Int J Clin Exp Pathol 2016;9(10):10290-10297
www.ijcep.com /ISSN:1936-2625/IJCEP0037767

Original Article

Down-regulation and relationship with clinicopathological parameters of LncRNA UCHL1-AS1 in hepatocellular carcinoma tissues

Ziling Huang[1*], Lanshan Huang[1*], Kanglai Wei[1], Siqiao Shen[1], Chunyao Li[1], Weijun Li[2], Jianjun Li[3], Yiwu Dang[1], Yan Wei[1], Gang Chen[1], Zhenbo Feng[1]

Departments of [1]Pathology, [2]Medical Record & Information, [3]General Surgery of Western Branch, The First Affiliated Hospital of Guangxi Medical University, Nanning, China. [*]Equal contributors.

Received August 12, 2016; Accepted August 24, 2016; Epub October 1, 2016; Published October 15, 2016

Abstract: Antisense ubiquitin carboxyl-terminal hydrolase L1 (antisense Uchl1, UCHL1-AS1) is a spliced antisense long noncoding RNAs (LncRNA) of Uchl1 which may influence UCHL1 protein level in human cancers. The aim of this study was to detect the expression of LncRNA UCHL1-AS1 in hepatocellular carcinoma (HCC) and reveal the correlation between UCHL1-AS1 level and clinicopathological parameters. In current study, the expression of UCHL1-AS1 was detected by RT-qPCR in 72 HCC and adjacent noncancerous liver tissues, as well as in five HCC cell lines and a normal liver epithelium cell line L-O2. The correlation of UCHL1-AS1 level with clinicopathological parameters of HCC was analyzed by the Chi-square test, Kaplan-Meier method and Cox proportional hazards model. We found that the relative expression of UCHL1-AS1 in HCC was significantly lower than that in adjacent noncancerous liver tissues (P<0.0001). The area under curve (AUC) of UCHL1-AS1 was 0.787 (95% CI 0.713 to 0.861, P<0.0001). On the other hand, lower expression of UCHL1-AS1 was found in three HCC cell lines compared to the normal liver epithelial cell line L-O2 (P<0.05). Of note, UCHL1-AS1 expression level showed significant correlation with hepatitis history (r=-0.336, P=0.002), portal vein tumor thrombus (PVTT) (r=-0.302, P=0.010) and distant metastasis (r=-0.235, P=0.047). To conclude, these findings support that UCHL1-AS1 is down-regulated in HCC and might be of value for the prediction of carcinogenic progression.

Keywords: Long non-coding RNA, UCHL1-AS1, hepatocellular carcinoma, metastasis, prognosis

Introduction

As one of the highly malignant tumors, hepatocellular carcinoma (HCC) is the second most lethal neoplasm in male and sixth in female patients which leads to an estimated 745500 deaths annually worldwide [1]. For developing countries like China, it is the second lethal factor in male and the fifth in female cancer patients [1, 2]. Less than half of HCC patients are initial diagnosed at an early stage, treatable with curative options such as local ablation, surgical resection and liver transplantation [3, 4]. Patients with unresectable or metastatic HCC may benefit from chemotherapy and target therapy rather than operations [5, 6]. However, negative effects have been verified in four-fifths phase 3 trials testing molecular target therapies from 2007 to 2012 according to a recent research in HCC [7]. There is an imperative necessity to investigate biomarkers and reveal functional mechanism of these predicted targets for novel treatment strategies against this lethal disease.

Long noncoding RNAs (LncRNAs) are non-protein coding transcripts containing more than 200 nucleic acids [8]. They are categorized into five groups including sense LncRNAs, antisense LncRNAs, bidirectional LncRNAs, intronic LncRNAs and intergenic LncRNAs. A significant number of LncRNAs have been verified to be dysregulated in human malignancies and being part of the oncogenesis [9-12]. It's indicated that LncRNA could be a potential target for HCC treatment and should be further investigated in HCC [13-17]. Antisense ubiquitin carboxyl-terminal hydrolase L1 (antisense Uchl1) is a spliced antisense LncRNA of ubiquitin carboxyl-terminal hydrolase L1 (Uchl1), a gene

