Original Article
Running Title: Prognostic Value of LncRNA PANDAR in Hepatocellular Carcinoma
Received: October 19, 2024; Accepted: December 30, 2024

Prognostic Significance of LncRNA PANDAR Expression Levels in Patients with Hepatocellular Carcinoma

Sahar Ravanshad[*], MD, Mohammadhossein Taherynejad[**], MD, Mohsen Aliakbarian[***], MD, Mojtaba Asgharian Rezaei[*], MD, Amin Dalili[***], MD, Hassan Mehrad-Majd[****♦], PhD

[*]*Department of Internal Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
[**]*Endoscopic and Minimally Invasive Surgery Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*
[***]*Surgical Oncology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*
[****]*Cancer Molecular Pathology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*

♦Corresponding Author
Hassan Mehrad-Majd, PhD
Clinical Research development Unit,
Ghaem Hospital, Faculty of Medicine,
Mashhad University of Medical Sciences,
Mashhad, Iran
Tel/Fax: 098 51 38012694
Email: Mehradmajd.h@gmail.com

Abstract

Background: Long non-coding RNAs (LncRNAs) have been implicated in various biological processes and tumorigenesis. PANDAR is one of the long non-coding RNAs implicated in the development of multiple cancers, which has yet to be investigated for its clinical significance in hepatocellular carcinoma (HCC). Therefore, this study aimed to investigate the clinical significance of PANDAR in a cohort of Iranian patients with HCC.

Method: In this cross-sectional study, real-time quantitative polymerase chain reaction (PCR) was employed to analyze the expression levels of PANDAR in 22 paired HCC and adjacent normal tissues. Patients were subsequently stratified into high- and low-expression subgroups based on PANDAR expression levels. The associations between PANDAR expression, clinicopathological features, and overall survival were evaluated and analyzed using appropriate statistical tests.

Results: The results showed that PANDAR expression was significantly increased in HCC tumor tissues compared with adjacent normal tissues (P = 0.03, fold change = 2.04). Although Kaplan-Meier analysis did not reveal a significant difference in mean overall survival between the high- and low-expression groups (P = 0.215), Cox regression analysis identified an independent predictor role of PANDAR expression in HCC, both in univariate (hazard ratio (HR)=1.74, 95% confidence interval (CI): 1.10-2.99, P = 0.04) and multivariate (HR=1.74, 95%CI: 1.01-3.901, P = 0.05) models.

Conclusion: This study suggests that elevated PANDAR expression is associated with poor prognosis in HCC patients, thereby highlighting its potential clinical utility as a promising prognostic biomarker and therapeutic target.

Keywords: Hepatocellular carcinoma, long non-coding RNA PANDAR, Biomarkers, Gene expression, Prognosis

Introduction

Hepatocellular carcinoma (HCC) ranks among the most common and lethal forms of cancer, and its incidence and mortality rates are currently increasing worldwide.[1, 2] Although strides are made in diagnostic and therapeutic strategies, prognosis remains poor for HCC patients, commonly showing a 5-year survival of less than 20%.[2] Many believe that the poor outcome is due to the incapability of ideal biomarkers that can help in early detection, risk stratification, and monitoring to minimize its burden and associated mortality.[3] Therefore, identifying and developing genomic biomarkers could vastly improve patient outcomes by making early detection and treatment possible.[4, 5]

Long non-coding RNAs (lncRNAs) are undoubtedly the most hotly debated genomic biomarker that has gained attention in recent years. LncRNAs are long-chain transcripts, over 200 nucleotide bases long, that do not code for proteins. However, they are involved in the regulation of gene expression through mechanisms including interaction with RNA binding proteins, chromatin modifying enzymes, and microRNAs. Moreover, studies have shown that lncRNAs are potential biomarkers for various diseases, including cancer, which involved in biological processes, such as cell proliferation, differentiation, and apoptosis.[6-9] Also, some lncRNAs have been found to function as oncogenes, promoting tumorigenesis, while others act as tumor suppressors, highlighting their potential as diagnostic and therapeutic targets.

