elegant graphics for data analysis. Journal of the Royal Statistical Society. 2011; 174(1):245–6
16. Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez JC, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics. 2011; 12:77. doi:10.1186/1471-2105-12-77.
17. Therneau TM, April. A Package for Survival Analysis in S. 1994
18. Therneau TM, Grambsch PM: Modeling Survival Data: Extending the Cox Model, vol. 97. New York: Springer; 2000.
19. Jiao Y, Li Y, Lu Z, Liu Y. High Trophinin-Associated Protein Expression Is an Independent Predictor of Poor Survival in Liver Cancer. Digestive diseases and sciences. 2019; 64(1):137-43. doi:10.1007/s10620-018-5315-x.

20. Jiao Y, Li Y, Liu S, Chen Q, Liu Y. ITGA3 serves as a diagnostic and prognostic biomarker for pancreatic cancer. OncoTargets and therapy. 2019; 12:4141-52. doi:10.2147/ott.S201675.
21. Jiao Y, Li Y, Jiang P, Han W, Liu Y. PGM5: a novel diagnostic and prognostic biomarker for liver cancer. PeerJ. 2019; 7:e7070. doi:10.7717/peerj.7070.
22. Jiao Y, Li Y, Jiang P, Fu Z, Liu Y. High MAST2 mRNA expression and its role in diagnosis and prognosis of liver cancer. Scientific reports. 2019; 9(1):19865. doi:10.1038/s41598-019-56476-x.
23. Jiao Y, Li Y, Jia B, Chen Q, Pan G, Hua F, et al. The prognostic value of lncRNA SNHG4 and its potential mechanism in liver cancer. Bioscience reports. 2020; 40(1). doi:10.1042/bsr20190729.
24. Jiao Y, Li Y, Ji B, Cai H, Liu Y. Clinical Value of lncRNA LUCAT1 Expression in Liver Cancer and its Potential Pathways. Journal of gastrointestinal and liver diseases : JGLD. 2019; 28(4):439-47. doi:10.15403/jgld-356.
25. Jiao Y, Li Y, Fu Z, Hou L, Chen Q, Cai Y, et al. OGDHL Expression as a Prognostic Biomarker for Liver Cancer Patients. Disease markers. 2019; 2019:9037131. doi:10.1155/2019/9037131.
26. Jiao Y, Fu Z, Li Y, Zhang W, Liu Y. Aberrant FAM64A mRNA expression is an independent predictor of poor survival in pancreatic cancer. PloS one. 2019; 14(1):e0211291. doi:10.1371/journal.pone.0211291.
27. Jiao Y, Fu Z, Li Y, Meng L, Liu Y. High EIF2B5 mRNA expression and its prognostic significance in liver cancer: a study based on the TCGA and GEO database. Cancer management and research. 2018; 10:6003-14. doi:10.2147/cmar.S185459.
28. Yang D, Jiao Y, Li Y, Fang X. Clinical characteristics and prognostic value of MEX3A mRNA in liver cancer. PeerJ. 2020; 8:e8252. doi:10.7717/peerj.8252.

29. Nie Y, Jiao Y, Li Y, Li W. Investigation of the Clinical Significance and Prognostic Value of the lncRNA ACVR2B-As1 in Liver Cancer. BioMed research international. 2019; 2019:4602371. doi:10.1155/2019/4602371.
30. Cui Y, Jiao Y, Wang K, He M, Yang Z. A new prognostic factor of breast cancer: High carboxyl ester lipase expression related to poor survival. Cancer genetics. 2019; 239:54-61. doi:10.1016/j.cancergen.2019.09.005.
31. Zhao YC, Jiao Y, Li YQ, Fu Z, Yang ZY, He M. Elevated high mobility group A2 expression in liver cancer predicts poor patient survival. Revista espanola de enfermedades digestivas : organo oficial de la Sociedad Espanola de Patologia Digestiva. 2020; 112(1):27-33. doi:10.17235/reed.2019.6365/2019.
32. Li Y, Jiao Y, Luo Z, Li Y, Liu Y. High peroxidasin-like expression is a potential and independent prognostic biomarker in breast cancer. Medicine. 2019; 98(44):e17703. doi:10.1097/md.0000000000017703.
33. Li Y, Jiao Y, Li Y, Liu Y. Expression of La Ribonucleoprotein Domain Family Member 4B (LARP4B) in Liver Cancer and Their Clinical and Prognostic Significance. Disease markers. 2019; 2019:1569049. doi:10.1155/2019/1569049.
34. Li Y, Jiao Y, Fu Z, Luo Z, Su J, Li Y. High miR-454-3p expression predicts poor prognosis in hepatocellular carcinoma. Cancer management and research. 2019; 11:2795-802. doi:10.2147/cmar.S196655.
35. Hou L, Jiao Y, Li Y, Luo Z, Zhang X, Pan G, et al. Low EIF2B5 expression predicts poor prognosis in ovarian cancer. Medicine. 2020; 99(5):e18666. doi:10.1097/md.0000000000018666.
36. Fu Z, Jiao Y, Li YQ, Ke JJ, Xu YH, Jia BX, et al. PES1 In Liver Cancer: A Prognostic Biomarker With Tumorigenic Roles. Cancer management and research. 2019; 11:9641-53. doi:10.2147/cmar.S226471.