associated with neurodegenerative diseases [18] and human cancer [19]. In mus musculus, the function of antisense Uchl1 is under the control of stress signaling pathways, as mTORC1 inhibition by rapamycin leads to an increase in UCHL1 protein that is related to the shuttling of antisense Uchl1 RNA from the nucleus to the cytoplasm. Antisense Uchl1 RNA is subsequently required for the relation with the overlapping sense protein-coding mRNA to active polysomes translation. Recently, antisense Uchl1 was found to control *Uchl1* translation through an embedded SINEB2 repeat [20], which brings a clue that the antisense Uchl1 could be a potential target in UCHL1-deficient disease like Parkinson's disease and Alzheimer's disease.

UCHL1 antisense RNA 1 (UCHL1-AS1), a spliced antisense LncRNA of ubiquitin carboxyl-terminal hydrolase L1 (*UCHL1*), is the name used in human beings. Previous study has showed that UCHL1 is a protein related to neurodegenerative diseases and human cancer, which acts as a deubiquitinating enzyme, ubiquitin ligase or monoubiquitin stabilizer [18, 19, 21]. UCHL1 was first found to control intracellular protein degradation as a member of the ubiquitin proteasome pathway, maintaining ubiquitin balance by releasing ubiquitin from tandem conjugated ubiquitin monomers [22]. The oxidative inactivation of UCHL1 protein has been observed in neurodegenerative diseases, such as Parkinson's disease and Alzheimer's disease [23]. Moreover, UCHL1 is gradually observed to function in tumorigenesis and has been identified as a potential TSG, methylated in human cancers [24-32]. But the role of UCHL1 anti- or pro-tumor depends on different cancer types [33, 34]. Yu et al. have verified that UCHL1 is silenced and associated with promoter CpG hypermethylation in liver cancer and other digestive cancers like colon, gastric, and esophageal cancers [30]. They also showed that UCHL1 functions as a TSG to suppress tumor cell growth via suppressing proliferation, inducing cellular apoptosis, and stabilizing p53 from the de-ubiquitination pathway.

Sense and antisense Uchl1 expression in mouse and human tissues showed similar patterns [20]. UCHL1 has been justified as a tumor suppressor in HCC [30]. However, the role of UCHL1-AS1 in HCC and the relationship between them remain unclear. In this study, we detected the expression of UCHL1-AS1 in HCC tissues and cell lines, and further assessed the correlation of UCHL1-AS1 level with clinicopathological features, as well as disease specific survival (DSS).

Materials and methods

Patients and tissue samples

Seventy-two fresh samples, which contained adjacent noncancerous liver tissues of histologically confirmed HCC patients, were collected from October, 2010 to January, 2014 in The First Affiliated Hospital of Guangxi Medical University (Guangxi, China). Clinicopathological features were analyzed, including age, gender, alpha-fetoprotein (AFP), hepatitis history, Hepatitis B Virus (HBV), tumor size, tumor number, histological grade, tumor node metastasis (TNM) stage, portal vein tumor thrombosis (PVTT), cirrhosis and distant metastasis. Post-surgery follow-up was performed till May 1, 2015. All patients had completed follow-up information. The DSS was defined as the length of time between the surgery and death. The study was approved by the Research Ethics Committee of the First Affiliated Hospital of Guangxi Medical University, China. Informed written consents were obtained from all patients who participated in this study.

Cell lines

Human normal liver cell line L-02, HCC cell lines HepG2, Hep3B, Bel-7402, Bel-7404 and Bel-7721 were obtained from Shanghai Institute of Cell Biology (Shanghai, China) and cultured in Dulbecco's modified Eagle's medium (DMEM) with high glucose (Wisent, Nanjing, China) supplemented with 10% fetal bovine serum (Si-Ji-Qing, Hangzhou, China) in a humidified incubator with 5% CO_2 at 37°C.