PANDAR - promoter of the cyclin dependent kinase inhibitor 1A (CDKN1A) antisense DNA damage-activated RNA - is a lncRNA located at chromosomal region 6p21.2 and spanned with 1506 nucleotides. There are various types of cancer associated with PANDAR including, gastric cancer, clear renal cell carcinoma, non-small-cell lung cancer, breast, bladder, colorectal, cervical, and liver cancer.[6, 9-14] It has been reported that PANDAR regulates gene expression by sponging microRNAs, inhibiting the translation of tumor suppressor genes, and promoting cell proliferation and migration. In HCC, PANDAR expression levels have been found to be significantly higher in tumor tissues compared with adjacent non-tumor tissues. However, the prognostic significance of PANDAR expression levels in HCC patients remains controversial, some studies suggest that high PANDAR expression is associated with poor overall survival and disease-free survival, while others have found no significant correlation between PANDAR expression and clinical outcomes.[15]

To address this knowledge gap, we conducted a cohort study to investigate the prognostic significance of PANDAR expression levels in a group of Iranian patients with HCC. Iran has one of the highest rates of liver cancer incidence in the world, with HCC being the most common type of liver cancer.[16] Specifically, our study aimed to determine whether PANDAR expression levels are associated with clinicopathological characteristics and patient outcomes in patients with HCC. By exploring the relationship between PANDAR expression and HCC prognosis, we hope to provide valuable insights into the potential

utility of PANDAR as a biomarker for early diagnosis and prognosis prediction in this devastating disease.

Materials and Methods

Patients and clinical tissue samples

This cross-sectional study was conducted between 2018 and 2021 at Imam Reza and Montaseriyeh hospitals in Mashhad, Iran. Tumor and non-tumor tissues were obtained from 24 patients with a definitive diagnosis of HCC. The inclusion criteria were: being newly diagnosed with HCC, as confirmed by imaging and histopathological evaluations, and having not received any preoperative treatments. Patients with a history of chemotherapy, radiotherapy, transarterial chemoembolization (TACE), radiofrequency ablation (RFA), or other locoregional therapies before surgical intervention and those concurrently diagnosed with malignancies in other organs were excluded from the study. Tissue samples used in the study were collected either from surgical hepatectomy specimens or liver tru-cut biopsies, depending on the patient's clinical situation and treatment plan. After surgery, all tissue specimens were immediately frozen and stored in RNA later (Thermo Fisher Scientific, Waltham, MA, USA) at 4 °C overnight and then moved at -80 °C till the extraction of RNA.

Informed consent was obtained from all patients. The study was approved by the organizational Ethics Committee of the faculty of medicine at Mashhad University of Medical Sciences (IR.MUMS.MEDICAL.REC.1397.196) and complied with the ethical standards outlined in the 1964 Helsinki Declaration, and its later amendments or equivalent ethical standards were ensured.

Quantitative real-time reverse transcriptase PCR

Total RNA was extracted from preserved tissue samples using the Trizol Reagent (Sangon Biotech Co., Ltd., Shanghai, China) and the quality and concentration of the extracted RNA were assessed using a Nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, cDNA synthesis was performed using a Reverse Transcription Kit (Wizbiosolutions, Seongnam, Gyeonggi, Korea) according to the manufacturer's protocol. Specific primers were designed and synthesized for the detection of PANDAR and GAPDH mRNA expression levels. The PANDAR primers used were 5'-CAATGCCTTGCTTCACAGTC-3' (forward) and 5'-TGGGGTTCTTAGAAGTGGTGA-3' (reverse), while those for GAPDH were 5'-CTCCTCCTCGTCGCAGTAGA-3' (forward) and 5'-GCTGCTTAGACGCTGGATTT-3' (reverse). The relative expression levels of PANDAR and GAPDH were quantified using quantitative real-time polymerase chain reaction (qRT-PCR) with SYBR Green dye (Takara) on a Roche LightCycler 96 Real-Time PCR System. The thermal cycling conditions consisted of an initial denaturation step at 95°C for 4 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing at 60°C for 1 minute, and extension at 95°C for 10 seconds. The $2^{-\Delta\Delta Ct}$ method was employed to analyze the data, normalizing the results to the internal control GAPDH.

