RESEARCH ARTICLE

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Plasma circular RNA hsa_circ_0001821 acts as a novel diagnostic biomarker for malignant tumors

Yulan Song[1] | Peng Cao[2] | Jipeng Li[3]

[1]Blood Purification Center, The Affiliated People's Hospital, Ningbo University, Ningbo, China

[2]Department of Laboratory Medicine, The Affiliated Hospital of Medical School, Ningbo University, Ningbo, China

[3]Department of Central Laboratory, The Affiliated People's Hospital, Ningbo University, Ningbo, China

Correspondence
Jipeng Li, Department of Central Laboratory, The Affiliated People's Hospital, Ningbo University, 251 East Baizhang Road, Ningbo 315040, China.
Email: lijipeng1109@163.com

Abstract

Background: Circular RNAs (circRNAs) can function as key regulators of oncogenic processes. The main purpose of this study was to evaluate the expression of hsa_circ_0001821 in plasma of patients with colorectal cancer (CRC) and other malignant tumors and analyze its correlations with clinical features and diagnostic values.

Methods: In total, 467 plasma samples, including samples from 80 healthy controls, were collected between 2015 and 2019 from patients at the Affiliated People's Hospital of Ningbo University. Plasma levels of hsa_circ_0001821 were analyzed by qRT-PCR. The diagnostic value was performed using receiver operating characteristic (ROC) curve.

Results: Plasma hsa_circ_0001821 was increased in CRC patients, and high hsa_circ_0001821 expression predicted advanced stage and unfavorable in overall survival. In addition, this study showed the upregulation of hsa_circ_0001821 in plasma of lung cancer and hepatocellular carcinoma (HCC). ROC curve showed that the region under the loop for the diagnosis of CRC, HCC, and lung cancer was 0.815, 0.692, and 0.792.

Conclusion: Plasma hsa_circ_0001821 possibly is a novel biological marker for malignant tumors.

KEYWORDS
biomarker, circular RNAs, malignant cancer, plasma

1 | INTRODUCTION

In recent years, the incidence and mortality of malignant tumors have been increasing. In China, the ratio of new tumor cases is more than 4.2 million, whereas every year 2.8 million deaths occur,[1] which brings great pain and burden to patients, families, and society. Deaths occurring due to malignant tumors are increasing, and it affects most people who are in the middle and late stages after they are discovered with malignant tumors.[2] One of the main reasons includes missing the appropriate treatment time. To reduce the incidence of cancer, diagnosis at early stages, and new treatment strategies are required. With the development of high-throughput omics (such as genomics, proteomics, and metabolomics) and the rise of various new detection methods, people have discovered some new tumor markers,[3–5] especially in the field of noncoding RNA. For example, plasma miR-221 can be used as an early diagnosis marker for hepatocellular carcinoma,[6] and circulating lncRNA SNHG11 is a biomarker for the prognosis and diagnosis of colorectal cancer at an early stage.[7] However, linear RNA has poor stability in vitro and is easily degraded, which limits its clinical application.

The structure of circular RNA (circRNA) is characterized by a covalently single-stranded loop structure except 3′ and 5′ terminals, which is more stable than linear RNA.[8–10] Researches that were conducted in the past few years suggested that in malignant tumors, a variety of circRNAs are often expressed abnormally, which play a significant role in the development and occurrence of malignant tumors. For instance, Zhu et al. determined that elevated serum hsa_circ_0081001 levels were associated with poor prognosis of osteosarcoma patients.[11] Lin et al. reported that a panel of three circRNAs (circ-STIL, circ-CCDC66, and circ-ABCC1) could serve as an independent diagnostic biomarker for colorectal cancer (CRC).[12]

Hsa_circ_0001821 has been reported as highly expressed in colorectal carcinoma and gastric and lung cancers and promotes the malignant progression of tumors.[13-15] However, whether hsa_circ_0001821 can be secreted into the circulatory system, the expression of circulating hsa_circ_0001821 and its clinical significance remain unclear. This study aims to determine the feasibility of using hsa_circ_0001821 as a noninvasive diagnostic marker for tumors.