RNA isolation and RT-qPCR

Total RNA was isolated from fresh tissues or cells using a TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and reverse transcribed into cDNA using Thermo Scientific RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA,USA) according to the manufacturer's instructions. RT-qPCR was performed in a Light Cycler 480 (Roche, Basel, Switzerland) with a SYBR Green Premix Ex Taq (Roche). ß-actin mRNA was used as an internal control. The primers

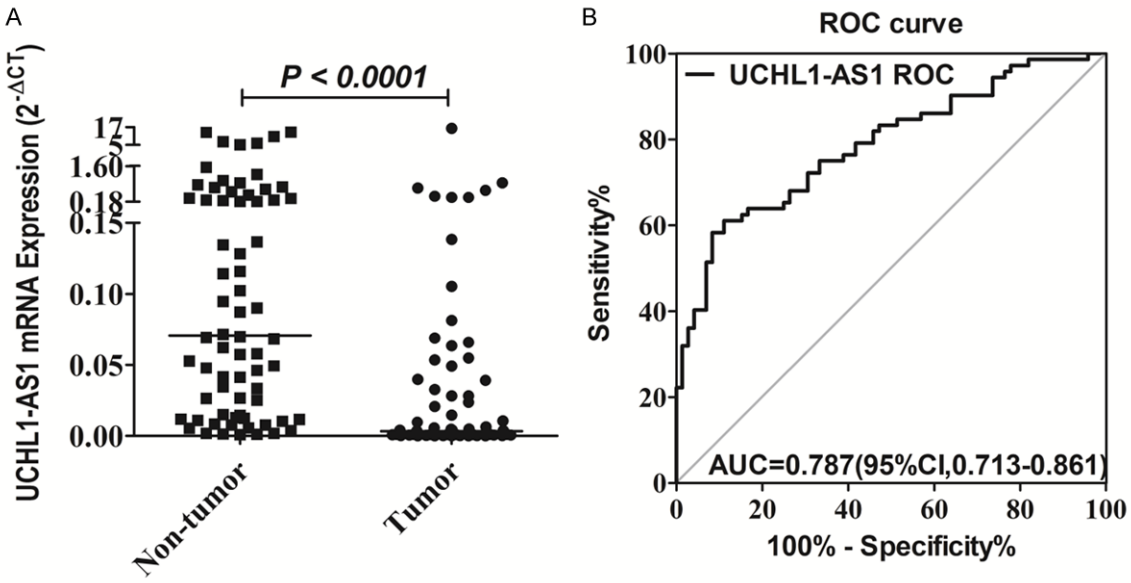

Figure 1. UCHL1 antisense RNA 1 (UCHL1-AS1) expression and its diagnostic value in hepatocellular carcinoma (HCC). The expression level of UCHL1-AS1 in 72 cases was measured in HCC and paired noncancerous liver tissues (A, *P*<0.0001). ROC curve of UCHL1-AS1 level in HCC (B). The area under curve (AUC) of UCHL1-AS1 was 0.787 (95% CI 0.713 to 0.861, *P*<0.0001).

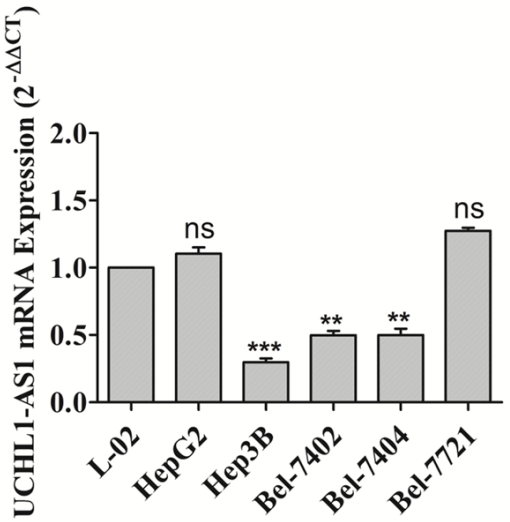

Figure 2. Expression of UCHL1 antisense RNA 1 (UCHL1-AS1) in five hepatocellular carcinoma (HCC) cell lines and a normal liver cell line L-02. A significant lower expression of UCHL1-AS1 was found in Hep3B, Bel-7402 and Bel-7404 than in L-02 (ns: no significance, **P*<0.05, ***P*<0.01, ****P*<0.001).

were used: UCHL1, the forward primer 5'-CCATACAGGCAGCCCATGA-3' and reverse primer 5'-GCCATCCACGTTGTTAAACAGAATA-3'; UCHL1-AS1, the forward primer 5'-TGGAGACGGGATTTGTTGGT-3' and reverse primer 5'-GGGATGGTGAAAGGATGGGTT-3'; ß-actin, the forward primer 5'-GCACCACACCTTCTACAATGAGC-3' and reverse primer 5'-GGAT-AGCACAGCCTGGATAGCAAC-3'.