Statistical analysis

IBM SPSS Statistics 22 software, was used to analyze the data, and a P-value<0.05 was considered statistically significant. The levels of PANDAR expression in tumor and non-tumor tissues were compared using a paired-sample t-test. Patients were then divided into two groups of high and low based on their PANDAR expression levels. To explore the relationship between PANDAR expression and various patient characteristics, such as age, gender, and cancer stage, the

independent t-test, Chi-square test, and Fisher's exact test were applied. Kaplan–Meier analysis and Log Rank (Mantel-Cox) tests, were used to perform the overall survival analysis. Finally, Cox proportional hazard analyses both in univariate and multivariate models were conducted to assess the impact of PANDAR expression levels and other variables on patients' overall survival.

Results

Baseline characteristics of patients

A total of 24 patients with a confirmed diagnosis of HCC were initially enrolled in this study. Of these, 22 patients reached the final data analysis phase. The majority of patients were male (86.36%), with a mean age of 52.95±15.37 years. The detailed clinicopathological features of patients are summarized in Table 1.

PANDAR expression was up-regulated in HCC tissues

Initially, we explored the relative expression level of lncRNA PANDAR in HCC (n = 22) compared with adjacent non-tumor tissue. The expression level of the PANDAR gene in tumor tissue was significantly higher than in normal tissue ($P = 0.04$) (Figure 1). In terms of fold change (ΔΔCT), the PANDAR gene expression in tumor tissue is more than double than in normal tissue (fold change = 2.04, $P = 0.04$).

PANDAR expression and clinicopathological characteristics in HCC

To further assess the correlation between PANDAR expression and clinicopathological features, patients were divided into high (n = 10) or low (n = 12) expression groups based on the Log2 fold changes of PANDAR expression in tumors and non-tumor tissues (Figure 2). As shown in Table 1, there was no significant difference in most clinicopathological features between the groups, except for liver status. Patients in the high PANDAR expression group were significantly more likely to have undergone liver transplantation compared with those in the low expression group (90% vs. 10%; $P = 0.045$). This difference shows a potential relationship between the expression of PANDAR and liver transplantation specimens.

PANDAR expression and prognosis of HCC patients

All patients' overall mean and median survival was 32.14 ± 3.31 months and 36 months, respectively. The prognostic significance of PANDAR expression was further evaluated in the context of liver transplant specimens. While Kaplan-Meier analysis showed that there was no significant difference in the mean overall survival between patients with high and low PANDAR expression group (33.47 ± 6.13 and 41.10 ± 4.77 months, Log-rank test, $P = 0.21$), univariate and multivariate Cox regression analyses identified PANDAR expression as an independent prognostic factor (hazard ratio (HR)=1.74, 95% confidence interval (CI): 1.01-3.01, $P = 0.05$). This suggests that PANDAR expression could play a role in predicting survival outcomes of patients (Table 2 and Figure 3).

Discussion

This study investigated the role of PANDAR in patients with HCC. Expression of PANDAR was significantly upregulated in the HCC tissues compared with adjacent normal tissues. Moreover, we found that patients with higher PANDAR expression were more likely to have undergone liver transplantation, and PANDAR was identified as an independent prognostic factor for overall survival.

HCC remains one of the leading causes of cancer-related mortality worldwide. LncRNAs have emerged as important players in cancer biology, influencing tumor initiation, progression, and prognosis.[9, 17, 18]