2 | MATERIALS AND METHODS

2.1 | Samples collection

There were 467 plasma samples in total, in which healthy controls were 80, and 20 paired CRC and matched normal adjacent tissue samples were collected between 2015 and 2019 from patients at the Affiliated People's Hospital of Ningbo University. The total number of patients suffering from malignant cancer participated in this study was 387, in which 51 had gastric cancer, 122 had CRC, 95 had lung cancer, 27 had ovarian cancer, and 92 had HCC. Samples were stored at −80℃ prior to use. The patients who participated in the current study had not undergone any cancer therapy, surgery, or radiology. The current research was conducted after the permission of the Ethical Committee of the Affiliated People's Hospital of Ningbo University. From both the patients and healthy controls, informed consent was taken.

2.2 | CRC cell lines

Colorectal cancer cancer cell lines HCT116, HT29, SW480, and SW620 and human normal colonic epithelial cells NCM460 were cultivated in DMEM (Dulbecco's modified Eagle medium) supplemented with 10% FBS (fetal bovine serum) (Gibco, NY, USA). The cells were incubated in an atmosphere that contained 5% CO2 and a temperature of 37°C.

2.3 | RNA preparation

The total RNA was taken out from colorectal cancer (CRC) cells and tissues by using TRIzol reagent (Invitrogen, CA, USA), whereas total RNA was taken out from plasma by using the TRIzol™ LS Reagent (Invitrogen) as per the instructions of the manufacturer. During the isopropanol precipitation, glycogen (ThermoFisher, final concentration: 100 µg/ml) was added to increase the RNA precipitation. ImProm-II Reverse Transcription System (Promega, WI, USA) was used as per the instructions of the manufacturer to reversely transcribe total RNA.

2.4 | qRT-PCR

For the CRC cells and tissues, GAPDH was used as the endogenous control, and $2^{-\Delta\Delta Cq}$ formula was employed to figure out the relative expression of hsa_circ_0001821. In the current study, absolute quantification was used as there is no recognized endogenous control for the quantification of circRNA in plasma.[16] Briefly, the hsa_circ_0001821 PCR product was cloned into the pUC57 vector and diluted from 1×10^5 copies/ml to 1×10^2 copies/ml. These diluted vectors were subjected to qPCR under the same conditions as the samples, and for the quantification of the expression of hsa_circ_0001821, a standard curve was drawn with vectors of known concentration (Figure S1). The following primers were used in this study: hsa_circ_0001821: 5′- GGTTCCACCAGCGTTATTC-3′ (forward) and 5′-CAACTTCCTTTGGGTCTCC-3′ (reverse); GAPDH: 5′-CAACGGGGAAGCTCACTGG-3′ (forward) and 5′-GCCTGCTTCACCACCTTCT-3′ (reverse).

2.5 | Statistical analysis

Statistical analysis was done by using GraphPad Prism 7.0 (GraphPad, Inc., CA, USA). Statistical procedures such as Chi-square test and Student's t-test were used for the comparison of data, as appropriate. Pearson's correlation analyses were employed to assess relationships between variables. MedCalc 11.0 (MedCalc, Ostend, Belgium) was used for ROC curve analyses. $p < 0.05$ was the significance threshold in these analyses.

