Available online at www.sciencedirect.com

ScienceDirect

Chronic Diseases and Translational Medicine 6 (2020) 62−68

www.keaipublishing.com/en/journals/cdtm/

www.cdatm.org

Original Article

Screening of long non-coding RNAs markers in plasma of children with chronic gastritis

Zhen Gu*, Hua-Qin Shen, Pei-Hua Fu, Mei Chen

Department of Pediatrics, Shanghai Pudong New District Zhoupu Hospital, Shanghai 201318, China

Received 24 October 2019

Available online 10 February 2020

Abstract

Objective: The study aimed to detect and analyze long non-coding RNAs (lncRNAs) in plasma of children diagnosed with chronic gastritis, and to explore its biological functions and involved signaling pathways.

Methods: The plasma samples were collected from six children that were diagnosed with chronic gastritis by physical examination, gastroscopy, and pathological examination and six healthy children. The plasma samples were assayed for determining the expression profiles of lncRNA based upon the gen chip detection. The specific expression of lcnRNA in plasma of children with chronic gastritis was analyzed and its biological functions were speculated.

Results: Five lncRNAs (*RP11-697M17.1*, *RP11-388M20.9*, *AFAP1-AS1*, *BC062758*, and *XLOC001406*) were significantly up-regulated, and five lncRNAs (*UNQ697*, *BX571672.5*, *CYP4F35P*, *ANKRD20A5P*, and *AL832737*) were observed to be significantly down-regulated. The lncRNAs *RP11-697M17.1,* and *UNQ697* were detected with the highest up-regulation and down-regulation, respectively. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis showed that the up-regulated lncRNAs were significantly enriched in 20 signaling pathways such as phosphoinositide-3-kinase−protein kinase B (PI3K-Akt) pathway, and the down-regulated lncRNAs target genes were significantly enriched in 20 signaling pathways such as the metabolic pathway.

Conclusion: The analysis of the lncRNA expression profiles in plasma of children with chronic gastritis revealed that the lncRNA *RP11-697M17.1,* and lncRNA *UNQ697* may act as plasma markers for predicting chronic gastritis in children.

Keywords: *RP11-697M17.1*; *UNQ697*; Chronic gastritis; Plasma markers

Introduction

Chronic gastritis is an inflammation of the stomach lining that is caused by a combination of various factors, including the *Helicobacter pylori* (*H. pylori*) infection.[1−7] It can lead to various conditions, such as

* Corresponding author.
E-mail address: Guzhen_2019@126.com (Z. Gu).

Peer review under responsibility of Chinese Medical Association.

Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.cdtm.2020.01.001

hernia, nausea, pain etc. In the current scenario, the common methods for the treatment of chronic gastritis include diet therapy, symptomatic treatment, and eradication of *H. pylori*.[8−13] However, an effective approach for the treatment of chronic gastritis is the eradication of *H. pylori* that can markedly reduce its recurrence rate. The long non-coding RNAs (lncRNAs) are a group of non-coding transcripts that are greater than 200 nucleotides (nt) in length but lack the protein-coding capacity. Recently, studies have reported that lncRNAs are widely implicated in various physiological and pathological processes in the body, and also involved in regulation of the progression of various diseases.[14−17] Specific lncRNAs, such as *H19* and HOX transcript antisense RNA (*HOTAIR*), have been shown to play important roles in digestive tract diseases.[18] For example, *H19* induces the production of microRNA-675 (miR-675) by the tumor suppressor runt-related transcription factor 1, and regulates the gastric cancer cell proliferation. Moreover, the overexpression of *HOTAIR* may be involved in tumor escape mechanisms. These observations strongly suggest that lncRNAs are a molecular etiology of digestive tract diseases. Notably, the circulating lncRNAs have been detected as novel biomarkers for the digestive tract diseases, which is promising for the monitoring of development and progression of digestive tract diseases, and screening of patients.[19−21]

The plasma RNA has been identified as a novel non-invasive diagnostic biomarker.[22] It has been reported that the circulating RNA in the blood is encapsulated within the vesicles, such as exosomes enabling it to persist for a longer time. Furthermore, it is more heterogeneous than endoscopy for detection of the disease. Previously, the circulating biomarkers have been screened mainly in adults for the detection of digestive tract diseases. In the present study, we report the analysis of expression profile of lncRNAs and screening of specific lncRNA markers in the plasma of children that were diagnosed with chronic gastritis.