The role of PANDAR in cancer is multifaceted and previous studies have explored both upregulation and downregulation of PANDAR expression in various types of cancer. In line with our findings, Peng W et al.[13] reported that PANDAR is remarkably upregulated in HCC samples and associated with poor prognosis, and it has diagnostic value as a biomarker for screening of HCC patients. Similarly, in cervical and colorectal cancer, studies have reported overexpression of the PANDAR gene, correlating with adverse of clinical outcomes, including shorter overall survival and aggressive tumor characteristics such as advanced tumor-node-metastasis (TNM) stage, histological grade, and tumor size.[6, 8] Furthermore, a comprehensive meta-analysis conducted by Mehrad-Majd H revealed that high PANDAR expression is significantly associated with poor prognosis in patients with different types of cancer.[15] On the other hand, there is conflicting evidence with our findings. Puvvula et al.[19] and Peng C et al.[20] reported that the PANDAR was downregulated in HCC patients compared with normal tissues. These studies suggested the lower PANDAR expression might be conducive to tumorigenesis by down-regulating the transcription of pro-inflammatory cytokines like IL8. Such discrepant observations further solidify the versatile aspect that PANDAR may have in different tumor types and attest to the requirement for more detailed clarifications regarding that dual nature of malignancies. Our study indicates that PANDAR expression may serve as a promising biomarker for predicting prognosis and clinical pathology including measurable aspects of tumor behavior and tumor progression, including the tumor size, histological differentiation, vascular invasion, and metastatic potential in cancer patients. Specifically, high expression levels of PANDAR showed an association with poor survival outcomes and a status of liver transplantation, suggesting its possible functionality as a marker of an advanced disease or a poor prognosis. PANDAR expression in previous studies was also linked with lymph node metastasis, TNM stage, and aggressiveness of the tumor in several cancers, indicating its relevance in making treatment decisions and predicting the outcome of patients.

However, despite the strengths of the present study, including the use of well-characterized patient cohorts and statistically robust analyses, an area of limitation remains open for discussion. A selection bias might have affected our findings. For instance, there were many patients with advanced HCC requiring liver transplantation who had high PANDAR expression. Secondly, the exclusion of patients without complete medical records may have contributed to a smaller subpopulation size of some clinical groups. Thirdly, the whole selection could have been biased because of limited access to a proper evaluation of the microvascular invasion and histological grade by liver biopsy. All these limitations may have introduced some bias since these histopathological parameters are well-known for their significant role in the prognosis of the disease and expressivity as a biomarker. We hope that this study may pave the way for more extensive research involving many different patient populations, so as to closely incorporate a correct pathological assessment and deal with the issues of future selection bias. Only through this will PANDAR be better situated, first to define its role, and then to become a possible candidate for clinical mastication.

Conclusion

Our study demonstrates that PANDAR expression is remarkably associated with the prognosis and overall survival of HCC patients. PANDAR is capable of being

applied as a biomarker to reveal the prognosis and progression of disease in HCC patients. However, while our findings highlight its clinical relevance. Further research is needed to fully understand the mechanisms underlying PANDAR's role in HCC development and progression.

Acknowledgments
The authors would like to express their gratitude to the Clinical Research Development Unit of Ghaem Hospital, Mashhad University of Medical Sciences, for their assistance in this manuscript. This study was supported by research project No. 961819 as a residency thesis at Mashhad University of Medical Sciences.

Funding
None declared.

Authors' Contributions
H.M: Study design, data gathering, drafting and reviewing the manuscript; S.R: Study design, and reviewing the manuscript; MH.T: Study design, and reviewing the manuscript; M.A: Data gathering, drafting; M.A: Data gathering, drafting; A.D: Study design, reviewing the manuscript; M.S: Study design, reviewing the manuscript.
All authors have read and approved the final manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest
None declared.