Statistics

All experiments were performed independently at least three times and the data were statistically analyzed with SPSS 20.0 software (Chicago, IL, USA). Data were presented as means ± standard deviations (SD) and analyzed by Mann-Whitney-test, Chi-square test, Student t-test, or one-way ANOVA. Spearman correlation was applied to study the association of gene expression with clinicopathological parameters. DSS rates were calculated by Kaplan-Meier method with the log-rank test. Univariate analysis was used on the basis of Cox proportional hazards mode. A *p* value ≤0.05 was considered statistically significant.

Results

Down-regulation of UCHL1-AS1 expression in HCC tissues

We first measured the expression level of UCHL1-AS1 in 72 cases. The expression in HCC was obviously lower than paired noncancerous liver tissues (*P*<0.0001; **Figure 1A**). In ROC curve, the area under curve (AUC) was 0.787 (*P*<0.0001) which showed diagnostic value of

Table 1. Correlation of UCHL1-AS1 expression with clinicopathological features in HCC patients

Characteristics	Numbers of cases	Low	High	*P* value*	R	*P* value#
Age (years)						
≤50	45	26	19	0.902	-0.015	0.903
>50	27	16	11			
Gender						
Female	7	4	3	1.000	-0.008	0.947
Male	65	38	27			
AFP (ng/ml)						
≤400	32	21	11	0.262	0.132	0.268
>400	40	21	19			
Hepatitis History						
Absent	28	10	18	0.002	-0.366	0.002
Present	44	32	12			
HBV						
-	16	7	9	0.180	-0.158	0.185
+	56	35	21			
Tumor size						
≤5	26	15	11	0.934	-0.010	0.935
>5	46	27	19			
Tumor number						
Single	46	28	18	0.561	0.068	0.568
Multiple	26	14	12			
Histological grade						
Well+moderate	50	30	20	0.665	0.051	0.671
Poor	22	12	10			
TNM						
I-II	17	9	8	0.606	-0.061	0.612
III-IV	55	33	22			
PVTT						
Absent	40	18	22	0.010	-0.302	0.010
Present	32	24	8			
Cirrhosis						
Absent	26	14	12	0.561	-0.068	0.568
Present	46	28	18			
Distant Metastasis						
Absent	38	18	20	0.046	-0.235	0.047
Present	34	24	10			

*The data was analyzed by Chi-square test, Continuity correction test or Fisher exact test; #The data was obtained by Spearman correlation. AFP, α-fetoprotein; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; PVTT, portal vein tumor thrombus; UCHL1-AS1, antisense ubiquitin carboxyl-terminal hydrolase L1.

UCHL1-AS1 level in HCC. The cutoff value was 0.005 at which the sensitivity and specificity were 58.33% (*P*<0.0001; 46.11% to 69.85%) and 91.67% (*P*<0.0001; 82.74% to 96.88%), respectively (**Figure 1B**).

Expression of UCHL1-AS1 in HCC cell lines and L-O2 cells

UCHL1-AS1 expression was detected in five HCC cell lines and human normal liver cell line L-O2. A significant lower expression of UCHL1-AS1 was found in Hep3B, Bel-7402 and Bel-7404 than L-02 (*P*<0.05). However, there was no significant difference of UCHL1-AS1 for HepG2 and Bel-7721 when compared with L-02 (*P*>0.05, **Figure 2**).