37. Fu Z, Jiao Y, Li Y, Ji B, Jia B, Liu B. TYMS presents a novel biomarker for diagnosis and prognosis in patients with pancreatic cancer. Medicine. 2019; 98(51):e18487. doi:10.1097/md.0000000000018487.
38. Cai H, Jiao Y, Li Y, Yang Z, He M, Liu Y. Low CYP24A1 mRNA expression and its role in prognosis of breast cancer. Scientific reports. 2019; 9(1):13714. doi:10.1038/s41598-019-50214-z.
39. Fitzgerald G, Soro-Arnaiz I, De Bock K. The Warburg Effect in Endothelial Cells and its Potential as an Anti-angiogenic Target in Cancer. Front Cell Dev Biol. 2018; 6:100. doi:10.3389/fcell.2018.00100.
40. Zhang W, Zhang SL, Hu X, Tam KY. Targeting Tumor Metabolism for Cancer Treatment: Is Pyruvate Dehydrogenase Kinases (PDKs) a Viable Anticancer Target? Int J Biol Sci. 2015; 11(12):1390-400. doi:10.7150/ijbs.13325.
41. Bonnet S, Archer SL, Allalunis-Turner J, Haromy A, Beaulieu C, Thompson R, et al. A mitochondria-K+ channel axis is suppressed in cancer and its normalization promotes apoptosis and inhibits cancer growth. Cancer Cell. 2007; 11(1):37-51. doi:10.1016/j.ccr.2006.10.020.
42. Michelakis ED, Sutendra G, Dromparis P, Webster L, Haromy A, Niven E, et al. Metabolic modulation of glioblastoma with dichloroacetate. Sci Transl Med. 2010; 2(31):31ra4. doi:10.1126/scitranslmed.3000677.
43. He Z, Li Z, Zhang X, Yin K, Wang W, Xu Z, et al. MiR-422a regulates cellular metabolism and malignancy by targeting pyruvate dehydrogenase kinase 2 in gastric cancer. Cell Death Dis. 2018; 9(5):505. doi:10.1038/s41419-018-0564-3.
44. Martin M, Beauvoit B, Voisin PJ, Canioni P, Guerin B, Rigoulet M. Energetic and morphological plasticity of C6 glioma cells grown on 3-D support; effect of transient glutamine deprivation. J Bioenerg Biomembr. 1998; 30(6):565-78.

doi:10.1023/a:1020584517588.

45. Moreno-Sanchez R, Rodriguez-Enriquez S, Marin-Hernandez A, Saavedra E. Energy metabolism in tumor cells. FEBS J. 2007; 274(6):1393-418. doi:10.1111/j.1742-4658.2007.05686.x.

46. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science. 2009; 324(5930):1029-33. doi:10.1126/science.1160809.

47. Mathupala SP, Ko YH, Pedersen PL. Hexokinase-2 bound to mitochondria: cancer's stygian link to the "Warburg Effect" and a pivotal target for effective therapy. Semin Cancer Biol. 2009; 19(1):17-24. doi:10.1016/j.semcancer.2008.11.006.

48. Potter M, Newport E, Morten KJ. The Warburg effect: 80 years on. Biochem Soc Trans. 2016; 44(5):1499-505. doi:10.1042/BST20160094.

49. Obre E, Rossignol R. Emerging concepts in bioenergetics and cancer research: metabolic flexibility, coupling, symbiosis, switch, oxidative tumors, metabolic remodeling, signaling and bioenergetic therapy. Int J Biochem Cell Biol. 2015; 59:167-81. doi:10.1016/j.biocel.2014.12.008.

50. Ma R, Zhang W, Tang K, Zhang H, Zhang Y, Li D, et al. Switch of glycolysis to gluconeogenesis by dexamethasone for treatment of hepatocarcinoma. Nat Commun. 2013; 4:2508. doi:10.1038/ncomms3508.

Tables

Table 1 Correlation of PDK2 mRNA expression in LC tissue with clinicopathologic variables.

Clinical characteristics	Variable	No. of patients	PDK2 expression				$\chi 2$	P value
			High	%	Low	%		
Age	<55	117	103	(31.12)	14	(34.15)	0.0465	0.7227
	>=55	255	228	(68.88)	27	(65.85)		