References
1. Foglia B, Turato C, Cannito S. Hepatocellular carcinoma: Latest research in pathogenesis, detection and treatment. *Int J Mol Sci.* 2023;24(15):12224. doi: 10.3390/ijms241512224. PMID: 37569600; PMCID: PMC10419038.
2. Asafo-Agyei KO, Samant H. Hepatocellular carcinoma. 2023 Jun 12. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan–. PMID: 32644603.
3. Chakraborty E, Sarkar D. Emerging therapies for hepatocellular carcinoma (HCC). *Cancers (Basel).* 2022;14(11):2798. doi: 10.3390/cancers14112798. PMID: 35681776; PMCID: PMC9179883.
4. Das S, Dey MK, Devireddy R, Gartia MR. Biomarkers in cancer detection, diagnosis, and prognosis. *Sensors (Basel).* 2023;24(1):37. doi: 10.3390/s24010037. PMID: 38202898; PMCID: PMC10780704.
5. Yuan D, Chen Y, Li X, Li J, Zhao Y, Shen J, et al. Long non-coding RNAs: Potential biomarkers and targets for hepatocellular carcinoma therapy and diagnosis. *Int J Biol Sci.* 2021;17(1):220-35. doi: 10.7150/ijbs.50730. PMID: 33390845; PMCID: PMC7757045.
6. Huang HW, Xie H, Ma X, Zhao F, Gao Y. Upregulation of LncRNA PANDAR predicts poor prognosis and promotes cell proliferation in cervical cancer. *Eur Rev Med Pharmacol Sci.* 2017;21(20):4529-35. PMID: 29131264.
7. Jiang Y, Feng E, Sun L, Jin W, You Y, Yao Y, et al. An increased expression of long non-coding RNA PANDAR promotes cell proliferation and inhibits cell apoptosis in pancreatic ductal adenocarcinoma. *Biomed Pharmacother.* 2017;95:685-91. doi: 10.1016/j.biopha.2017.08.124. PMID: 28886528.
8. Lu M, Liu Z, Li B, Wang G, Li D, Zhu Y. The high expression of long non-coding RNA PANDAR indicates a poor prognosis for colorectal cancer and promotes metastasis by EMT pathway. *J Cancer Res Clin Oncol.* 2017;143(1):71-81. doi: 10.1007/s00432-016-2252-y. PMID: 27629879.

9. Li X, Wang F, Sun Y, Fan Q, Cui G. Expression of long non-coding RNA PANDAR and its prognostic value in colorectal cancer patients. *Int J Biol Markers.* 2017;32(2):e218-e223. doi: 10.5301/jbm.5000249. PMID: 28106228.
10. Han L, Zhang EB, Yin DD, Kong R, Xu TP, Chen WM, et al. Low expression of long noncoding RNA PANDAR predicts a poor prognosis of non-small cell lung cancer and affects cell apoptosis by regulating Bcl-2. *Cell Death Dis.* 2015;6(2):e1665. doi: 10.1038/cddis.2015.30. PMID: 25719249; PMCID: PMC4669812.
11. Zhan Y, Lin J, Liu Y, Chen M, Chen X, Zhuang C, et al. Correction: Up-regulation of long non-coding RNA PANDAR is associated with poor prognosis and promotes tumorigenesis in bladder cancer. *J Exp Clin Cancer Res.* 2023;42(1):185. doi: 10.1186/s13046-023-02773-3. Erratum for: *J Exp Clin Cancer Res.* 2016;35(1):83. doi: 10.1186/s13046-016-0354-7. PMID: 37507730; PMCID: PMC10385883.
12. Ma P, Xu T, Huang M, Shu Y. Increased expression of LncRNA PANDAR predicts a poor prognosis in gastric cancer. *Biomed Pharmacother*. 2016;78:172-6. doi: 10.1016/j.biopha.2016.01.025. PMID: 26898439.
13. Peng W, Fan H. Long non-coding RNA PANDAR correlates with poor prognosis and promotes tumorigenesis in hepatocellular carcinoma. *Biomed Pharmacother*. 2015;72:113-8. doi: 10.1016/j.biopha.2015.04.014. PMID: 26054684.
14. Xu Y, Tong Y, Zhu J, Lei Z, Wan L, Zhu X, et al. An increase in long non-coding RNA PANDAR is associated with poor prognosis in clear cell renal cell carcinoma. *BMC Cancer*. 2017;17(1):373. doi: 10.1186/s12885-017-3339-9. PMID: 28545465; PMCID: PMC5445460.
15. Mehrad-Majd H, Akhtari J, Haerian MS, Ravanshad Y. Clinicopathological and prognostic value of lncRNA PANDAR expression in solid tumors: Evidence from a systematic review and meta-analysis. *J Cell Physiol.* 2019;234(4):4206-16. doi: 10.1002/jcp.27179. PMID: 30191982.
16. Fattahi N, Rezaei N, Asadi-Lari M, Yousefi M, Madadi Z, Gohari K, et al. Hepatocellular carcinoma incidence at national and provincial levels in Iran from 2000 to 2016: A meta-regression analysis. *PLoS One.* 2021;16(1):e0245468. doi: 10.1371/journal.pone.0245468. PMID: 33481849; PMCID: PMC7822534..
17. Galicia-Moreno M, Silva-Gomez JA, Lucano-Landeros S, Santos A, Monroy-Ramirez HC, Armendariz-Borunda J. Liver cancer: Therapeutic challenges and the importance of experimental models. *Can J Gastroenterol Hepatol.* 2021;2021:8837811. doi: 10.1155/2021/8837811. PMID: 33728291; PMCID: PMC7937489.
18. Lee JT. Epigenetic regulation by long noncoding RNAs. *Science.* 2012;338(6113):1435-9. doi: 10.1126/science.1231776. PMID: 23239728.
19. Puvvula PK, Desetty RD, Pineau P, Marchio A, Moon A, Dejean A, et al. Long noncoding RNA PANDA and scaffold-attachment-factor SAFA control senescence entry and exit. *Nat Commun.* 2014;5:5323. doi: 10.1038/ncomms6323. PMID: 25406515; PMCID: PMC4263151.
20. Peng C, Hu W, Weng X, Tong R, Cheng S, Ding C, et al. Over expression of long non-coding RNA PANDA promotes hepatocellular carcinoma by inhibiting senescence associated inflammatory factor IL8. *Sci Rep.* 2017;7(1):4186. doi: 10.1038/s41598-017-04045-5. PMID: 28646235; PMCID: PMC5482898.