3 | RESULTS

3.1 | Highly expressed Hsa_circ_0001821 in plasma and tissues of CRC patients

The current study tested hsa_circ_0001821 levels in CRC tissues, plasma samples (screening cohort, $n = 20$), and CRC cell lines. The data revealed that in the CRC tissues hsa_circ_0001821 was significantly upregulated as compared to normal tissues that are adjacent to it (Figure 1A). Consistently, the hsa_circ_0001821 expression in all CRC cell lines was significantly upregulated as compared with normal colon epithelial cell line NCM460 (Figure 1B). Furthermore, in CRC patients, the hsa_circ_0001821 level in plasma samples was excessive than that in healthy controls (Figure 1C). The current study revealed a positive correlation between the level of

FIGURE 1 The expression of hsa_circ_0001821 was high in CRC. (A) qRT-PCR identified expression of Hsa_circ_0001821 in 20 paired CRC samples. As a control, GAPDH was adapted. (B) Hsa_circ_0001821 expression in CRC cell lines and human normal colorectal cell line NCM460. (C) qRT-PCR showed the absolute RNA levels of hsa_circ_0001821 ($n = 20$). (D) The correlation between the relative RNA level in CRC tissues and the absolute RNA level in plasma ($n = 20$)
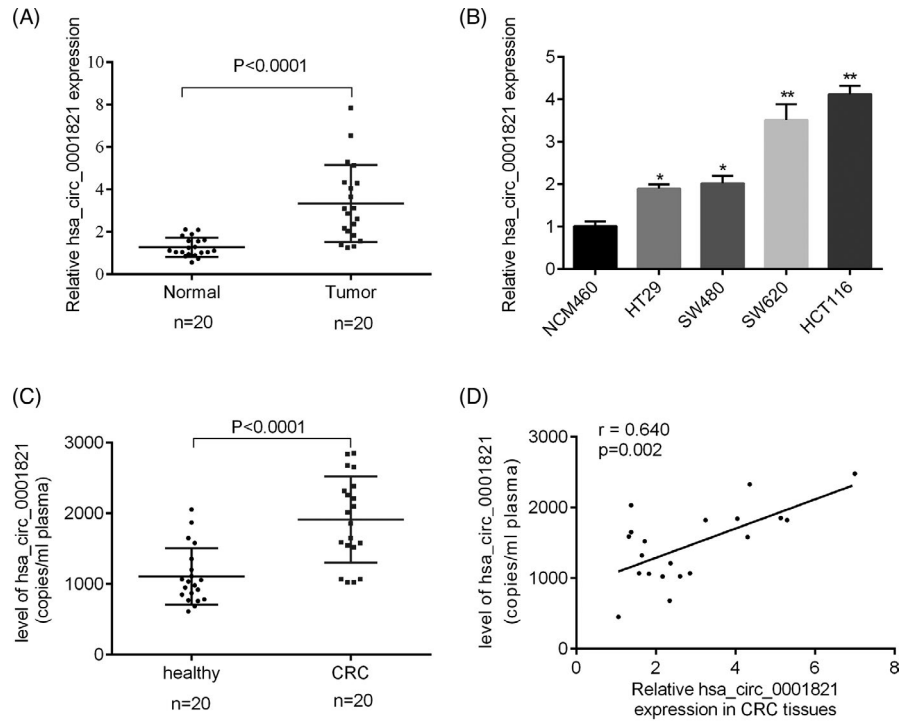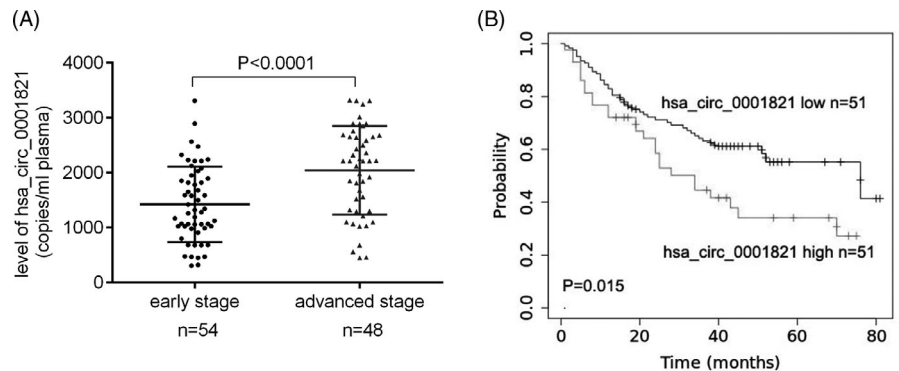

