Original Paper

Circulating LncRNAs Serve as Diagnostic Markers for Hepatocellular Carcinoma

Weidong Yuan Yong Sun Ling Liu Bing Zhou Shuming Wang Dianhua Gu

Department of Hepatobiliary Surgery, Huai'an First People's Hospital, Huai'an, Jiangsu, P. R. China

Key Words

Hcc • Fingerprint • AUC • LncRNA • Plasma

Abstract

Background/Aims: Circulating (serous or plasmic) long non-coding RNA (lncRNA) as biomarkers for predicting the diagnosis or prognosis of human disease have been well documented. Due to the sensibility or specificity limitation of Alpha Fetoprotein (AFP), a cluster lncRNAs were revealed as fingerprints for hepatocellular carcinoma (HCC). In this study, we enrolled all the reported circulating lncRNAs in HCC as candidate targets and examined in an independent cohort. **Methods:** The candidate lncRNAs were determined by qRT-PCR divided into training and validation sets. The risk score analysis was employed to evaluate the potential diagnosis ability of the lncRNAs independently or combining with AFP value. The receiver operating characteristic curve (ROC) was applied for presentation of sensibility or specificity. **Results:** Among the ten candidate circulating lncRNA, LINC00152, RP11-160H22.5, XLOC014172 and LOC149086 were screened with significant difference in training set. Further investigation in validation set indicated LINC00152, RP11-160H22.5 and XLOC014172 might be the fingerprints for HCC comparing with chronic hepatitis (CH) patients or healthy controls. The risk score analysis revealed the combination of three lncRNAs with AFP could distinguish the HCC from either CH or healthy control with the area under curve value (AUC) of 0.986 and 0.985, respectively. **Conclusion:** The three lncRNAs may act as novel biomarkers for acting as fingerprint in HCC combining with AFP.

© 2017 The Author(s)
Published by S. Karger AG, Basel

Introduction

As one of the most common life-threatening malignancies worldwide, hepatocellular carcinoma (HCC) was estimated to cause 748, 300 new liver cancer cases and 695, 900 cancer deaths worldwide per year and nearly half of these cases and deaths were estimated to take place in China [1-3]. Although the major risk factors have been identified, including hepatitis C (HCV) and B virus (HBV) infection, toxins (aflatoxin B1), chronic alcohol abuse and nonalcoholic fatty liver disease [4-6]. The early diagnosis of HCC still remain a crucial issue currently due the low sensibility or specificity limitation of Alpha Fetoprotein (AFP)[7-9]. Hepatitis B virus (HBV) infection is an important factor for HCC occurrence. The association

Dianhua Gu

Department of Hepatobiliary Surgery, Huai'an First People's Hospital,
6 Beijing Road West, Huai'an, Jiangsu, (China)
Tel. +86-0517-84952313, Fax +86-0517-84952313, E-Mail ywdhayy@yeah.net

between HBV and HCC has been demonstrated by multiple epidemiological studies: High HBV prevalence may result in a high frequency of occurrence of HCC, and the risk of HCC in HBV carriers is significantly increased compared with that of non-carriers [10]. Thus, the occurrence of hepatocellular carcinoma (HCC) is decreased but not eliminated. The molecular mechanisms as well as the biomarkers for HCC that contributing to the occurrence and development of HCC remain elusive, hindering the development of clinic diagnostic or therapeutic approaches.

To date, the long non-coding has been proved as important role in the pathogenesis of human disease including HCC[11-13]. lncRNAs were considered as non-protein-coding transcripts longer than 200 nucleotides and widely expressed in various organs and tissues of the body [14-16]. Functional lncRNA was involved in various cancer biological processes, such as proliferation, apoptosis, invasion and metastasis through modulation of chromatin remodeling, epigenetics modification, regulation of gene transcription, post-transcriptional mRNA processing, protein function and localization, and intercellular signal transduction regulation [17]. Many previous studies showed that lncRNAs might be as biomarkers in multiple cancers. For example, The MD-miniRNA, derived from MALAT-1, has been explored, and was available for clinical detection for human prostate cancer [18]. The fragment of Linc00974 could be also detectable and might improve the prognosis of HCC by comparing with CYFRA-21-1[19].