Correlation of UCHL1-AS1 expression with clinicopathological features in HCC patients

To investigate the correlation of UCHL1-AS1 expression with clinicopathological parameters, clinical and laboratory data of 72 patients was collected (**Table 1**). The cutoff value to distinguish low or high expression of UCHL1-AS1 in HCC tissues was acquired from ROC curve. The lower expression of UCHL1-AS1 was observed in group of hepatitis history, PVTT and distant metastasis than in the corresponding groups (all *P*<0.05, **Table 1**; **Figure 3A-C**). In addition, UCHL1-AS1 expression level showed negative correlation with hepatitis history (r=-0.336, *P*=0.002), PVTT (r=-0.302, *P*=0.010) and distant metastasis (r=-0.235, *P*=0.047) analyzed by Spearman coefficient of correlation. However, no significant correlation was identified between UCHL1-AS1 expression and other clinicopathological parameters, such as age, gender, AFP, HBV, tumor size, tumor number, histological grade, TNM stage and cirrhosis (all *P*>0.05, **Table 1**).

Correlation of UCHL1-AS1 expression with survival in HCC patients

The mean survival time was 12.765 ± 13.862 months for all patients followed-up. The longest time of follow-up was 58.9 months and the shortest was 2.9 months. Patients with low UCHL1-AS1 expression showed worse prognosis as compared to those with high expression while there was no statistic significance (high UCHL1-AS1 group: 13.487 ± 15.522 and low group:

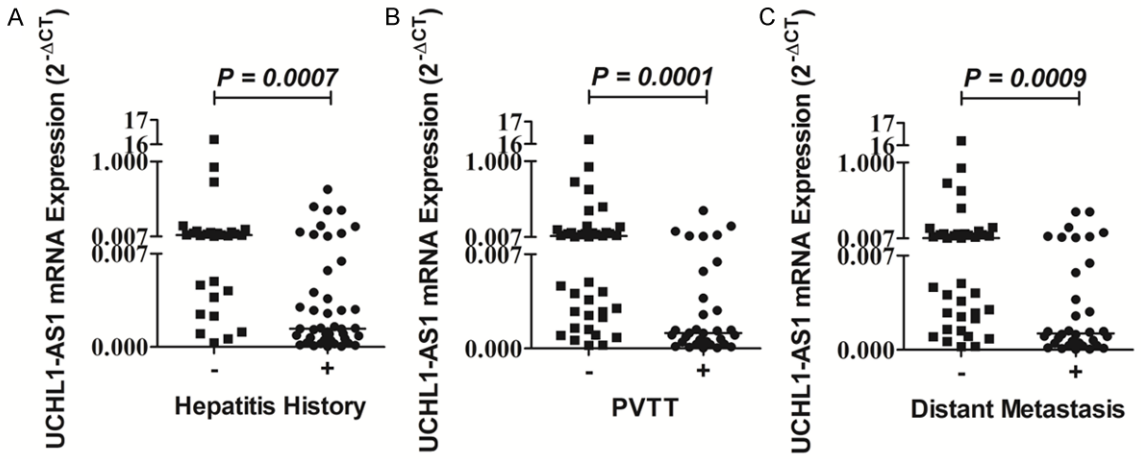

Figure 3. Relationship between UCHL1 antisense RNA 1 (UCHL1-AS1) expression and clinicopathological features in hepatocellular carcinoma (HCC). UCHL1-AS1 expression and Hepatitis History (A), portal vein tumor thrombus (PVTT) (B), Distant Metastasis (C).

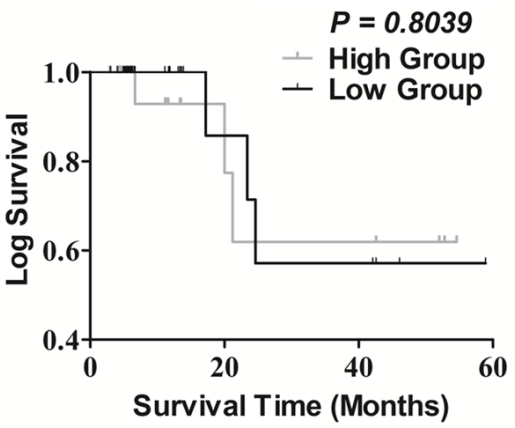

Figure 4. Kaplan-Meyer Curves of UCHL1 antisense RNA 1 (UCHL1-AS1) expression in hepatocellular carcinoma (HCC).

