

# Proliferating cell nuclear antigen (PCNA) overexpression in hepatocellular carcinoma predicts poor prognosis as determined by bioinformatic analysis

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*To the Editor:* Nowadays, many methods have been taken in early diagnosis and clinical therapy, but the overall survival (OS) of hepatocellular carcinoma (HCC) patients remains unsatisfactory.<sup>[1]</sup> Therefore, it is seriously significant to identify novel prognostic biomarkers and better to find the promising therapeutic targets.

Proliferating cell nuclear antigen (PCNA) is a protein with molecular weight of 36,000 that works as a DNA sliding clamp and functions well in regulating cell proliferation.<sup>[2]</sup> It was reported that the high expression level of PCNA or over-expression of p53 have a negative effect on tumor recurrence, tumor growth, and survival. Therefore, it is meaningful to do the related research about PCNA and find out whether it may potentially be utilized as a prognosis biomarker.

In order to analyze the expression of PCNA, available RNA-sequencing (RNA-seq) data from 465 cases (work flow type: HTSeq-FPKM), comprised of 407 HCC samples and 58 adjacent nontumorous samples (including 58 paired HCC samples), as well as corresponding clinical information from 379 HCC patients, were downloaded from The Cancer Genome Atlas (TCGA, <https://gdc-portal.nci.nih.gov/>) database on November 1, 2019. After removing missing values such as follow-up time, survival status, gender, and age, a total of 369 HCC patients with clinical characteristics were retained and further analyzed [Supplementary Table 1, <http://links.lww.com/CM9/A371>]. Four liver cancer-related datasets (GSE54236, GSE76427, GSE14520 and GSE64041) were downloaded from the Gene Expression Omnibus (GEO, <http://www.ncbi.nlm.nih.gov/geo/>) database [Supplementary Table 2, <http://links.lww.com/CM9/A371>].

We combined the four microarrays, including 272 HCC patients and 208 controls to validate differential expression of PCNA genes. GSE14520 (19 paired samples) and GSE64041 (60 paired samples) were also used to validate PCNA genes with differential expression in 58 paired samples in the TCGA database. We also tested and compared the expression of PCNA in an HCC cell line Hep3B (ATCC, Manassas, VA, USA) and a normal hepatocyte HL-7702 (acquired from Shanghai Xin Yu Biotech Co., Ltd, China). Additionally, gene set enrichment analysis (GSEA) was performed to gain further insight into the biological pathways in HCC related PCNA regulatory networks.

The results of PCNA expression are shown in Figure 1. The expression of PCNA in 407 HCC patients was higher than in the 58 normal patients ( $P < 0.001$ ) [Figure 1A], based on the TCGA database. From the four microarrays (GSE54236; GSE76427; GSE14520; GSE64041) combined from the GEO database, including 272 HCC and 208 normal patients, the expression of PCNA was also higher in the tumor group than in the normal group ( $P < 0.001$ ) [Figure 1B]. There were also 58 paired samples from TCGA [Figure 1C], 19 paired samples from GSE14520 [Figure 1D], and 60 paired samples from GSE64041 [Figure 1E], whereby the levels of PCNA expression were all higher in the tumor groups compared with the normal groups (all  $P < 0.001$ ). The verification cell lines result of PCNA expression showed that the expression level in Hep3B cell was about 1.12 fold (1.10–1.15) higher than that in HL-7702 cell ( $P < 0.001$ ) [Supplementary Figure 1, <http://links.lww.com/CM9/A371>], which was accordance with the results of TCGA and GEO database. The expression of PCNA correlated