FIGURE 2 Plasma hsa_circ_0001821 expression correlation with prognosis of patients. (A) qRT-PCR detected a comparison of hsa_circ_0001821 expression between the final stage (III + IV) and initial stage (I+II) patients. (B) By using logrank test, Kaplan-Meier survival analysis of CRC sufferers according to the hsa_circ_0001821 expression level ($n = 102$)

hsa_circ_0001821 expression in CRC tissues with levels in plasma of the patient (Figure 1D), suggesting that these hsa_circ_0001821 may be secreted from tumors into circulation.

3.2 | Clinical implications of plasma hsa_circ_0001821 in CRC

The research examined plasma hsa_circ_0001821 expression in another verification cohort in samples of 102 CRC patients, and it was found that in plasma samples of advanced stage CRC, hsa_circ_0001821 was significantly and consistently increased (Figure 2A). Besides, Kaplan–Meier analysis revealed a higher expression of hsa_circ_0001821 in the plasma of CRC samples and was closely correlated with the poorer prognosis of CRC patients (Figure 2B), and by using Cox regression analysis, hsa_circ_0001821 was proved to be an independent predictor (Table 1). Furthermore, the correlation of the plasma hsa_circ_0001821 expression was found to be positively correlated by correlation analysis with tumor size ($p = 0.001$), distant metastasis ($p = 0.016$), and lymph node

metastasis ($p = 0.012$). However, the research did not identify any association between its levels with other clinic pathological features such as age, gender, and tumor location (Table 2).

3.3 | Hsa_circ_0001821 had high expression in plasma of patients with lung cancer or HCC

For the verification of hsa_circ_0001821 expression in these patients, additional plasma specimens were collected from patients having hepatocellular carcinoma (HCC), gastric, lung, or ovarian cancer. It was shown by the results of the study that significantly higher hsa_circ_0001821 was found in the plasma of patients with hepatocellular carcinoma as compared to healthy controls (Figure 3A). Importantly, increased hsa_circ_0001821 was significantly correlated with larger tumor size and advanced clinical stage in HCC patients (Table S1). Multivariate Cox regression analysis demonstrated that only tumor size was independent prognostic factors for HCC patients (Table S2). It was also found that the expression of hsa_circ_0001821 was distinctive when comparing plasma samples from

Variable	Subset	Hazard ratio for OS (95% CI)	p value
Univariate analysis (n = 102)			
Age (years)	<60 vs ≥60	–	0.403
Gender	Male vs Female	–	0.562
pT status	T1+2 vs T3+4	–	0.205
pN status	N0 vs N1	0.126 (0.051–0.223)	<0.001
pM status	M0 vs M1	0.056 (0.028–0.168)	<0.001
Clinical stage	I + II vs III + IV	0.052 (0.017–0.171)	<0.001
hsa_circ_0001821 expression	Low vs high	0.462 (0.183–1.124)	0.037
Multivariate analysis (n = 102)			
pN status	N0 vs N1	26.382 (8.068–85.360)	<0.001
pM status	M0 vs M1	9.580 (2.651–28.048)	<0.001
Clinical stage	I + II vs III + IV	–	0.436
hsa_circ_0001821 expression	Low vs high	0.351 (0.098–0.971)	0.034

healthy controls to those from patients with lung cancer (Figure 3B). Patients with advanced T stage and clinical stage exhibited a higher expression of hsa_circ_0001821 (Table S3), and by using Cox regression analysis, advanced T stage was proved to be an independent predictor (Table S4). However, no significant difference was found in the expression of hsa_circ_0001821 between the plasma of ovarian cancer or gastric patients and healthy controls (Figure 3C,D).