12.250 ± 12.716; *P*>0.05, **Figure 4**). Univariate analysis of DSS revealed that the relative level of UCHL1-AS1 expression and all the clinicopathological features were not statistically significant prognosis factors (all *P*>0.05, **Table 2**).

Discussion

LncRNAs are noncoding RNAs containing more than 200 nucleic acids [8], divided into sense, antisense, bidirectional, intronic and intergenic LncRNAs [35]. Antisense LncRNA is one subtype of five groups in LncRNAs. In mus musculus, antisense Uchl1 is a spliced antisense LncRNA of Uchl1. It promotes Uchl1 translation via an embedded SINEB2 repeat [20]. In human beings, *UCHL1* is a spliced antisense mRNA of LncRNA UCHL1-AS1. Its protein was reported to act as a tumor suppressor in various cancers including HCC [19, 30]. Our hypothesis was that UCHL1-AS1 may also influence UCHL1 protein level in tumor.

In our current study, we found that UCHL1-AS1 expression was lower in HCC tissues than adjacent noncancerous liver tissues. As UCHL1 protein functions as a tumor suppressor in HCC, our result about UCHL1-AS1 expression is in accord with our previous hypothesis that UCHL1-AS1 may be down regulated in HCC. Moreover, significant correlations were found between UCHL1-AS1 expression and three clinicopathological features, including hepatitis history, PVTT and distant metastasis. However, no association of UCHL1-AS1 expression was found with age, gender, AFP, HBV, tumor size, tumor number, histological grade, TNM stage and cirrhosis. Further large-scale studies will be needed to confirm our findings. Also, advanced studies will be needed to confirm whether UCHL1-AS1 is a potential factor in HCC metastasis or not.

On the other hand, we further studied the effect of UCHL1-AS1 expression on disease survival. No significant correlation of UCHL1-AS1 expression with patient survival was revealed by Kaplan-Meier analysis or univariate analysis of DSS. There lacked enough evidence to verify that patients with low UCHL1-AS1 expression had a worse disease free survival than those

Table 2. Univariate Analysis of Clinicopathological Features for Disease-specific Survival in HCC patients

Characteristics	Numbers of cases	HR	95% CI	*P* value
Age (years)				
≤50	45	1	0.453-11.807	0.314
>50	27	2.312		
Gender				
Female	7	1	0.082-2.545	0.372
Male	65	0.458		
AFP (ng/ml)				
≤400	32	1	0.445-13.721	0.301
>400	40	2.471		
Hepatitis History				
Absent	28	1	0.252-7.547	0.711
Present	44	1.379		
HBV				
-	16	1	4.467E-8-1.948E10	0.744
+	56	29.499		
Tumor size				
≤5	26	1	0.219-6.601	0.831
>5	46	1.203		
Tumor number				
Single	46	1	0.137-4.119	0.742
Multiple	26	0.751		
Histological grade				
Well + moderate	50	1	0.338-8.349	0.526
Poor	22	1.680		
TNM				
I-II	17	1	0.163-12.192	0.756
III-IV	55	1.408		
PVTT				
Absent	40	1	0.468-11.697	0.301
Present	32	2.339		
Cirrhosis				
Absent	26	1	0.233-6.996	0.779
Present	46	1.276		
Distant Metastasis				
Absent	38	1	0.834-25.329	0.080
Present	34	4.597		
UCHL1-AS1 Expression				
Low	42	1	0.246-6.086	0.804
High	30	1.225		

AFP, α-fetoprotein; CI, confidence interval; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HR, hazard ratio; PVTT, portal vein tumor thrombus; UCHL1-AS1, antisense ubiquitin carboxyl-terminal hydrolase L1.

with high UCHL1-AS1 expression in HCC. Longer follow-up time and larger patient size are eagerly required to further investigate the correlation of UCHL1-AS1 level with patient survival of HCC. This is the primary study to investigate the role of UCHL1-AS1 in human HCC. Low level of UCHL1-AS1 may influence the development and progression of HCC. However, the molecular mechanism involved is unclear. It was revealed that antisense Uchl1 expression inside cell nuclear was related to UCHL1 protein level in mus musculus, and this regulation on Uchl1 translation could be induced by mTOR inhibitor [20]. Although UCHL1 in HCC is suppressed as well as UCHL1-AS1, the association between them remains unclarified. And mechanism of UCHL1-AS1 in human tumor may differ from that in mus musculus tissue. The specific target genes and signal-pathway controlled by UCHL1-AS1 in HCC require detailed investigation in the future. The mechanism involved in UCHL1-AS1 regulation in HCC still remains to be elucidated.