Gender	Female	121	108	(32.53)	13	(31.71)	0.0000	1.0000
	Male	252	224	(67.47)	28	(68.29)		
Histological type	Fibrolamellar carcinoma	3	2	(0.60)	1	(2.44)	3.8433	0.1001
	Hepatocellular carcinoma	363	325	(97.89)	38	(92.68)		
	Hepatocholangiocarcinoma (Mixed)	7	5	(1.51)	2	(4.88)		
Histologic grade	G1	55	54	(16.51)	1	(2.44)	20.5437	**0.0001**
	G2	178	165	(50.46)	13	(31.71)		
	G3	123	100	(30.58)	23	(56.1)		
	G4	12	8	(2.45)	4	(9.76)		
Stage	I	172	159	(51.13)	13	(34.21)	5.5831	0.1476
	II	87	76	(24.44)	11	(28.95)		
	III	85	71	(22.83)	14	(36.84)		
	IV	5	5	(1.61)	0	(0.00)		
T classification	T1	182	168	(50.91)	14	(34.15)	5.9882	0.1728
	T2	95	83	(25.15)	12	(29.27)		
	T3	80	66	(20.00)	14	(34.15)		
	T4	13	12	(3.64)	1	(2.44)		
	TX	1	1	(0.30)	0	(0.00)		
N classification	N0	253	224	(67.67)	29	(70.73)	1.0958	0.4190
	N1	4	3	(0.91)	1	(2.44)		
	NX	115	104	(31.42)	11	(26.83)		
M classification	M0	267	239	(71.99)	28	(68.29)	0.884	0.7364
	M1	4	4	(1.20)	0	(0.00)		
	MX	102	89	(26.81)	13	(31.71)		
Radiation therapy	No	340	304	(98.38)	36	(92.31)	3.3056	**0.0490**
	Yes	8	5	(1.62)	3	(7.69)		
Residual tumor	R0	326	292	(89.57)	34	(85.00)	1.4073	0.4755
	R1	17	15	(4.60)	2	(5.00)		
	R2	1	1	(0.31)	0	(0.00)		
	RX	22	18	(5.52)	4	(10.00)		
Vital status	Deceased	130	109	(32.83)	21	(51.22)	4.6548	**0.0240**
	Living	243	223	(67.17)	20	(48.78)		
Sample type	Primary tumor	371	330	(99.40)	41	(100.00)	0.0000	1.0000
	Recurrent tumor	2	2	(0.60)	0	(0.00)		
OS	No	237	218	(66.67)	19	(47.50)	4.9165	**0.0222**
	Yes	130	109	(33.33)	21	(52.50)		
RFS	No	179	163	(56.60)	16	(50.00)	0.2761	0.5741
	Yes	141	125	(43.40)	16	(50.00)		

Figure captions

Figure 1. Boxplots based on patient groups. Differences in PDK2 expression based on clinicopathological characteristics such as LC histological type, histologic grade, stage, TNM classification, residual tumor and radiation therapy.

Figure 2. ROC analysis of PDK2 expression in LC. Normal and tumor samples; Normal and stage I tumor samples; Normal and stage II tumor samples; Normal and stage III tumor samples;

Normal and stage IV tumor samples.

Figure 3. Relationship of hepatic PDK2 expression with OS and RFS. Relationship of hepatic PDK2 expression with OS and RFS in all patients.

Figure 4. Relationship of hepatic PDK2 expression with OS in subgroup analysis. Subgroup analysis of OS was performed according to patient gender, age, clinical stage and G stage.

Figure 5. Relationship of hepatic PDK2 expression with RFS in subgroup analysis. Subgroup analysis of RFS was performed according to patient gender, age, clinical stage and G stage.

Figure 6. Univariate and multivariate analyses of OS in LC patients.

Figure 7. Univariate and multivariate analyses of RFS in LC patients.