3.4 | Data of ROC (receiver operating characteristic) curve for the clinical diagnostic value of hsa_circ_0001821

To evaluate the clinical value of novel biomarkers in the diagnosis of cancer at an early stage, ROC curves can be adapted. ROC curves were used for the evaluation of the clinical diagnostic value of hsa_circ_0001821, traditional biomarkers, or a combination of these biomarkers (Panel) in the plasma of patients with HCC, CRC, or lung cancer. The AUCs of hsa_circ_0001821 in patients with CRC (Figure 4A), HCC (Figure 4B), or lung cancer (Figure 4C) were 0.815 (95% CI 0.751–0.869), 0.692 (95% CI 0.617–0.760), and 0.792 (95% CI 0.724–0.850), respectively, indicating that in these cancers, hsa_circ_0001821 may act as an effective diagnostic biomarker. Moreover, we also found that the Panel was more accurate than hsa_circ_0001821 alone as a means of differentiating between malignant tumors and healthy samples (Table S5).

4 | DISCUSSION

In clinical diagnosis, plasma is the most widely used sample, so researchers are interested to identify effective biomarkers in blood.

Many research works that were conducted in the past decades reported that circulating miRNA/lncRNA in plasma/serum may become a reliable biomarker for cancer.[17–20] Due to the unique structure of circRNA, it has high stability in peripheral blood. In recent years, the expression characteristics and clinical significance of circulating circRNA in malignant tumors have become a research hotspot in tumor diagnosis.[21–23] Luo et al.[24] reported that highly expressed circulating circAKT3 is correlated with tumor size, recurrence, and prognosis of HCC patients. Fan et al.[25] verified that serum circMAN1A2 is highly expressed in tumors such as thyroid cancer, ovarian cancer, and oral cancer, and its diagnostic value was found to be good. Here, our results identified that high expression of hsa_circ_0001821 in colorectal cancer plasma and tissues and the expression level is positively correlated, suggesting that hsa_circ_0001821 in plasma may be derived from tumor cells. More importantly, it was shown by Kaplan–Meier analysis that high expression of plasma hsa_circ_0001821 in patients had a poor prognosis, and further COX regression suggested that in the prognosis of colorectal cancer, high hsa_circ_0001821 expression is an independent risk factor. Hsa_circ_0001821 is derived from the noncoding region of the oncogene PVT1, with a length of 410 bp,[26] and has been identified to be upregulated in many types of cancers, including gastric cancer,[13] CRC,[14] lung cancer,[15] HCC,[27] and ovarian cancer.[28] These studies have revealed the oncogenic roles of hsa_circ_0001821 in the progression of cancer. Therefore, this study revealed plasma hsa_circ_0001821 expression in some solid tumors and found that hsa_circ_0001821 also increased to varying degrees in HCC and lung cancer. ROC curves showed that plasma hsa_circ_001821 has a high diagnostic value for CRC, HCC, and lung cancer, suggesting that for the early diagnosis of the tumor, plasma hsa_circ_001821 may also be adapted widely as a tumor biological marker.

TABLE 2 Correlation analysis between has_circ_0001821 expression and clinicopathological parameters of CRC