In this study, we conducted our experiment based on the identified lncRNAs which was certain diagnostic value in HCC according to previous report. We enrolled ten candidate lncRNAs for screening. We hypothesized that these HCC-related lncRNAs might be utilized to detect and early monitor HCC by combining with the well-known marker AFP. We aimed to explore the potential lncRNA acting as fingerprint for the early identification of HCC.

Materials and Methods

Samples and Screening phase

100 fresh HCC plasma samples were obtained from the patients received therapy at Huai'an First People's Hospital. Controls were from healthy volunteers without any health problems during their health check-ups at the Huai'an First People's Hospital. The study was approved by our Institutional Ethics Committee of Huai'an First People's Hospital. All patients provided their written informed consent to participate in the study. The HCC patients were confirmed by the histopathological examination. Peripheral blood samples of patients were collected before surgery. Blood samples were collected in a separate vacuum cube, followed by centrifugation at 3,000 rpm for 10 min. All samples were stored at -80°C until further analysis. The clinical characteristics of all patients are summarized in Table 1. All candidates were tested in an independent cohort of 20 plasma samples obtained from patients regarding as training set. The rest 80 samples was labelled as validation set randomly.

Table 1. Clinicopathological features analysis of hepatocellular carcinoma (HCC) patients, patients with HBV-positive chronic hepatitis (CH) and cancer-free control samples. ^a Student t-test. ^b Chi-square test

	HCC	CH	Control	P value
N	100	100	100	
Age Mean (SE) year	54.31(0.36)	53.98(0.51)	54.13(0.42)	0.37 ^a
Sex (male/female)	87/13	85/15	88/12	0.22 ^b
Smoking history				
No	23	31	67	<0.001 ^b
Yes	77	69	33	
Tumor Size(cm)				
≤3cm	66			
>3cm	34			
Tumor Number				
Solitary	41			
Multiple	59			
Tumor Capsular				
Incomplete	32			
Complete	68			
TNM stage				
I-II	71			
III-IV	29			
AFP(ng/ml)				
<20	11	35	99	
≥20	89	65	1	

Quantitative real-time PCR (qRT-PCR)

Total RNAs were obtained from plasmic samples using TRIzol reagent as described by the manufacturer (Invitrogen Life Technologies Co, Carlsbad, CA, USA). For mRNA detection, total RNAs (500ng) were reverse transcribed using the reverse transcription kit (Takara, Tokyo, Japan). The quality of RNA samples was assessed by a UV spectrophotometer (Bio-Rad, Hercules, CA, USA), and the 260/280nm absorbance ratio of samples were limited to 1.8-2.0. QRT-PCR was performed by using ABI Prism 7900HT (Applied Biosystems, CA, USA). The expression levels of these candidates were analyzed in all samples, and comparative $2^{-\Delta\Delta Ct}$ method algorithms were used to analyze the difference between patients and health controls.

Risk score analysis

Risk score analysis was performed to evaluate the associations between the concentrations of the plasma lncRNA expression levels. The upper 95% reference interval of each lncRNA value in controls or CH group was set as the threshold to code the expression level of the corresponding lncRNA for each sample as 0 and 1 in the training set. A risk score function (RSF) to predict HCC group was defined according to a linear combination of the expression level for each lncRNA. For example, the RSF for sample i using information from three lncRNAs was: $rs_i = \sum 3j - 1W_j.s_{ij}$. In the above equation, s_{ij} is the risk score for lncRNA j on sample i , and W_j is the weight of the risk score of lncRNA j . The risk score of three lncRNAs was calculated using the weight by the regression coefficient that was derived from the univariate logistic regression analysis of each lncRNAs. Samples were ranked according to their RSF and then divided into a high-risk group, representing the HCC patients, and a low-risk group, representing the predicted control individuals or CH patients. Frequency tables and ROC curves were then used to evaluate the diagnostic effects of the profiling and to find the appropriate cutoff point, and to validate the procedure and cutoffs in the next validation sample set.