Figures

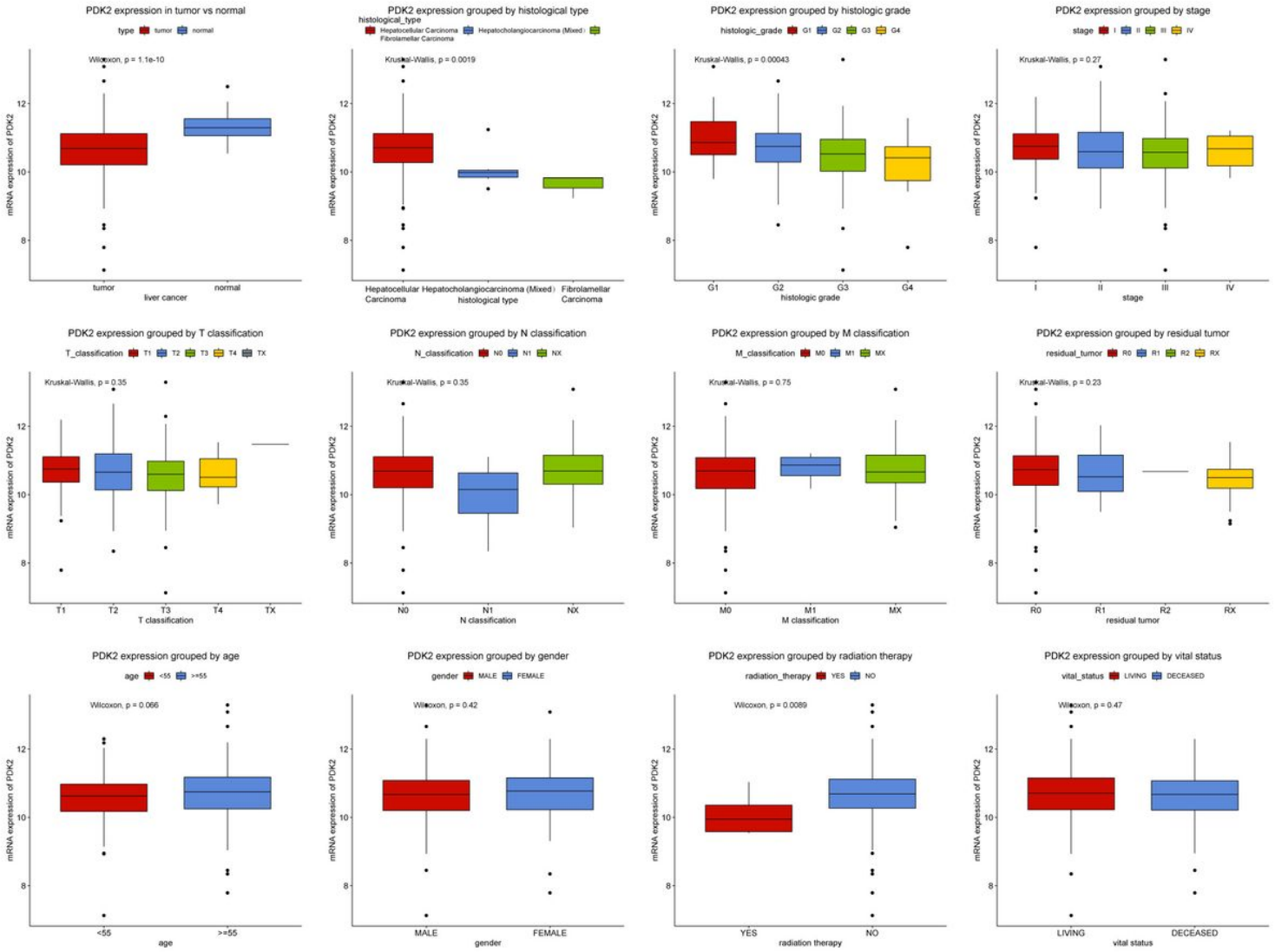


Figure 1

Boxplots based on patient groups. Differences in PDK2 expression based on clinicopathological characteristics such as LC histological type, histologic grade, stage, TNM classification, residual tumor and radiation therapy.

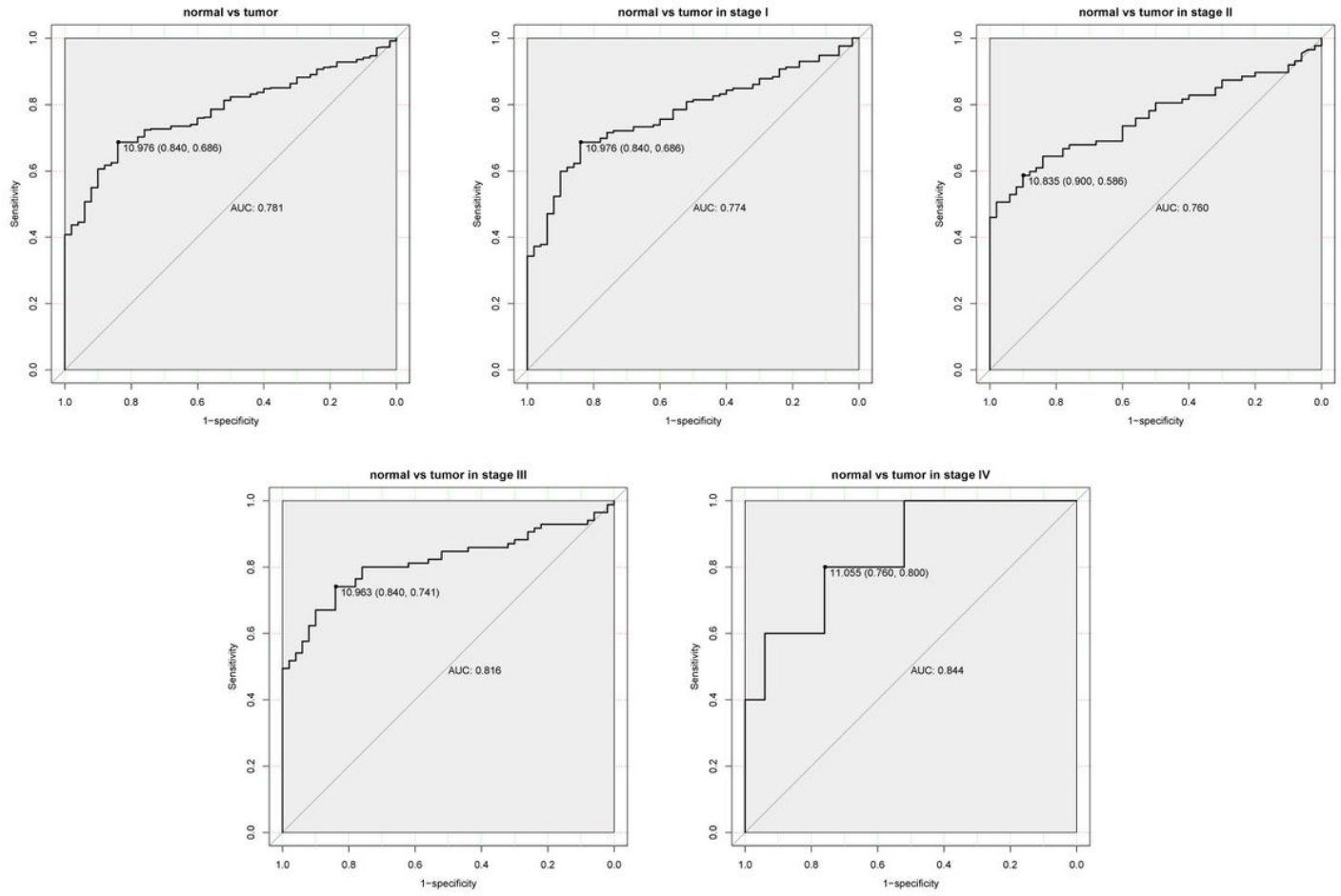


Figure 2

ROC analysis of PDK2 expression in LC. Normal and tumor samples; Normal and stage I tumor samples; Normal and stage II tumor samples; Normal and stage III tumor samples; Normal and stage IV tumor samples.