Methods

Ethical approval

The study was approved by the ethics committee of Shanghai Pudong New District Zhoupu Hospital. All clinical practices and observations were conducted in accordance with the *Declaration of Helsinki*. Informed consent was obtained from each patient before the study was conducted.

Patients

The study included six children (8.0−12.0 years old) from Shanghai Pudong New District Zhoupu Hospital from December 2018 to August 2019. The patients were selected for the study on the basis of clinical symptoms, physical examination, gastroscopy, and pathological examination of chronic gastritis. Additionally, six blood samples were collected as control group from children that were not diagnosed with chronic gastritis by the same clinical examination.

Plasma sample collection and preservation

The plasma samples were collected from six children that were diagnosed with chronic gastritis, and six healthy children. The samples were collected in BD EDTA (Becton Dickinson [BD] Ethylenediaminetetraacetic acid) tubes. The plasma samples were initially centrifuged at a speed of $1500\times g$ at 4 °C for 30 min, and then at $3000\times g$ at 4 °C for 10 min. The supernatant was collected for each sample and stored in TRIzol® reagent (Qiagen, Inc. Valencia, California, USA) at −80 °C.

Total RNA extraction and reverse transcription

Total RNA was extracted from the plasma samples using the miRNeasy Serum/Plasma kit (REF 217184, Germany) according to the manufacturer's instructions. Total RNA concentration and purity were measured using the NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific). Total RNA was then dissolved in RNase-free water and then immediately used for reverse transcription using the PrimeScript RT-PCR kit (REF RR036A, Japan) according to the manufacturer's instructions.

lncRNA gene chip detection

Twelve plasma samples were assayed for determining the expression profiles of lncRNA using the Agilent Human LncRNA Array v3.0 (8 × 60K, Arraystar, USA).

GO and KEGG analysis

Gene ontology was classified into three groups by Gene Ontology (GO) database: BP (biological process), CC (cell component), and MF (molecular

function). KEGG pathway analysis was performed to determine the involvement of differentially expressed lncRNA genes in different biological pathways. The cut off *P* value (hypergeometric *P* value) was set at 0.05.

Statistical analysis

Numerical data were expressed as mean ± standard deviation (SD) and measured in triplicates. The comparison between the statistically normalized data sets was performed using the t-test or the Mann-Whitney U test. For statistical significance, *P* value was set at 0.05 and the confidence level was set at 95%.

Results

Differentially expressed lncRNAs in chronic gastritis

In gene chip detection, a specific lncRNA expression profile was obtained in children with chronic gastritis. A differential expression pattern of 2053 lncRNAs was observed in children with chronic gastritis (fold change $\geq$ 2.0, $P \leq 0.05$) as compared to the healthy control group. Out of these, 934 and 1119 lncRNAs were significantly up-regulated and down-regulated, respectively in diseased children as compared to the healthy control group (Fig. 1). Among these, five lncRNAs (*RP11-697M17.1*, *RP11-388M20.9*, *AFAP1-AS1*, *BC062758*, and *XLOC001406*) were observed to be significantly up-regulated (Table 1), and five lncRNAs (*UNQ697*, *BX571672.5*, *CYP4F35P*, *ANKRD20A5P*, and *AL832737*) were observed to be significantly down-regulated (Table 2). The lncRNAs *RP11-697M17.1* (Fig. 2A) and *UNQ697* (Fig. 2B) were identified with the highest up-regulation and down-regulation, respectively.

GO annotation and KEGG enrichment of differential genes

The information about GO functions was used to annotate 934 up-regulated and 1119 down-regulated genes, and each GO group was enriched separately. The results showed that 2683 up-regulated lncRNAs were enriched in BP, 435 were enriched in CC, and 675 were enriched in MF ($P < 0.05$). The GO analysis showed that 1418 down-regulated genes were enriched in BP, 261 were enriched in CC, and 428 were enriched in MF ($P < 0.05$). The functions of top 20 up-regulated and down-regulated genes are described in Figs. 3 and 4, respectively.