Statistical analysis

Data were presented as mean (S.E.M.). The Student's t-test and Mann-Whitney unpaired test analysis of variance were used to evaluate statistical differences between patients and controls. Analysis of area under the ROC curve (AUC) was used to estimate the effectiveness of lncRNA. Statistical analysis was performed using softwares of STATA 10.0 and GraphPad prism. In all cases, $P < 0.05$ was considered to be significant. All P values were two-sided.

Results

Screening the HCC-associated lncRNAs in training set

The ten candidate circulating lncRNAs was enrolled including DANCER, SPRY4-IT1, HULC, LINC00152, LINC00974, MALAT1, lncRNA-CTBP, RP11-160H22.5, XLOC014172 and LOC149086. The candidates were first detected in the 20 plasma samples as training set. We found that the four lncRNAs (LINC00152, RP11-160H22.5, XLOC014172 and LOC149086) were significant different in HCC patients comparing with either patients with CH or healthy control (Fig. 1)

Confirming the candidate biomarker in validation set

The following investigation was conducted by detecting the expression of the four lncRNAs in the validation set. As presented in Fig. 2, LINC00152, RP11-160H22.5 and XLOC014172 were confirmed in the larger individual samples indicating as the potential biomarker for predicting HCC, while the LOC149086 present no significant difference.

Diagnostic potency prediction by risk score analysis

Researcher has reported the three lncRNAs might be potential biomarkers for HCC; however, they only focused on predictive value of these lncRNAs through healthy controls, no evidence was given for these lncRNAs to predict the HCC from patients with CH. This might be a useful factor for clinical application. Here, in this study, to validate the accuracy and specificity of these 3 lncRNA as a HCC potential signature, the risk score formula was applied to assess the diagnostic value of the three lncRNAs profiling system by combining with AFP.

Fig. 1. Relative expression of ten candidate lncRNAs in training set. The 20 paired plasma from HCC patients and 20 cancer-free controls were applied by using RT-qPCR. Data was presented as mean $\pm$ SEM. Data was analyzed by student t test. * indicated $p < 0.05$, ** indicated $p < 0.01$.

Fig. 2. Relative expression of lncRNAs in validation set. Data were presented as plot of the median and range of log-transformed relative expression level and was analyzed with student t test. ** indicated $p < 0.01$.

Table 2. Risk score analysis of in HCC and cancer-free control plasma samples. ^a PPV, positive predictive value, ^b NPV, negative predictive value

Score	0-8.12	8.12-13.31	PPV ^a	NPV ^b
Training set			0.70	0.80
HCC	6	14		
Control	16	4		
Validation set			0.88	0.81
HCC	12	88		
Control	81	19		

First, we analyzed the predictive value of these three lncRNA and AFP comparing with the healthy control. The risk score of each plasma sample in the training set was calculated, as the basis of their risk scores and a set cut off, plasma samples were then divided into a high-risk group, representing the possible HCC group, and a low-risk group, representing the predicted controls. At the optimal cutoff value (Value=8.12) with the value of sensitivity + specificity considered to be maximal, the positive predictive value (PPV) and negative predictive value (NPV) was 70% and 80% in the training set, respectively. Similarly, when the same cutoff value was applied to calculate the risk score of samples in the larger validation sets, the PPV and NPV were 88%, 81%, respectively (Table 2). ROC curve analysis was used to evaluate the predicting diagnosis value of lncRNAs and AFP for HCC. Areas under the ROC curves of the validated three lncRNAs signature were 0.869, 0.884 and 0.759, respectively while the combination of the three factors with AFP possessed a moderate ability for discrimination between HCC patients and controls with an area under the ROC curve of 0.986 (Fig. 3A).