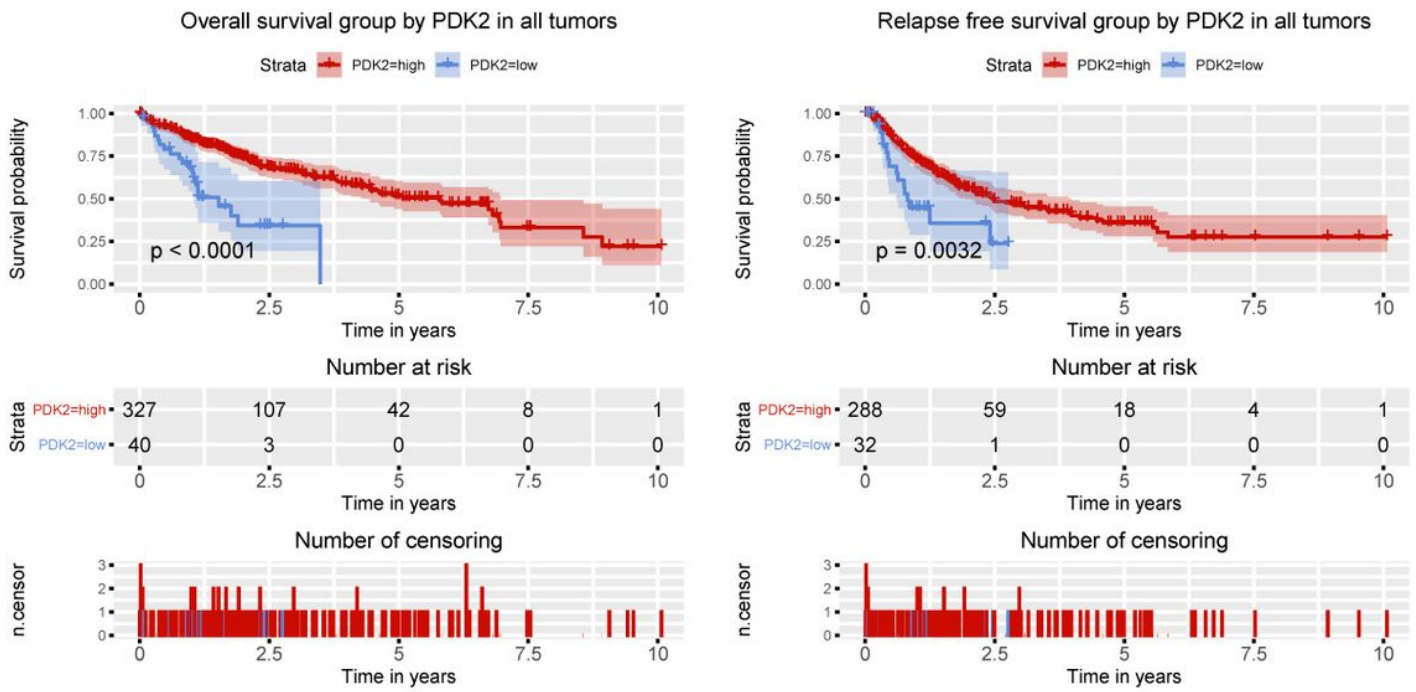


Figure 3

Relationship of hepatic PDK2 expression with OS and RFS. Relationship of hepatic PDK2 expression with OS and RFS in all patients.