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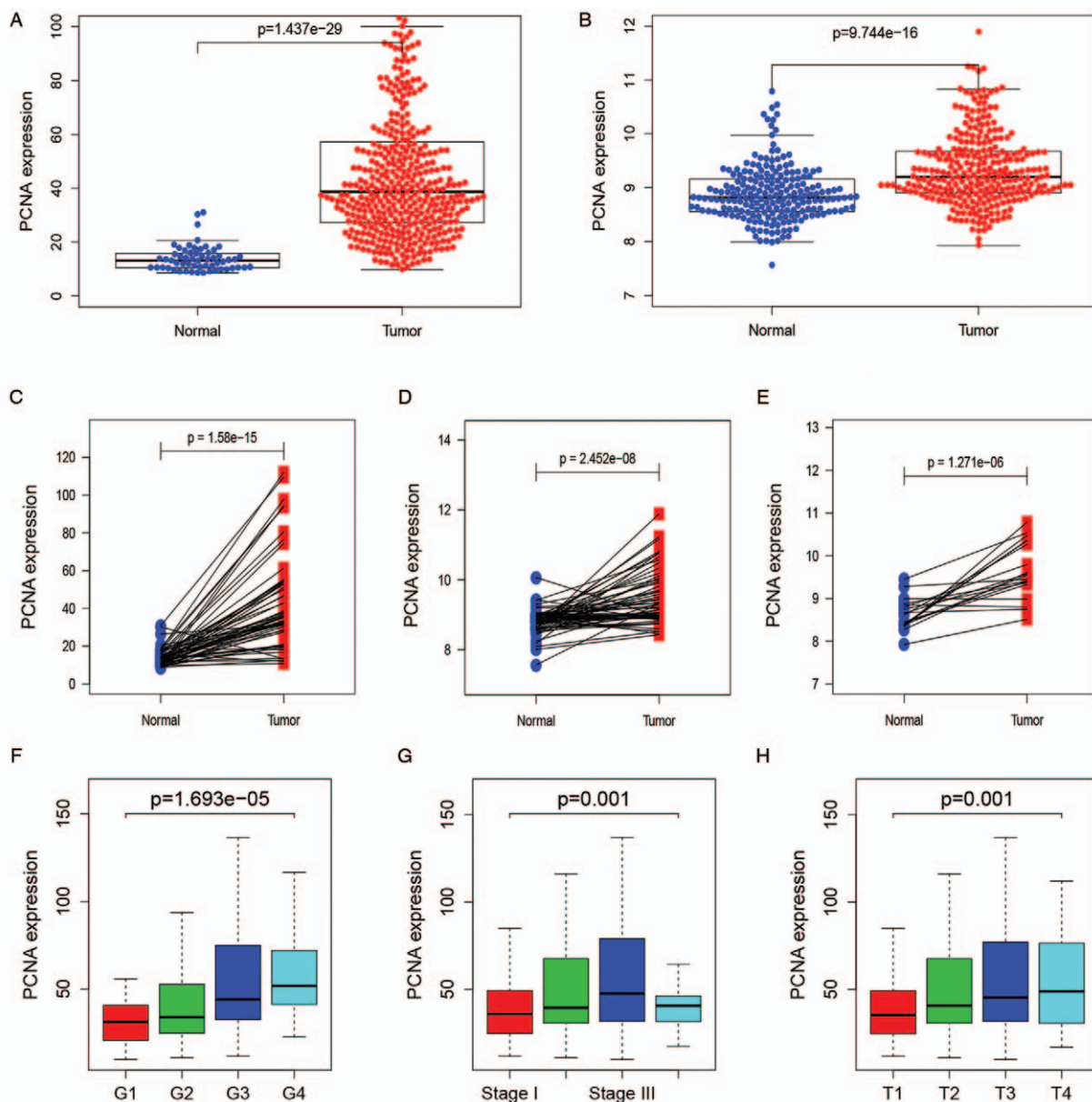
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**Figure 1:** The expression of PCNA in (A) 58 normal and 407 tumor groups from TCGA, (B) 208 normal and 272 tumor groups from GEO, (C) 58 paired groups from TCGA, (D) 60 paired groups from GEO (GSE64041), and (E) 19 paired groups from GEO (GSE14520). (F) The expression of PCNA correlated significantly with the clinicopathological features. (G) The association of PCNA expression with clinical grade. (H) The association of PCNA expression with tumor size. PCNA: Proliferating cell nuclear antigen; TCGA: The Cancer Genome Atlas.

significantly with the histologic grade, clinical stage, and tumor size (all  $P < 0.001$ ) [Figure 1F–H]. With the increase of histologic stage and tumor size, the level of PCNA expression also increased.