Fig. 3. ROC analysis of the three potential biomarkers for HCC by using risk score analysis combining with AFP. A: The ROC analysis of lncRNAs combining with AFP to predict HCC from healthy controls. B: The ROC analysis of lncRNAs to predict HCC from patient with CH.

Table 3. Risk score analysis of in HCC and CH control plasma samples. ^a PPV, positive predictive value, ^b NPV, negative predictive value

Score	0-5.88	5.88-11.25	PPV ^a	NPV ^b
Training set			0.65	0.80
HCC	7	13		
CH	16	4		
Validation set			0.87	0.80
HCC	13	87		
CH	80	20		

The similar strategy was applied for predicting the HCC diagnostic value of lncRNAs by comparing with patients with CH. At the optimal cutoff value (Value=5.88) with the value of sensitivity + specificity considered to be maximal, the positive predictive value (PPV) and negative predictive value (NPV) was 65% and 80% in the training set, respectively. Similarly, when the same cutoff value was applied to calculate the risk score of samples in the larger validation sets, the PPV and NPV were 87%, 80%, respectively (Table 3). ROC curve analysis was used to evaluate the predicting diagnosis value of lncRNAs and AFP for HCC. Areas under the ROC curves of the validated three lncRNAs signature were 0.826, 0.859 and 0.735, respectively While the combination of the three factors possessed a moderate ability for discrimination between HCC patients and controls with an area under the ROC curve of 0.985 (Fig. 3B).

Discussion

Hepatocellular carcinoma (HCC) is the sixth most common malignant tumor with the third leading cause of cancer-related death in the world [20, 21]. HCC is chartered by high malignancy, easy invasion especially towards intrahepatic invasion and frequent reoccurrence after surgical resection [22]. Increasing evidence indicated lncRNAs as biomarker for predicting survival and metastasis or diagnosing in multiple diseases which was confirmed contributing to many biological processes through diverse mechanisms [11].

Mechanism study have been described for selected lncRNA involved in liver diseases include widely diverse functions such as DNA imprinting, X inactivation, DNA demethylation, gene transcription and generation of other RNA molecules [23].

Not only the miRNA, but also the lncRNA have been annotated acting as biomarkers in predicting the feature of tumor. For example, circulating serum lncRNA RP11-445H22.4 as biomarker for the detection of breast cancer [24]. In this study, the ten lncRNA was enrolled as candidates based on the important function reported previously. These tissues different expressed lncRNA might present an alternated level in patients' bodily fluid. Research has speculated that the aberrant expressed circulation lncRNAs might be derived from the tumor. Thus, we examined the expression of the 10 lncRNAs in patients and healthy control. Accordingly, Long noncoding RNA DANCR could increase stemness features of hepatocellular carcinoma by derepression of CTNNB1 [25]. Overexpression of the long non-coding RNA SPRY4-IT1 promotes tumor cell proliferation and invasion by activating EZH2 in hepatocellular carcinoma [26]. HULC and linc00152 Act as Novel Biomarkers in Predicting Diagnosis of Hepatocellular Carcinoma [27]. A novel biomarker linc00974 interacting with KRT19 promotes proliferation and metastasis in hepatocellular carcinoma [19]. Long Non-Coding RNA MALAT1 Regulates ZEB1 Expression by Sponging miR-143-3p and Promotes Hepatocellular Carcinoma Progression [28]. The lncRNA-CTBP has also been identified had high sensitivity and specificity for discriminating HCC from healthy controls and also from CH patients [29]. Tang et al. also published the results that lncRNAs might act as biomarker for the diagnosis of HCC from healthy control and CH patients [30]. In this study, by employing a multiple screening phase, we found that LINC00152, RP11-160H22.5, XLOC014172 and LOC149086 were screened with significant difference in training set. Further investigation in validation set indicated LINC00152, RP11-160H22.5 and XLOC014172 might be the fingerprints for HCC comparing with patients with chronic hepatitis (CH) or healthy controls.