In summary, our results indicated that UCHL1-AS1 RNA level was significantly lower in HCC tissues and HCC cell line Hep3B, Bel-7402 and Bel-7404. The low expression of UCHL1-AS1 was associated with positive hepatitis history, PVTT and distance metastasis. In addition, the current data was not sufficient to assess the role of UCHL1-AS1 in prognosis prediction due to a short follow-up duration, which remained to be investigated in the following research. Overall, these findings suggest that UCHL1-AS1 might be a potential target to predict the carcinogenic progression of HCC. Further research in vitro and in vivo is under performance by our group to investigate the effect of UCHL1-AS1 on HCC.

Acknowledgements

This work was supported by grants from the National Natural Scientific Research Fund

(81560386, 81560489), Guangxi provincial Science and Technology Development Projects (#1298003-2-5 and #10124001A-1), and Guangxi provincial Talent Projects for Graduate Students (#YCSZ2014099).

Disclosure of conflict of interest

None.

Address correspondence to: Drs. Zhenbo Feng and Gang Chen, Department of Pathology, The First Affiliated Hospital of Guangxi Medical University, 6 Shuang Yong Road, Nanning 530021, Guangxi, China. Tel: +86-771-5356534; E-mail: guanghu1963@126.com (ZBF); chen_gang_triones@163.com (GC)

References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.

[2] Wang R, Chen XZ, Zhang MG, Tang L and Wu H. Incidence and mortality of liver cancer in mainland China: changes in first decade of 21st century. Hepatogastroenterology 2015; 62: 118-121.

[3] Chu KK and Cheung TT. Update in management of hepatocellular carcinoma in Eastern population. World J Hepatol 2015; 7: 1562-1571.

[4] Wang CH, Wey KC, Mo LR, Chang KK, Lin RC and Kuo JJ. Current trends and recent advances in diagnosis, therapy, and prevention of hepatocellular carcinoma. Asian Pac J Cancer Prev 2015; 16: 3595-3604.

[5] Deng GL, Zeng S and Shen H. Chemotherapy and target therapy for hepatocellular carcinoma: New advances and challenges. World J Hepatol 2015; 7: 787-798.

[6] Gadaleta-Caldarola G, Divella R, Mazzocca A, Infusino S, Ferraro E, Filippelli G, Daniele A, Sabba C, Abbate I and Brandi M. Sorafenib: the gold standard therapy in advanced hepatocellular carcinoma and beyond. Future Oncol 2015; 11: 2263-2266.

[7] Villanueva A. Rethinking future development of molecular therapies in hepatocellular carcinoma: a bottom-up approach. J Hepatol 2013; 59: 392-395.

[8] Kapranov P, Willingham AT and Gingeras TR. Genome-wide transcription and the implications for genomic organization. Nat Rev Genet 2007; 8: 413-423.

[9] Lau E. Non-coding RNA: Zooming in on lncRNA functions. Nat Rev Genet 2014; 15: 574-575.

[10] Ponting CP, Oliver PL and Reik W. Evolution and functions of long noncoding RNAs. Cell 2009; 136: 629-641.

[11] Ponjavic J, Ponting CP and Lunter G. Functionality or transcriptional noise? Evidence for selection within long noncoding RNAs. Genome Res 2007; 17: 556-565.

[12] Yang G, Lu X and Yuan L. LncRNA: a link between RNA and cancer. Biochim Biophys Acta 2014; 1839: 1097-1109.

[13] Zhang L, Yang F, Yuan JH, Yuan SX, Zhou WP, Huo XS, Xu D, Bi HS, Wang F and Sun SH. Epigenetic activation of the MiR-200 family contributes to H19-mediated metastasis suppression in hepatocellular carcinoma. Carcinogenesis 2013; 34: 577-586.