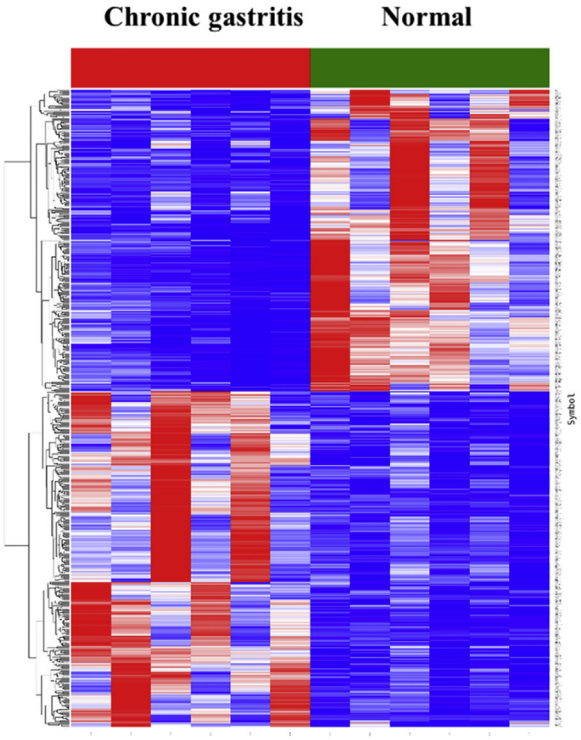

Fig. 1. Expression profiles of specific lncRNAs in children with chronic gastritis. 934 lncRNAs were significantly up-regulated and 1119 lncRNAs were significantly down-regulated.

KEGG signal pathway analysis

Based on the classification of GO annotations, KEGG pathway enrichment analysis was done for all

Table 1
Up-regulated lncRNAs in plasma of children with chronic gastritis.

Symbols	Fold change	*P*	Threshold
RP11-697M17.1	188.73	0.029	Up
RP11-388M20.9	76.68	0.032	Up
AFAP1-AS1	50.07	0.0075	Up
BC062758	43.63	0.039	Up
XLOC_001406	34.32	0.026	Up

Table 2
Down-regulated lncRNAs in children with chronic gastritis.

Symbols	Fold change	*P*	Threshold
AL832737	0.035	3.8E-05	Down
ANKRD20A5P	0.027	0.04	Down
CYP4F35P	0.025	0.027	Down
BX571672.5	0.017	0.025	Down
UNQ697	0.0089	0.00049	Down

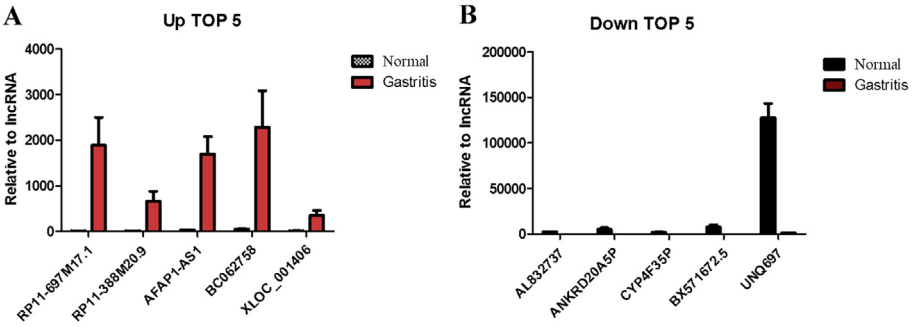

Fig. 2. Five lncRNAs (*RP11-697M17.1, RP11-388M20.9, AFAP1-AS1, BC062758*, and *XLOC001406*) were significantly up-regulated (A), and 5 lncRNAs (*UNQ697, BX571672.5, CYP4F35P, ANKRD20A5P*, and *AL832737*) were significantly down-regulated (B).