For the biomarkers screening study in HCC, researchers has identified multiple factors that might be the potential fingerprint; however, most of this study was conducted by comparing the expression of these lncRNAs in a case-control study. The control group enrolled was healthy volunteers. This kind of biomarker might be useful for distinguish the HCC patients from healthy human; however, the HCC patients was developed from HBV positive patients, most of them might be suffering with CH or liver cirrhosis. Biomarkers for predicting HCC from patients with CH or liver cirrhosis might be useful for clinical approach especially for the early diagnosis of HCC.

Researchers has found that cigarette smoke exposure will cause an aberrant expression profiling of lncRNA in lung cancer associated cell lines [31]. Based on the different cigarette exposure in the three groups, we thought it was necessary to identify the expression of circulating lncRNA in patients with the cigarette exposure or not. This might give us a novel point both for the pathogenesis and diagnosis of HCC.

Conclusion

We identified three lncRNAs, LINC00152, RP11-160H22.5 and XLOC014172, as the potential biomarker for the tumorigenesis prediction of HCC from both healthy controls and patients with CH combining with AFP in this study. These factors might serve as a better biomarker for the early diagnosis of HCC especially for patients with HBV infection background.

Disclosure Statement

The authors declare that they have no financial conflict of interest.

References

- 1 Chaisaingmongkol J, Budhu A, Dang H, Rabibhadana S, Pupacdi B, Kwon SM, Forgues M, Pomyen Y, Bhudhisawasdi V, Lertprasertsuke N, Chotirosniramit A, Pairojkul C, Auewarakul CU, Sricharunrat T, Phornphutkul K, Sangrajang S, Cam M, He P, Hewitt SM, Ylaya K, Wu X, Andersen JB, Thorgeirsson SS, Waterfall JJ, Zhu YJ, Walling J, Stevenson HS, Edelman D, Meltzer PS, Loffredo CA, Hama N, Shibata T, Wiltout RH, Harris CC, Mahidol C, Ruchirawat M, Wang XW, Consortium T-L: Common Molecular Subtypes Among Asian Hepatocellular Carcinoma and Cholangiocarcinoma. *Cancer Cell* 2017;32:57-60.
- 2 Logan GJ, Dane AP, Hallwirth CV, Smyth CM, Wilkie EE, Amaya AK, Zhu E, Khandekar N, Ginn SL, Liao SHY, Cunningham SC, Sasaki N, Cabanes-Creus M, Tam PPL, Russell DW, Lisowski L, Alexander IE: Identification of liver-specific enhancer-promoter activity in the 3' untranslated region of the wild-type AAV2 genome. *Nat Genet* 2017;49:1268-1273.
- 3 Cancer Genome Atlas Research Network. Electronic address wbe, Cancer Genome Atlas Research N: Comprehensive and Integrative Genomic Characterization of Hepatocellular Carcinoma. *Cell* 2017;169:1327-1341.
- 4 El-Khoueiry AB, Sangro B, Yau T, Crocenzi TS, Kudo M, Hsu C, Kim TY, Choo SP, Trojan J, Welling THR, Meyer T, Kang YK, Yeo W, Chopra A, Anderson J, Dela Cruz C, Lang L, Neely J, Tang H, Dastani HB, Melero I: Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet* 2017;389:2492-2502.