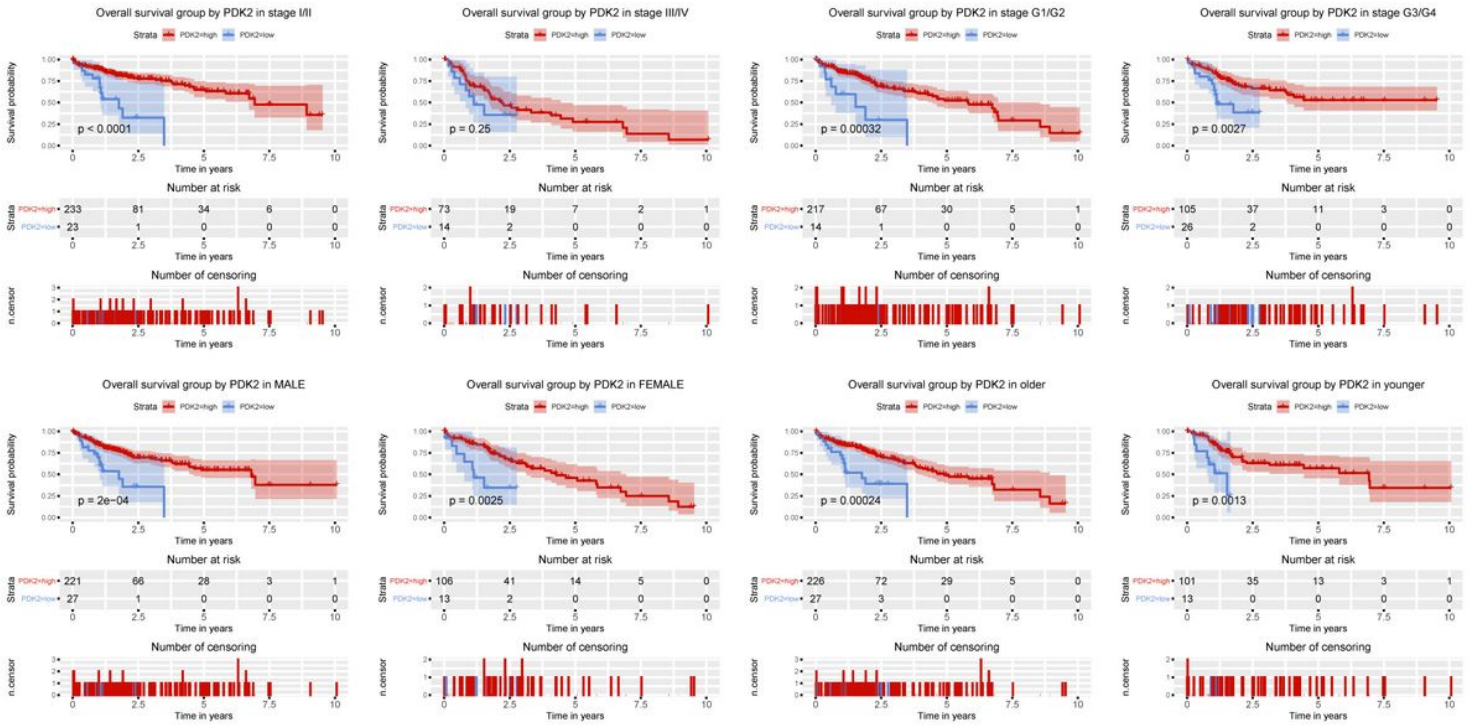


Figure 4

Relationship of hepatic PDK2 expression with OS in subgroup analysis. Subgroup analysis of OS was performed according to patient gender, age, clinical stage and G stage.