| Variable | Number of cases | hsa_circ_0001821 expression | | p value |
		High expression (N = 51)	Low expression (N = 51)	
Age				
≥60	42	20	22	0.687
<60	60	31	29	
Gender				
Female	40	18	22	0.417
Male	62	33	29	
Tumor location				
Colon	50	26	24	0.692
Rectum	52	25	27	
pT status				
T1–T2	15	7	8	0.780
T3–T4	87	44	43	
pN status				
N0	68	28	40	0.012
N1–N2	34	23	11	
pM status				
M0	80	35	45	0.016
M1	22	16	6	
Clinical stage				
I + II	54	18	36	<0.0001
III + IV	48	33	15	
Tumor size (cm)				
<5	68	26	42	0.001
≥5	34	25	9	
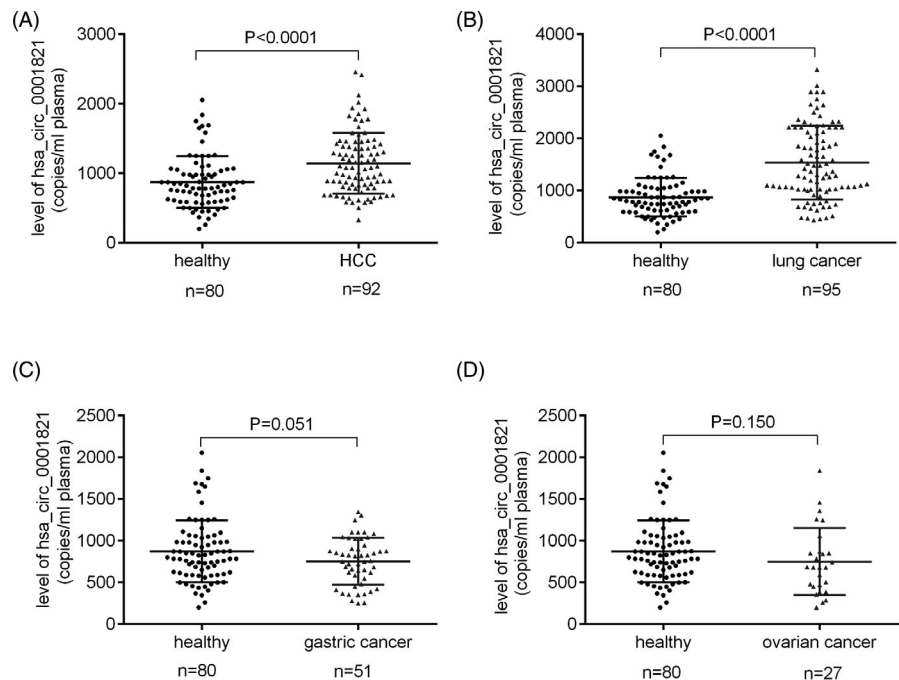
FIGURE 3 Hsa_circ_0001821 high expression in plasma of gastric cancer, lung cancer, or HCC patients. qRTPCR showed the absolute levels of RNA of hsa_circ_0001821 in 92 HCC plasma (A), 95 lung cancer (B), 51 gastric cancer (C), 27 ovarian cancer (D) and 80 healthy plasma
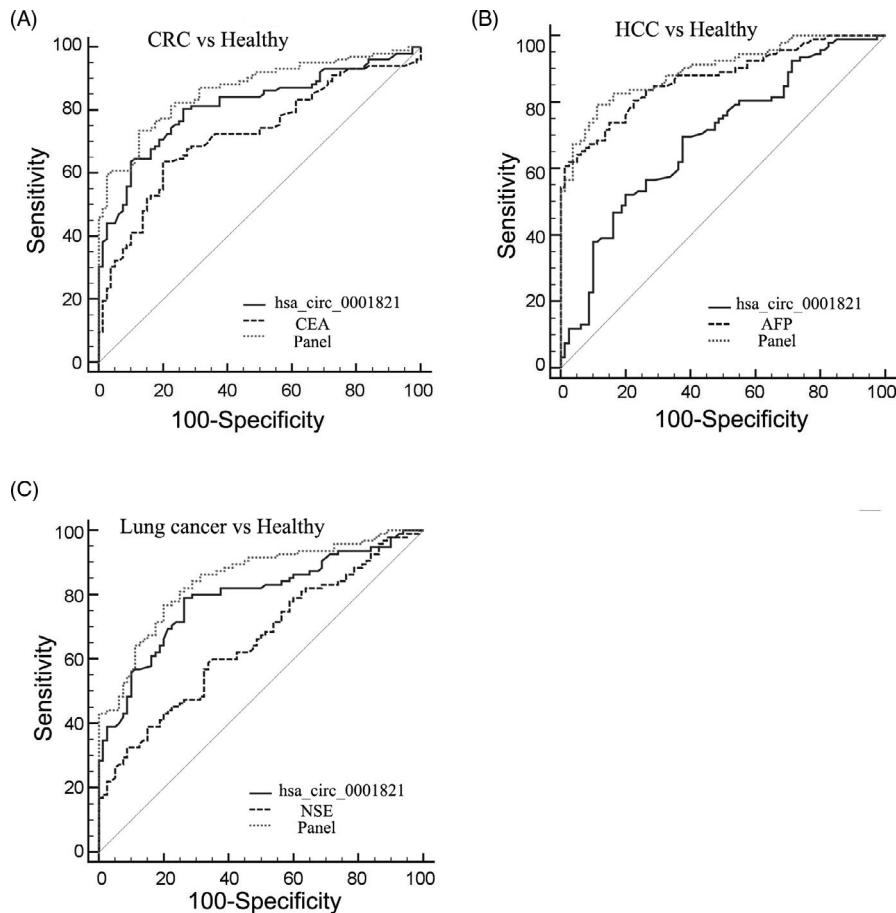
FIGURE 4 Assessment of the diagnostic utility of hsa_circ_0001821, traditional biomarkers, and the combination thereof. (A) ROC curve corresponding to hsa_circ_0001821, CEA and a combination of these two biomarkers (Panel). (B) ROC curve corresponding to hsa_circ_0001821, AFP and a combination of these two biomarkers (Panel). (C) ROC curve corresponding to hsa_circ_0001821, NSE and a combination of these two biomarkers (Panel)