Kaplan-Meier survival analysis showed that HCC patients with higher expression of PCNA had a worse prognosis than patients with lower expression of PCNA ( $P = 0.01$ ) [Supplementary Figure 2A, <http://links.lww.com/CM9/A371>]. As shown in Supplementary Table 3, <http://links.lww.com/CM9/A371>, the univariate cox regression analysis revealed that clinical stage (hazard ratio [HR]: 1.86; 95% Confidence interval [CI]: 1.46–2.39), tumor size (HR: 1.80; 95% CI: 1.43–2.27), and PCNA expression (HR: 1.013; 95% CI: 1.008–1.019) were significantly

associated with poor prognosis of HCC (all  $P < 0.001$ ). From the multivariate Cox regression analysis result, it is easy to find that the expression of PCNA can be recognized as an independent factor for predicting the prognosis of HCC with a HR of 1.654 (95% CI: 1.234–2.218,  $P < 0.001$ ) [Supplementary Table 3, <http://links.lww.com/CM9/A371>, Supplementary Figure 2B, <http://links.lww.com/CM9/A371>].

GSEA revealed significant differences with Nominal  $P$  value  $< 0.05$  and false discovery rate (FDR)  $Q$ -value  $< 0.05$  [Supplementary Table 4, <http://links.lww.com/CM9/A371>]. The most significantly enriched signaling pathways were selected based on their normalized enrichment score (NES). Supplementary Figure 3, <http://links.lww.com/CM9/A371>.

A371 showed that “Cell cycle,” “DNA replication,” “P53 signaling pathway,” “Thyroid cancer,” “Bladder cancer,” and “Pancreatic cancer” were differentially enriched in the PCNA high expression phenotype. Furthermore, “Complement and coagulation cascades,” “Primary bile acid biosynthesis,” “Fatty acid metabolism,” “Valine leucine and isoleucine degradation,” “Retinal metabolism,” and “PPAR signaling pathway” were differentially enriched in the low PCNA expression phenotype.

In our results, PCNA expression in the tumor group was higher. PCNA expression was discovered to be an important predictor of disease-free survival and OS.<sup>[3]</sup> Kong *et al*<sup>[4]</sup> found that, compared to its expression in nontumor samples, PCNA was dysregulated in HCC samples, and its aberrant expression was significantly associated with tumor stage and the outcomes of HCC. In our study, the expression of PCNA correlated significantly with histologic grade, clinical stage, and tumor size (all  $P < 0.001$ ). The univariate Cox analysis in our study showed that TNM stage influenced the prognosis of HCC patients. After the multivariate Cox analysis, our results showed that PCNA can be an independent factor for predicting the prognosis of HCC patients.

GSEA showed that “Cell cycle,” “DNA replication,” and “P53 signaling pathway,” and many tumor-related pathways including “Thyroid cancer,” “Bladder cancer,” and “Pancreatic cancer” were differentially enriched in the high PCNA expression phenotype. Cell cycle dysregulation has been demonstrated to be a common feature in the development and progression of many tumors, including HCC. The expression of PCNA or over-expression of p53 resulted in high risk of tumor recurrence, more aggressive growth, and poor survival. In addition, dysregulation of fatty acid metabolism is an emerging hallmark of cancer cells, which has been proved to be involved in HCC development and progression.<sup>[5]</sup>

A good prognostic indicator for HCC is highly desirable. The expression of PCNA in the tumor group was higher than that in the control group. GSEA results showed that

PCNA might play a role in the development of HCC through “Cell cycle” and “P53 signaling pathway” and the dysregulation of “Fatty acid metabolism” and “PPAR signaling pathway”. Clearly, we should focus more on the cell cycle pathway and related genes in order to explore the mechanism of HCC progression and HCC treatment. PCNA could be used as an independent prognostic indicator for HCC. Therefore, it is beneficial to monitor the prognosis of HCC patients using PCNA.

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### Conflicts of interest

None.

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