Table 1. Clinicopathological characteristics of patients with HCC based on PANDAR gene expression

Variable		PANDAR expression		*P*-value
		Low (n = 12)	High (n = 10)	
Age (years)		50.42 ± 16.63	56.0 ± 13.91	0.41
Tumor size (cm)		5.13 ± 2.88	5.23 ± 2.36	0.93
Alpha-fetoprotein (ng/ml)		3.86 ± 2.29	3.08 ± 1.61	0.38
Gender**	**Male (%)**	9 (75.00)	10 (100.00)	0.22
	Female (%)	3 (25.00)	0 (0)	
Cirrhosis **	**Yes**	6 (50.00)	8 (80.00)	0.20
	No	6 (50.00)	2 (20.00)	
Hepatitis B virus (%)	**Yes**	5 (41.67)	5 (50.00)	0.70
	No	7 (58.33)	5 (50.00)	
Hepatitis C virus (%)	**Yes**	1 (8.33)	1 (10.00)	0.09
	No	11 (91.67)	9 (90.00)	
Tumor differentiation (%)	**Well**	5 (41.67)	5 (50.0)	0.73
	Moderate	4 (33.33)	4 (40.00)	
	Poor	3 (25.00)	1 (10.00)	
TNM stage (%)	**T1**	6 (50.00)	2 (20.00)	0.41
	T2	1 (8.33)	2 (20.00)	
	T3	1 (8.00)	3 (30.00)	
	T4	4 (33.33)	3 (30.00)	
Tumor nodule (%)	**Single**	10 (83.33)	5 (50.00)	0.09
	Multiple	2 (16.67)	5 (50.00)	
Liver status (%)	**Recipient**	6 (50.00)	9 (90.00)	0.04
	Lobectomy	6 (50.00)	1 (10.00)	
Tumor encapsulation (%)	**Complete**	8 (66.67)	7 (70.00)	0.87
	Perforated	4 (33.33)	3 (30.00)	
Vascular invasion (%)	**Yes**	3 (25.00)	2 (00.00)	0.78
	No	9 (75.00)	8 (80.00)	
Patient outcome (%)	**Alive**	10 (83.33)	5 (50.00)	0.09
	Expired	2 (16.67)	5 (50.00)	

T-test (*), Chi-square test (**), and Fisher's exact test (***) were used to compare the two groups; HCC: Hepatocellular carcinoma; PANDAR: Promoter of the cyclin dependent kinase inhibitor 1A (CDKN1A) antisense DNA damage-activated RNA

Table 2. Univariate and multivariate analyses for variables associated with the overall survival of HCC patients