5 | CONCLUSION

In summary, the study revealed that the expression of plasma hsa_circ_0001821 is elevated in HCC, CRC, and lung cancer and has a high diagnostic value, suggesting that plasma hsa_circ_0001821 may be a possible pan-cancer diagnostic marker.

CONFLICT OF INTEREST
None.

AUTHOR CONTRIBUTIONS
LJ and SY designed and performed the study. LJ and CP were involved in gaining ethical approval, sample collection, and data analysis. SY wrote the first draft of the study. All authors reviewed and approved the final version of the study.

DATA AVAILABILITY STATEMENT
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ORCID
Jipeng Li https://orcid.org/0000-0001-5091-8432

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin*. 2020;70:7-30.
2. Li A, Lewison G. Chinese cancer research in 2009–18 and the disease Burden. *Cancer Manag Res*. 2020;12:5031-5040.
3. Takayasu T, Shah M, Dono A, et al. Cerebrospinal fluid ctDNA and metabolites are informative biomarkers for the evaluation of CNS germ cell tumors. *Sci Rep*. 2020;10:14326.
4. Guo LY, Wu AH, Wang YX, Zhang LP, Chai H, Liang XF. Deep learning-based ovarian cancer subtypes identification using multi-omics data. *BioData Min*. 2020;13:10.
5. Rahat B, Ali T, Sapehia D, Mahajan A, Kaur J. Circulating cell-free nucleic acids as epigenetic biomarkers in precision medicine. *Front Genet*. 2020;11:844.
6. Li J, Wang Y, Yu W, Chen J, Luo J. Expression of serum miR-221 in human hepatocellular carcinoma and its prognostic significance. *Biochem Biophys Res Commun*. 2011;406:70-73.
7. Xu W, Zhou G, Wang H, et al. Circulating lncRNA SNHG11 as a novel biomarker for early diagnosis and prognosis of colorectal cancer. *Int J Cancer*. 2020;146:2901-2912.
8. Jeck WR, Sorrentino JA, Wang K, et al. Circular RNAs are abundant, conserved, and associated with ALU repeats. *RNA*. 2013;19:141-157.
9. Barrett SP, Salzman J. Circular RNAs: analysis, expression and potential functions. *Development*. 2016;143:1838-1847.
10. Memczak S, Papavasileiou P, Peters O, Rajewsky N. Identification and characterization of circular RNAs as a new class of putative biomarkers in human blood. *PLoS ONE*. 2015;10:e0141214.