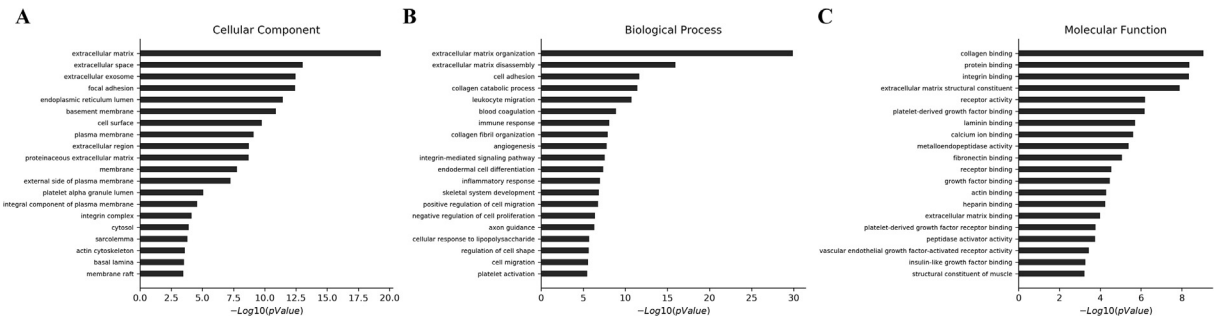

Fig. 3. The top 20 gene functions enriched in up-regulated lncRNAs in BP (biological processes), CC (cell components) and MF (molecular function) ($P < 0.05$).

identified 934 up-regulated and 1119 down-regulated genes. The up-regulated lncRNAs were mainly enriched in 20 signaling pathways, such as PI3K-Akt (Fig. 5) and the down-regulated lncRNAs target genes are significantly enriched in 20 signaling pathways, such as metabolic pathways (Fig. 6).

Discussion

So far, the gold standard for the diagnosis of chronic gastritis still depends upon the endoscopy and histopathological evaluation.[23] Various studies have shown that RNA in the blood plays an important role in the processes of cell-to-cell communication i.e. the inflammatory reactions, and tissue regeneration.[24–27] Circulating RNA in the blood may act as a potential biomarker for various diseases, which can be more heterogeneous than endoscopy in detecting various diseases.[28–34]

In the present study, we have determined the specific expression profiles of lncRNAs in plasma of children with chronic gastritis and normal healthy

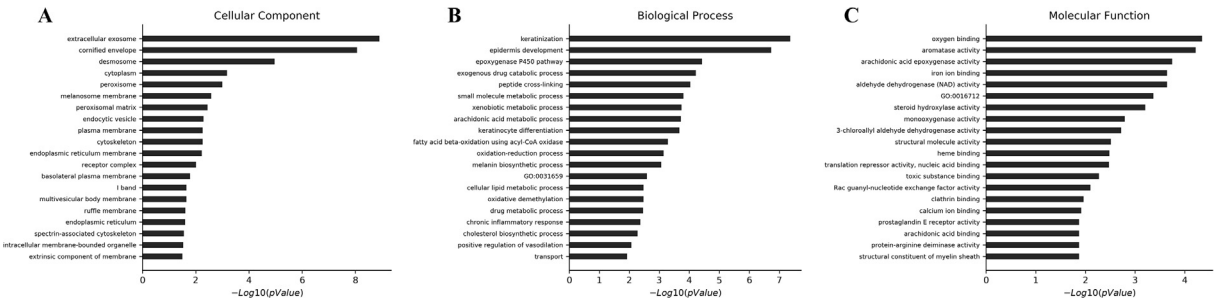

Fig. 4. The top 20 gene functions enriched in down-regulated lncRNAs in BP (biological processes), CC (cell components) and MF (molecular function) ($P < 0.05$).