- 5 Chan SL, Wong VW, Qin S, Chan HL: Infection and Cancer: The Case of Hepatitis B. *J Clin Oncol* 2016;34:83-90.
- 6 Liu Y, Chai Y, Zhang J, Tang J: A Function Variant at miR-501 Alters Susceptibility to Hepatocellular Carcinoma in a Chinese Han Population. *Cell Physiol Biochem* 2016;38:2500-2508.
- 7 Wang Y, He L, Du Y, Zhu P, Huang G, Luo J, Yan X, Ye B, Li C, Xia P, Zhang G, Tian Y, Chen R, Fan Z: The long noncoding RNA lncTCF7 promotes self-renewal of human liver cancer stem cells through activation of Wnt signaling. *Cell Stem Cell* 2015;16:413-425.
- 8 Lau CC, Sun T, Ching AK, He M, Li JW, Wong AM, Co NN, Chan AW, Li PS, Lung RW, Tong JH, Lai PB, Chan HL, To KF, Chan TF, Wong N: Viral-human chimeric transcript predisposes risk to liver cancer development and progression. *Cancer Cell* 2014;25:335-349.
- 9 Cao C, Sun J, Zhang D, Guo X, Xie L, Li X, Wu D, Liu L: The long intergenic noncoding RNA UFC1, a target of MicroRNA 34a, interacts with the mRNA stabilizing protein HuR to increase levels of beta-catenin in HCC cells. *Gastroenterology* 2015;148:415-426 e418.
- 10 Hong X, Kim ES, Guo H: Epigenetic Regulation of Hepatitis B Virus Covalently Closed Circular DNA: Implications for Epigenetic Therapy against Chronic Hepatitis B. *Hepatology* 2017. Doi:10.1002/hep.29479
- 11 Yang Y, Chen L, Gu J, Zhang H, Yuan J, Lian Q, Lv G, Wang S, Wu Y, Yang YT, Wang D, Liu Y, Tang J, Luo G, Li Y, Hu L, Sun X, Wang D, Guo M, Xi Q, Xi J, Wang H, Zhang MQ, Lu ZJ: Recurrently deregulated lncRNAs in hepatocellular carcinoma. *Nat Commun* 2017;8:14421.
- 12 Li D, Liu X, Zhou J, Hu J, Zhang D, Liu J, Qiao Y, Zhan Q: Long noncoding RNA HULC modulates the phosphorylation of YB-1 through serving as a scaffold of extracellular signal-regulated kinase and YB-1 to enhance hepatocarcinogenesis. *Hepatology* 2017;65:1612-1627.
- 13 Yang F, Zhang L, Huo XS, Yuan JH, Xu D, Yuan SX, Zhu N, Zhou WP, Yang GS, Wang YZ, Shang JL, Gao CF, Zhang FR, Wang F, Sun SH: Long noncoding RNA high expression in hepatocellular carcinoma facilitates tumor growth through enhancer of zeste homolog 2 in humans. *Hepatology* 2011;54:1679-1689.
- 14 Yuan JH, Liu XN, Wang TT, Pan W, Tao QF, Zhou WP, Wang F, Sun SH: The MBNL3 splicing factor promotes hepatocellular carcinoma by increasing PXN expression through the alternative splicing of lncRNA-PXN-AS1. *Nat Cell Biol* 2017;19:820-832.
- 15 Rizvi AH, Camara PG, Kandror EK, Roberts TJ, Schieren I, Maniatis T, Rabadan R: Single-cell topological RNA-seq analysis reveals insights into cellular differentiation and development. *Nat Biotechnol* 2017;35:551-560.
- 16 Liu M, Xing LQ, Liu YJ: A three-long noncoding RNA signature as a diagnostic biomarker for differentiating between triple-negative and non-triple-negative breast cancers. *Medicine (Baltimore)* 2017;96:e6222.
- 17 Abraham JM, Meltzer SJ: Long Noncoding RNAs in the Pathogenesis of Barrett's Esophagus and Esophageal Carcinoma. *Gastroenterology* 2017;153:27-34.