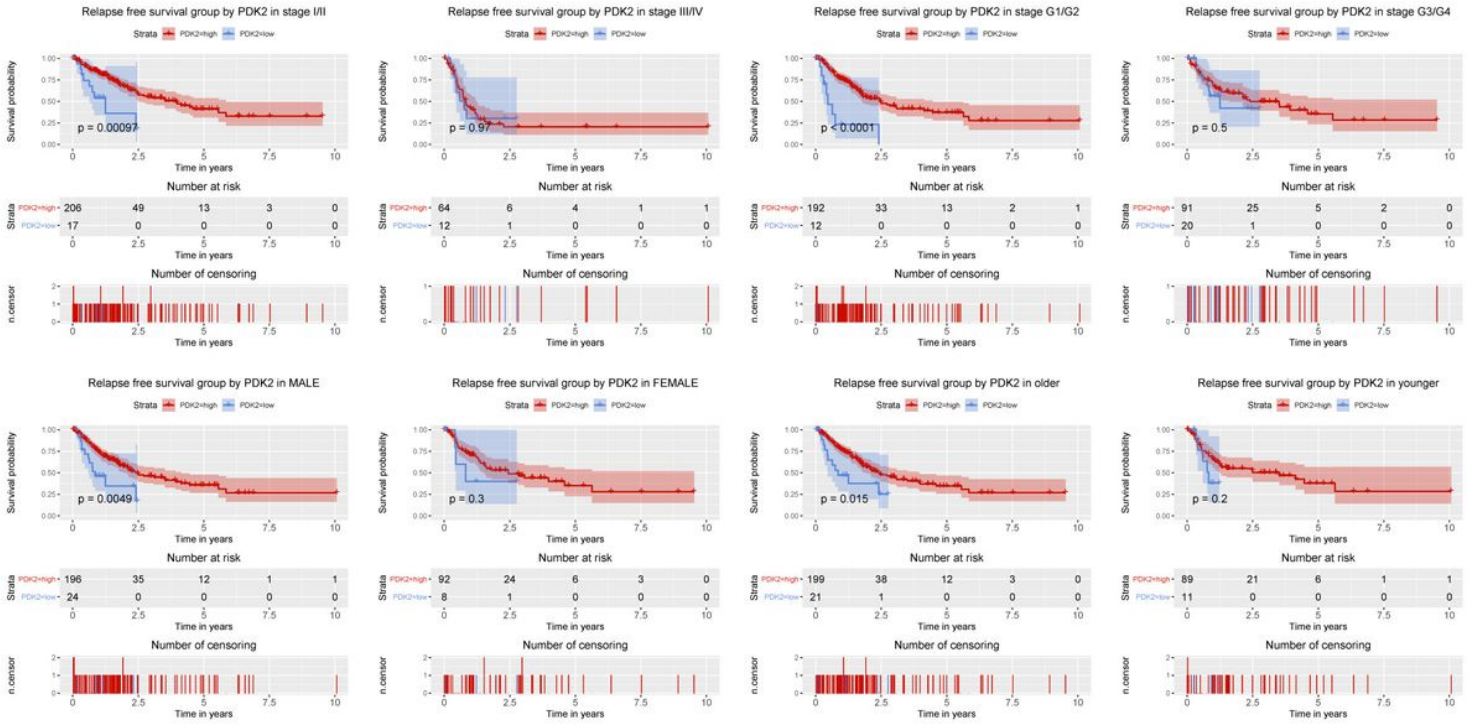
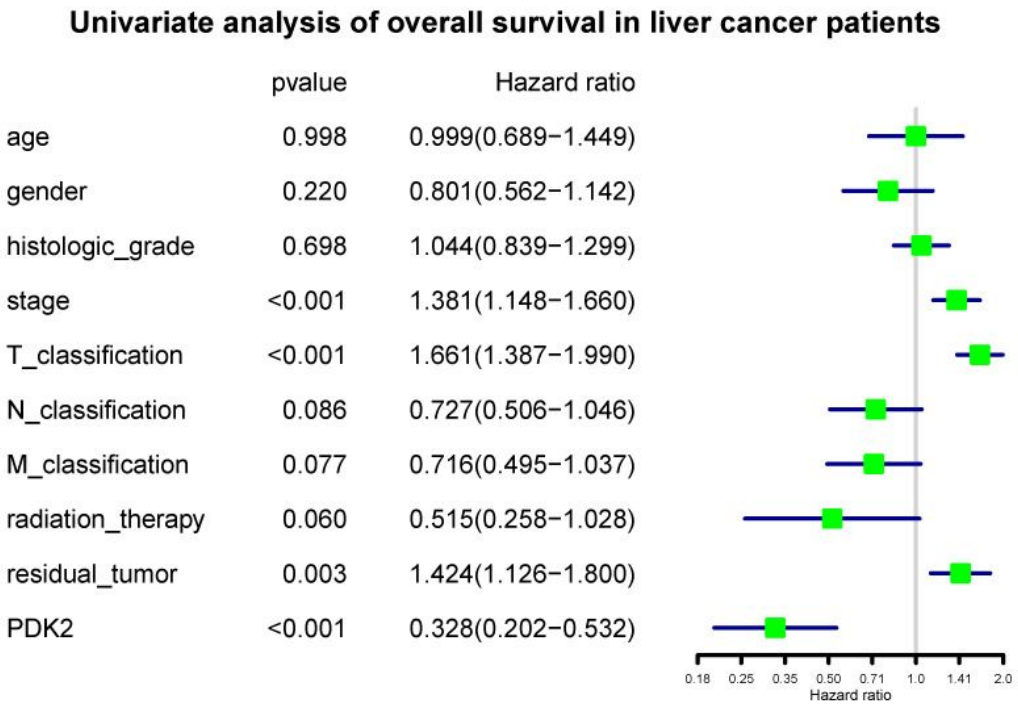


Figure 5

Relationship of hepatic PDK2 expression with RFS in subgroup analysis. Subgroup analysis of RFS was performed according to patient gender, age, clinical stage and G stage.