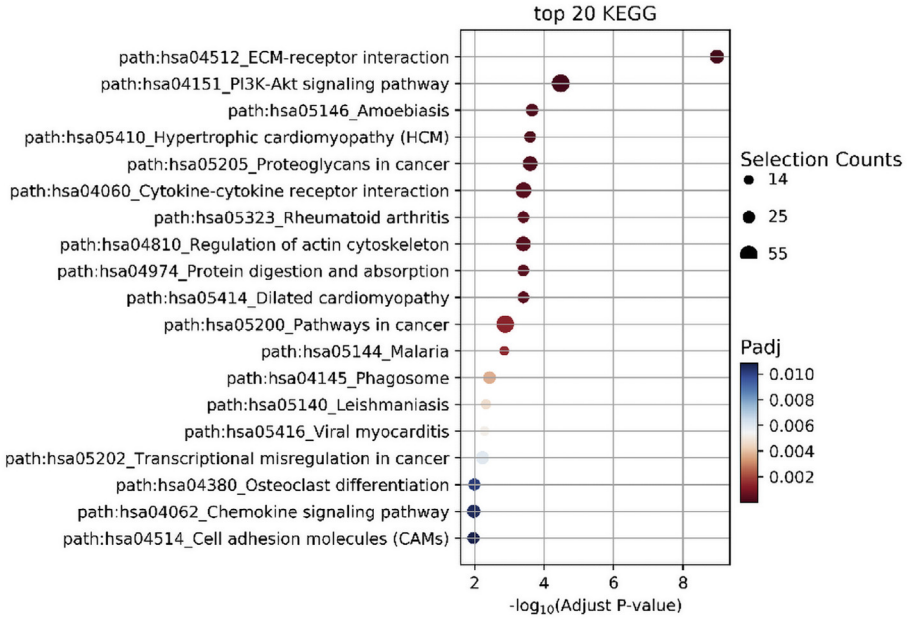

Fig. 5. Up-regulated lncRNAs are significantly enriched to the PI3K-Akt signaling pathway.

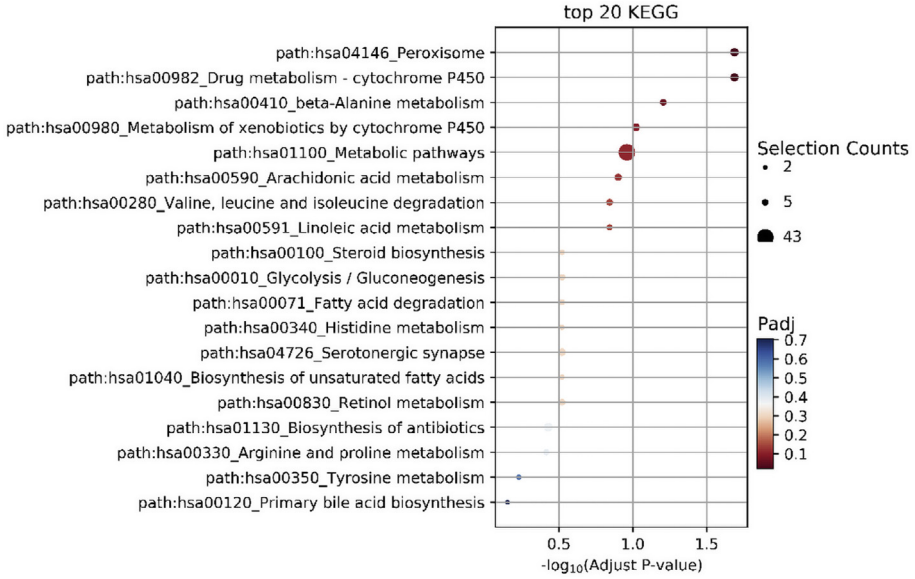

Fig. 6. Down-regulated lncRNAs target genes are significantly enriched into Metabolic signaling pathway.

children. The results showed that 2053 lncRNAs were specifically expressed in children with chronic gastritis. It was determined for the first time that the lncRNA *RP11-697M17.1*, *BC062758*, *XLOC001406*, lncRNAs *UNQ697*, *BX571672.5*, *CYP4F35P*, *ANKRD20A5P* and *AL832737*. In addition, lncRNA *RP11-388M20.9* and lncRNA *AFAP1-AS1* have been reported. It found that lncRNA *RP11-388M20.9* were significantly up-regulated with increasing concentrations of Cr(VI) in human bronchial epithelial cells. In gastric cancer, overexpression of lncRNA *AFAP1-AS1* promotes cell proliferation and invasion.

Studies have found that PI3K/Akt is the main signaling pathway in gastrointestinal diseases gastrointestinal

disorders. Helicobacter pylori (HP) mediated PI3K/AKT/GSK3β signal pathways in the occurrence of gastric cancer. In addition H. pylori causes a significant increase of PI3K/Akt in gastritis. We know that metabolic activities are also very rich in gastritis. So bioinformatics predicts that metabolic pathways are enriched more, which is consistent with previous reports. In the present study, it has been detected that lncRNA *RP11-697M17.1,* and lncRNA *UNQ697* were significantly expressed in gastritis patients as compared to the healthy control group. Moreover, that the bioinformatics analysis revealed that the lncRNA *RP11-697M17.1,* and lncRNA *UNQ69*7 might participate in PI3K-Akt and metabolic pathways. Further, it was speculated in our study that the lncRNA *RP11-697M17.1* and lncRNA *UNQ697,* may be involved in regulation rather than protein-coding.