Variables	Univariate analysis		Multivariate analysis	
	HR (95 % CI)	***P*-value**	**HR (95 % CI)**	***P*-value**
PANDAR	1.74 (1.10-2.99)	0.04	1.74 (1.01-3.01)	0.05
Age	1.02 (0.96-1.08)	0.62	-	-
Gender	0.04 (0.01-771.52)	0.52	-	-
Hepatitis B virus	1.27 (0.28-5.75)	0.76	-	-
Hepatitis C virus	0.66 (0.07-5.72)	0.71	-	-
Alpha-fetoprotein (ng/ml)	0.99 (0.97-1.01)	0.28	-	-
Cirrhosis	0.42 (0.05-3.71)	0.44	-	-
Tumor nodule	2.69 (0.59-12.20)	0.20	-	-
TNM stage	1.37 (0.71-2.64)	0.35	-	-
Tumor differentiation	0.79 (0.26-2.39)	0.68	-	-
Tumor encapsulation	1.57 (0.28-8.69)	0.60	-	-
Vascular invasion	0.58 (0.12-3.20)	0.54	-	-

HCC: Hepatocellular carcinoma; HR: Hazard ratio; CI: Confidence interval; PANDAR: promoter of the cyclin dependent kinase inhibitor 1A (CDKN1A) antisense DNA damage-activated RNA

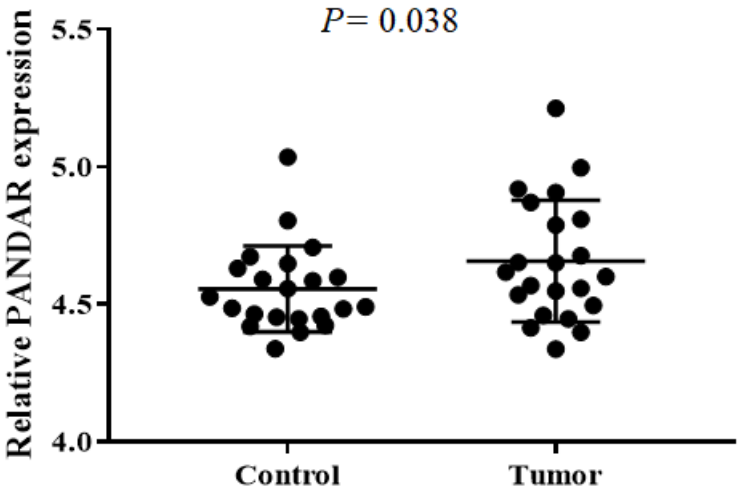

Figure 1. This figure shows the PANDAR expression levels in 22 HCC tissues and their corresponding non-tumor tissues.

HCC: Hepatocellular carcinoma; PANDAR: promoter of the cyclin dependent kinase inhibitor 1A (CDKN1A) antisense DNA damage-activated RNA

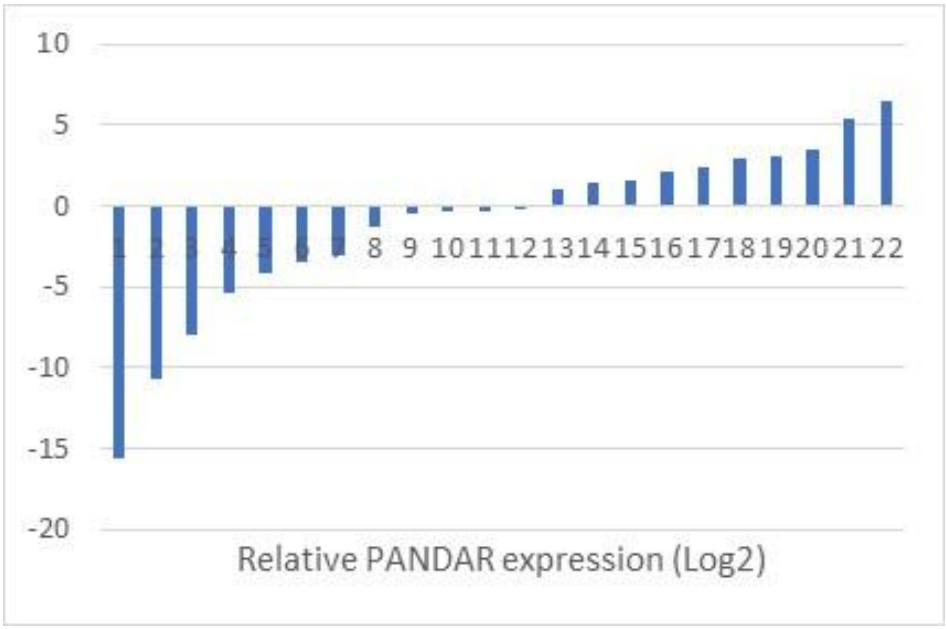

Figure 2. The Log2 fold changes of PANDAR expression based on tumors non-malignant samples in each of 22 HCC patients.