11. Kun-Peng Z, Chun-Lin Z, Jian-Ping H, Lei Z. A novel circulating hsa_circ_0081001 act as a potential biomarker for diagnosis and prognosis of osteosarcoma. *Int J Biol Sci*. 2018;14:1513-1520.

12. Lin J, Cai D, Li W, et al. Plasma circular RNA panel acts as a novel diagnostic biomarker for colorectal cancer. *Clin Biochem*. 2019;74:60-68.

13. Chen J, Li Y, Zheng Q, et al. Circular RNA profile identifies circPVT1 as a proliferative factor and prognostic marker in gastric cancer. *Cancer Lett*. 2017;388:208-219.

14. Wang Z, Su M, Xiang B, Zhao K, Qin B. Circular RNA PVT1 promotes metastasis via miR-145 sponging in CRC. *Biochem Biophys Res Commun*. 2019;512:716-722.

15. Wang C, Tan S, Liu WR, et al. RNA-Seq profiling of circular RNA in human lung adenocarcinoma and squamous cell carcinoma. *Mol Cancer*. 2019;18:134.

16. Yu J, Ding WB, Wang MC, et al. Plasma circular RNA panel to diagnose hepatitis B virus-related hepatocellular carcinoma: a large-scale, multicenter study. *Int J Cancer*. 2020;146:1754-1763.

17. Dufresne S, Rébillard A, Muti P, Friedenreich CM, Brenner DR. A Review of physical activity and circulating miRNA expression: implications in cancer risk and progression. *Cancer Epidemiol Biomarkers Prev*. 2018;27:11-24.

18. Nakamura K, Sawada K, Yoshimura A, Kinose Y, Nakatsuka E, Kimura T. Clinical relevance of circulating cell-free microRNAs in ovarian cancer. *Mol Cancer*. 2016;15:48.

19. Zhang K, Shi H, Xi H, et al. Genome-wide lncRNA microarray profiling identifies novel circulating lncRNAs for detection of gastric cancer. *Theranostics*. 2017;7:213-227.

20. Qi P, Zhou XY, Du X. Circulating long non-coding RNAs in cancer: current status and future perspectives. *Mol Cancer*. 2016;15:39.

21. Tang W, Fu K, Sun H, Rong D, Wang H, Cao H. CircRNA microarray profiling identifies a novel circulating biomarker for detection of gastric cancer. *Mol Cancer*. 2018;17:137.

22. Fang S, Pan J, Zhou C, et al. Circular RNAs serve as novel biomarkers and therapeutic targets in cancers. *Curr Gene Ther*. 2019;19:125-133.

23. Liu XX, Yang YE, Liu X, et al. A two-circular RNA signature as a noninvasive diagnostic biomarker for lung adenocarcinoma. *J Transl Med*. 2019;17:50.

24. Luo Y, Liu F, Gui R. High expression of circulating exosomal circAKT3 is associated with higher recurrence in HCC patients undergoing surgical treatment. *Surg Oncol*. 2020;33:276-281.

25. Fan CM, Wang JP, Tang YY, et al. circMAN1A2 could serve as a novel serum biomarker for malignant tumors. *Cancer Sci*. 2019;110:2180-2188.

26. Panda AC, Grammatikakis I, Kim KM, et al. Identification of senescence-associated circular RNAs (SAC-RNAs) reveals senescence suppressor CircPVT1. *Nucleic Acids Res*. 2017;45:4021-4035.

27. Zhu Y, Liu Y, Xiao B, et al. The circular RNA PVT1/miR-203/HOXD3 pathway promotes the progression of human hepatocellular carcinoma. *Biol Open*. 2019;8:bio043687.

28. Sun XF, Luo L, Gao YQ. Circular RNA PVT1 enhances cell proliferation but inhibits apoptosis through sponging microRNA-149 in epithelial ovarian cancer. *J Obstet Gynaecol Res*. 2020;46:625-635.

SUPPORTING INFORMATION

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