One main limitation of the present study is the small sample size, where only six samples of children with chronic gastritis were included for the analysis. Further validation experiments should be performed using the large patient population to explore the role of circulating lncRNAs in gastritis disease.

Conclusively, the plasma lncRNA *RP11-697M17.1,* and lncRNA *UNQ697* are the most promising biomarkers for the diagnosis of chronic gastritis in children.

Conflict of interest

None.

Funding

This work was supported by a grant from Science and Technology Development Fund of Shanghai Pudong New Area (PKJ2016-Y12).

References

1. Bacha D, Walha M, Ben Slama S, et al. Chronic gastritis classifications. *Tunis Med*. 2018;96:405−410.
2. Zhang F, Wang F, Chen C, et al. Prediction of progression of chronic atrophic gastritis with Helicobacter pylori and poor prognosis of gastric cancer by CYP3A4. *J Gastroenterol Hepatol*. 2019. https://doi.org/10.1111/jgh.14844.
3. Efficacy and safety of acupuncture therapy for chronic atrophic gastritis: a meta-analysis and trial sequential analysis protocol: Erratum. *Medicine (Baltim)*. 2019;98, e17514.
4. Castaneda CA, Castillo M, Chavez I, et al. Prevalence of Helicobacter pylori infection, its virulent genotypes, and Epstein-Barr virus in Peruvian patients with chronic gastritis and gastric cancer. *J Glob Oncol*. 2019;5:1−9.
5. Chandra P, Nath S, Jain D. Pancreatitis, cholangitis, and gastritis: the triumvirate of immunoglobulin G4-related disease identified simultaneously on 18F-fluorodeoxyglucose positron emission tomography/computed tomography. *Indian J Nucl Med*. 2019;34:335−337.
6. Collins K, Mnayer L, Shen P. Burkitt-like lymphoma with 11q aberration. *Clin Case Rep*. 2019;7:1823−1824.
7. Dohi O, Majima A, Naito Y, et al. Can image-enhanced endoscopy improve the diagnosis of Kyoto classification of gastritis in the clinical setting? *Dig Endosc*. 2019;32:191−203.
8. Rodriguez-Castro KI, Franceschi M, Noto A, et al. Clinical manifestations of chronic atrophic gastritis. *Acta Biomed*. 2018;89:88−92.
9. Li JA, Tian YL, Cong L, Fan S, Duan LW. Endoscopic submucosal dissection for treating gastritis cystica profunda. *J Biol Regul Homeost Agents*. 2019;33:1577−1580.
10. Liu XQ, Wang L. Effect of modified Zhengqi Powder in treating chronic gastritis and on patients' life quality and inflammatory factors (in Chinese). *China J Chin Mater Med*. 2019;44:181−185.
11. Liu XF, , Li DG, Liu JP, Du YR, Bai HY. Medicine-syndrome research and analysis of professor Li Dian-gui in treating chronic atrophic gastritis with intestinal metaplasia (in Chinese). *China J Chin Mater Med*. 2017;42:1792−1796.
12. Wei W, Yang Y, Shi HX. Status, challenges, and prospects of treating chronic atrophic gastritis by Chinese medical diagnosis and treatment (in Chinese). *Chin J Integr Tradit West Med*. 2015;35:1424−1426.
13. Meltzer AC, Winter LE, Kulie P, et al. Treating gastritis, peptic ulcer disease, and dyspepsia in the emergency department: the feasibility and patient-reported outcomes of testing and treating for Helicobacter pylori infection. *Ann Emerg Med*. 2015;66:131−139.
14. Pyfrom SC, Luo H, Payton JE. PLAIDOH: a novel method for functional prediction of long non-coding RNAs identifies cancer-specific LncRNA activities. *BMC Genomics*. 2019;20:137.
15. Qian W, Cai X, Qian Q, et al. lncRNA ZEB1-AS1 promotes pulmonary fibrosis through ZEB1-mediated epithelial-mesenchymal transition by competitively binding miR-141-3p. *Cell Death Dis*. 2019;10:129.
16. Ye ZM, Yang S, Xia YP, et al. LncRNA MIAT sponges miR-149-5p to inhibit efferocytosis in advanced atherosclerosis through CD47 upregulation. *Cell Death Dis*. 2019;10:138.
17. Zhang L, Wang Y, Zhang L, et al. ZBTB7A, a miR-663a target gene, protects osteosarcoma from endoplasmic reticulum stress-induced apoptosis by suppressing LncRNA GAS5 expression. *Cancer Lett*. 2019;448:105−116.
18. Lin Y, Xu L, Wei W, Zhang X, Ying R. Long noncoding RNA H19 in digestive system cancers: a meta-analysis of its association with pathological features. *BioMed Res Int*. 2016;2016:4863609.
19. Zhang S, Du L, Wang L, et al. Evaluation of serum exosomal LncRNA-based biomarker panel for diagnosis and recurrence prediction of bladder cancer. *J Cell Mol Med*. 2019;23:1396−1405.
20. Zhang C, Yang X, Qi Q, Gao Y, Wei Q, Han S. lncRNA-HEIH in serum and exosomes as a potential biomarker in the HCV-related hepatocellular carcinoma. *Cancer Biomark*. 2018;21:651−659.
21. Shen X, Zhang Y, Wu X, et al. Upregulated lncRNA-PCAT1 is closely related to clinical diagnosis of multiple myeloma as a predictive biomarker in serum. *Cancer Biomark*. 2017;18:257−263.
22. Cai Y, Yang Y, Chen X, et al. Circulating 'lncRNA OTTHUMT00000387022' from monocytes as a novel