HCC: Hepatocellular carcinoma; PANDAR: promoter of the cyclin dependent kinase inhibitor 1A (CDKN1A) antisense DNA damage-activated RNA

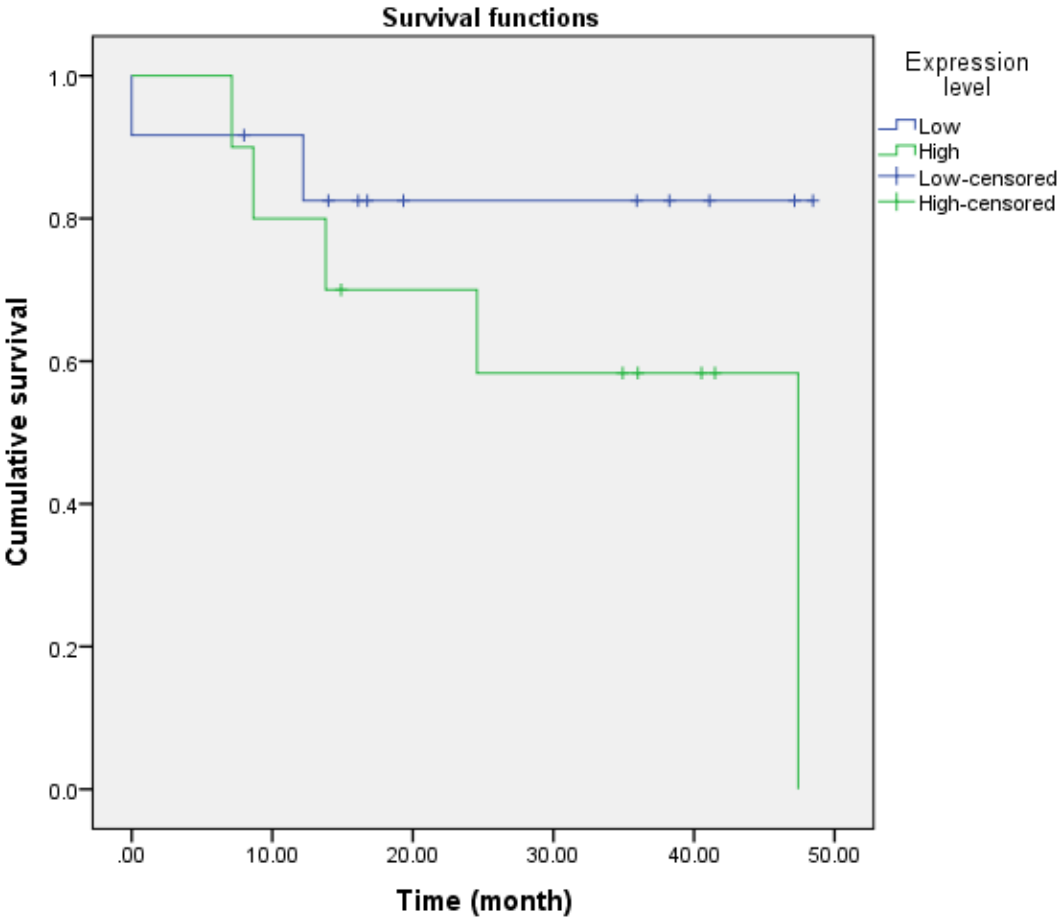

Figure 3. Kaplan-Meier analysis curve showing the correlation between PANDAR expression levels and the patients' overall survival.

PANDAR: promoter of the cyclin dependent kinase inhibitor 1A (CDKN1A) antisense DNA damage-activated RNA