- 18 Ren S, Wang F, Shen J, Sun Y, Xu W, Lu J, Wei M, Xu C, Wu C, Zhang Z, Gao X, Liu Z, Hou J, Huang J, Sun Y: Long non-coding RNA metastasis associated in lung adenocarcinoma transcript 1 derived miniRNA as a novel plasma-based biomarker for diagnosing prostate cancer. *Eur J Cancer* 2013;49:2949-2959.
- 19 Tang J, Zhuo H, Zhang X, Jiang R, Ji J, Deng L, Qian X, Zhang F, Sun B: A novel biomarker Linc00974 interacting with KRT19 promotes proliferation and metastasis in hepatocellular carcinoma. *Cell Death Dis* 2014;5:e1549.
- 20 Yip TC, Chan HL, Wong VW, Tse YK, Lam KL, Wong GL: Impact of age and gender on risk of hepatocellular carcinoma after hepatitis B surface antigen seroclearance. *J Hepatol* 2017. Doi:10.1016/j.jhep.2017.06.019
- 21 Lai Q, Vitale A, Iesari S, Finkenstedt A, Mennini G, Spoletini G, Hoppe-Lotichius M, Vennarecci G, Manzia TM, Nicolini D, Avolio AW, Frigo AC, Cillo U, Graziadei I, Rossi M, Tsochatzis E, Otto G, Ettorre GM, Tisone G, Vivarelli M, Agnes S, Lerut J, European Hepatocellular Cancer Liver Transplant Study G: Intention-to-treat survival benefit of liver transplantation in patients with hepatocellular cancer. *Hepatology* 2017. Doi:10.1002/hep.29342
- 22 Dutta R, Mahato RI: Recent advances in hepatocellular carcinoma therapy. *Pharmacol Ther* 2017;173:106-117.
- 23 Devaux Y, Zangrando J, Schroen B, Creemers EE, Pedrazzini T, Chang CP, Dorn GW, 2nd, Thum T, Heymans S, Cardioline n: Long noncoding RNAs in cardiac development and ageing. *Nat Rev Cardiol* 2015;12:415-425.
- 24 Xu N, Chen F, Wang F, Lu X, Wang X, Lv M, Lu C: Clinical significance of high expression of circulating serum lncRNA RP11-445H22.4 in breast cancer patients: a Chinese population-based study. *Tumour Biol* 2015;36:7659-7665.
- 25 Yuan SX, Wang J, Yang F, Tao QF, Zhang J, Wang LL, Yang Y, Liu H, Wang ZG, Xu QG, Fan J, Liu L, Sun SH, Zhou WP: Long noncoding RNA DANCER increases stemness features of hepatocellular carcinoma by derepression of CTNNB1. *Hepatology* 2016;63:499-511.
- 26 Zhou M, Zhang XY, Yu X: Overexpression of the long non-coding RNA SPRY4-IT1 promotes tumor cell proliferation and invasion by activating EZH2 in hepatocellular carcinoma. *Biomed Pharmacother* 2017;85:348-354.
- 27 Li J, Wang X, Tang J, Jiang R, Zhang W, Ji J, Sun B: HULC and Linc00152 Act as Novel Biomarkers in Predicting Diagnosis of Hepatocellular Carcinoma. *Cell Physiol Biochem* 2015;37:687-696.
- 28 Konishi H, Ichikawa D, Yamamoto Y, Arita T, Shoda K, Hiramoto H, Hamada J, Itoh H, Fujita Y, Komatsu S, Shiozaki A, Ikoma H, Ochiai T, Otsuji E: Plasma level of metastasis-associated lung adenocarcinoma transcript 1 is associated with liver damage and predicts development of hepatocellular carcinoma. *Cancer Sci* 2016;107:149-154.
- 29 El-Tawdi AH, Matboli M, Shehata HH, Tash F, El-Khazragy N, Azazy Ael S, Abdel-Rahman O: Evaluation of Circulatory RNA-Based Biomarker Panel in Hepatocellular Carcinoma. *Mol Diagn Ther* 2016;20:265-277.
- 30 Tang J, Jiang R, Deng L, Zhang X, Wang K, Sun B: Circulation long non-coding RNAs act as biomarkers for predicting tumorigenesis and metastasis in hepatocellular carcinoma. *Oncotarget* 2015;6:4505-4515.
- 31 Thai P, Statt S, Chen CH, Liang E, Campbell C, Wu R: Characterization of a novel long noncoding RNA, SCAL1, induced by cigarette smoke and elevated in lung cancer cell lines. *Am J Respir Cell Mol Biol* 2013;49:204-211.