biomarker for coronary artery disease. *Cardiovasc Res*. 2016;112:714−724.
23. Cao ZM, You WZ, Chen HB. A long-term follow-up study with endoscope in chronic gastritis (in Chinese). *Chin J Intern Med*. 1993;32:743−745.
24. Simonian M, Mosallayi M, Mirzaei H. Circulating miR-21 as novel biomarker in gastric cancer: diagnostic and prognostic biomarker. *J Cancer Res Ther*. 2018;14:475.
25. Xie JF, Xie NS, Tang JH, Gu QP, Luo QT. Circulating SHIP2 mRNA as a novel biomarker in the diagnosis and prognosis of gastric cancer. *Eur Rev Med Pharmacol Sci*. 2017;21:5129−5134.
26. Wu J, Li G, Wang Z, et al. Circulating MicroRNA-21 is a potential diagnostic biomarker in gastric cancer. *Dis Markers*. 2015;2015:435656.
27. Valladares-Ayerbes M, Reboredo M, Medina-Villaamil V, et al. Circulating miR-200c as a diagnostic and prognostic biomarker for gastric cancer. *J Transl Med*. 2012;10:186.
28. Penyige A, É Márton, Soltész B, et al. Circulating miRNA profiling in plasma samples of ovarian cancer patients. *Int J Mol Sci*. 2019;20:4533.
29. Kwee LC, Neely ML, Grass E, et al. Associations of osteopontin and NT-proBNP with circulating miRNA levels in acute coronary syndrome. *Physiol Genom*. 2019;51:506−515.
30. Chan HN, Ho SL, He D, Li HW. Direct and sensitive detection of circulating miRNA in human serum by ligase-mediated amplification. *Talanta*. 2020;206:120217.
31. Hao QQ, Wang QH, Xia W, Qian HZ. Circulating miRNA expression profile and bioinformatics analysis in patients with occult hepatitis B virus infection. *J Med Virol*. 2020;92:191−200.
32. Zhou X, Fang S, Wang M, et al. Diagnostic value of circulating miRNA-122 for hepatitis B virus and/or hepatitis C virus-associated chronic viral hepatitis. *Biosci Rep*. 2019;39, BSR20190900.
33. Swellam M, Ezz El Arab L, Al-Posttany AS, B Said S. Clinical impact of circulating oncogenic MiRNA-221 and MiRNA-222 in glioblastoma multiform. *J Neuro Oncol*. 2019;144:545−551.
34. Santos AS, Cunha Neto E, Fukui RT, Ferreira L, Silva M. Increased expression of circulating microRNA 101-3p in type 1 diabetes patients: New insights into miRNA-regulated pathophysiological pathways for type 1 diabetes. *Front Immunol*. 2019;10:1637.

Edtied by Yan-Gang Ren and Yi Cui