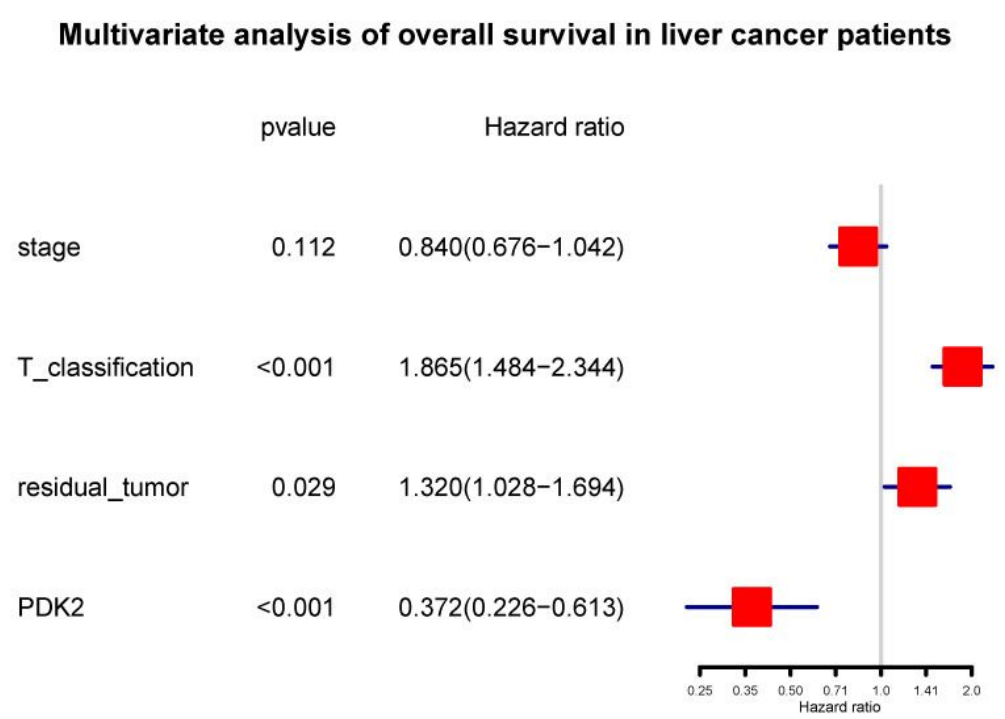
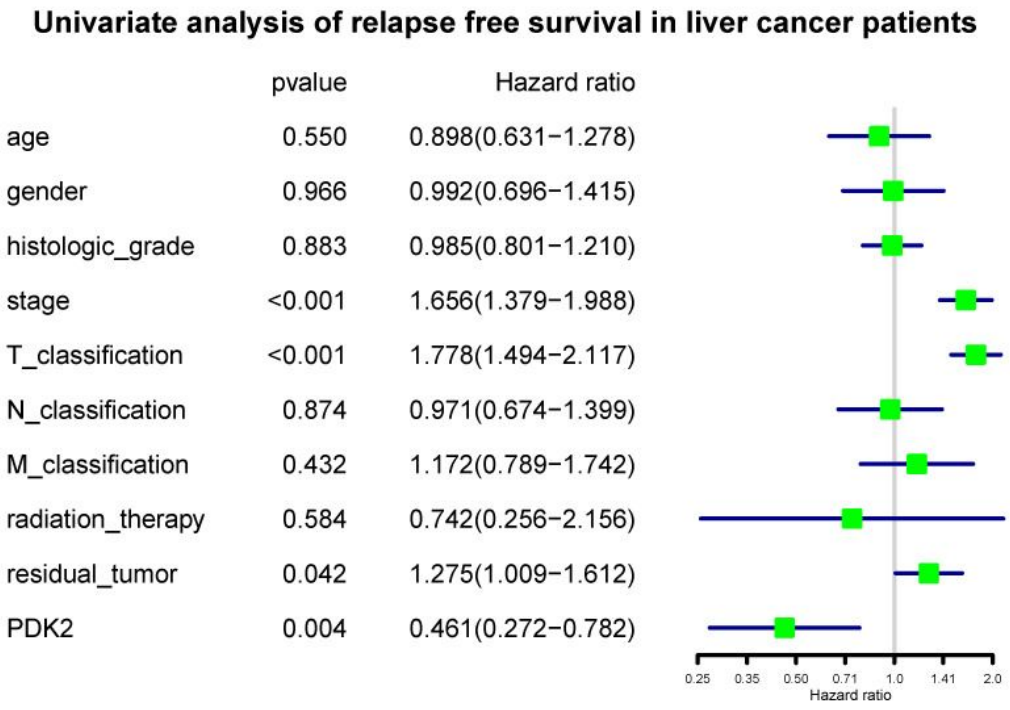


Figure 6

Univariate and multivariate analyses of OS in LC patients.



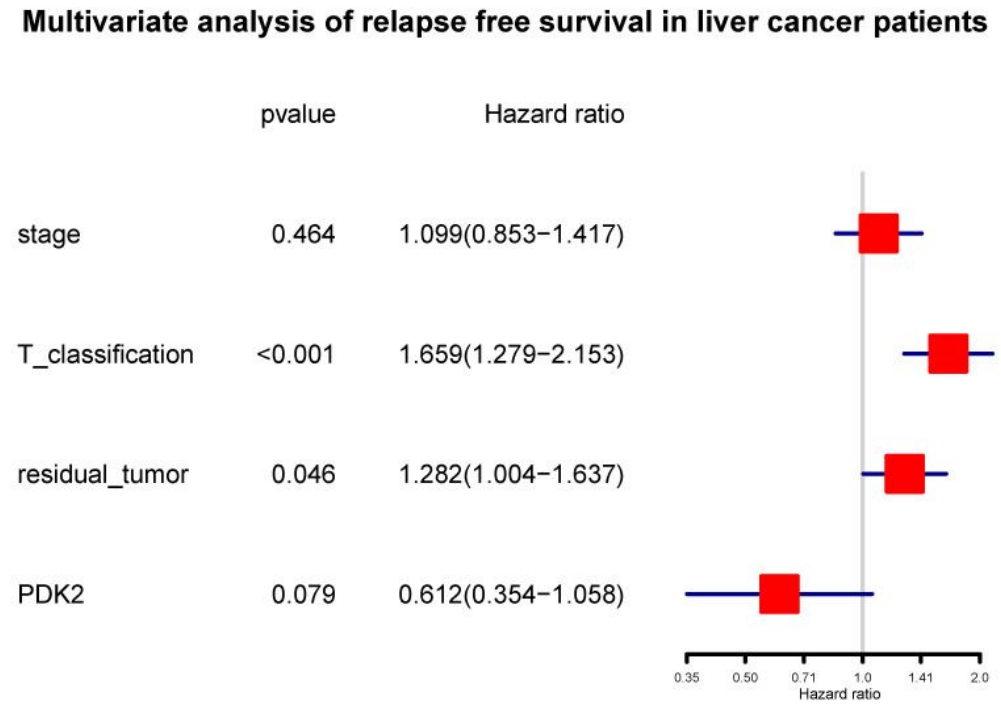


Figure 7

Univariate and multivariate analyses of RFS in LC patients.