[14] Huang JF, Guo YJ, Zhao CX, Yuan SX, Wang Y, Tang GN, Zhou WP and Sun SH. Hepatitis B virus X protein (HBx)-related long noncoding RNA (lncRNA) down-regulated expression by HBx (Dreh) inhibits hepatocellular carcinoma metastasis by targeting the intermediate filament protein vimentin. Hepatology 2013; 57: 1882-1892.

[15] Du Y, Kong G, You X, Zhang S, Zhang T, Gao Y, Ye L and Zhang X. Elevation of highly up-regulated in liver cancer (HULC) by hepatitis B virus X protein promotes hepatoma cell proliferation via down-regulating p18. J Biol Chem 2012; 287: 26302-26311.

[16] Yang Z, Zhou L, Wu LM, Lai MC, Xie HY, Zhang F and Zheng SS. Overexpression of long noncoding RNA HOTAIR predicts tumor recurrence in hepatocellular carcinoma patients following liver transplantation. Ann Surg Oncol 2011; 18: 1243-1250.

[17] Takahashi K, Yan I, Haga H and Patel T. Long noncoding RNA in liver diseases. Hepatology 2014; 60: 744-753.

[18] Setsuie R and Wada K. The functions of UCH-L1 and its relation to neurodegenerative diseases. Neurochem Int 2007; 51: 105-111.

[19] Jara JH, Frank DD and Ozdinler PH. Could dysregulation of UPS be a common underlying mechanism for cancer and neurodegeneration? Lessons from UCHL1. Cell Biochem Biophys 2013; 67: 45-53.

[20] Carrieri C, Cimatti L, Biagioli M, Beugnet A, Zucchelli S, Fedele S, Pesce E, Ferrer I, Collavin L, Santoro C, Forrest AR, Carninci P, Biffo S, Stupka E and Gustincich S. Long non-coding antisense RNA controls Uchl1 translation through an embedded SINEB2 repeat. Nature 2012; 491: 454-457.

[21] Liu Y, Fallon L, Lashuel HA, Liu Z and Lansbury PT Jr. The UCH-L1 gene encodes two opposing enzymatic activities that affect alpha-synuclein degradation and Parkinson's disease susceptibility. Cell 2002; 111: 209-218.

[22] Osaka H, Wang YL, Takada K, Takizawa S, Setsuie R, Li H, Sato Y, Nishikawa K, Sun YJ, Sakurai M, Harada T, Hara Y, Kimura I, Chiba S, Namikawa K, Kiyama H, Noda M, Aoki S and

Wada K. Ubiquitin carboxy-terminal hydrolase L1 binds to and stabilizes monoubiquitin in neuron. Hum Mol Genet 2003; 12: 1945-1958.

[23] Choi J, Levey AI, Weintraub ST, Rees HD, Gearing M, Chin LS and Li L. Oxidative modifications and down-regulation of ubiquitin carboxyl-terminal hydrolase L1 associated with idiopathic Parkinson's and Alzheimer's diseases. J Biol Chem 2004; 279: 13256-13264.

[24] Sato N, Fukushima N, Maitra A, Matsubayashi H, Yeo CJ, Cameron JL, Hruban RH and Goggins M. Discovery of novel targets for aberrant methylation in pancreatic carcinoma using high-throughput microarrays. Cancer Res 2003; 63: 3735-3742.

[25] Mandelker DL, Yamashita K, Tokumaru Y, Mimori K, Howard DL, Tanaka Y, Carvalho AL, Jiang WW, Park HL, Kim MS, Osada M, Mori M and Sidransky D. PGP9.5 promoter methylation is an independent prognostic factor for esophageal squamous cell carcinoma. Cancer Res 2005; 65: 4963-4968.

[26] Yamashita K, Park HL, Kim MS, Osada M, Tokumaru Y, Inoue H, Mori M and Sidransky D. PGP9.5 methylation in diffuse-type gastric cancer. Cancer Res 2006; 66: 3921-3927.

[27] Lee YM, Lee JY, Kim MJ, Bae HI, Park JY, Kim SG and Kim DS. Hypomethylation of the protein gene product 9.5 promoter region in gallbladder cancer and its relationship with clinicopathological features. Cancer Sci 2006; 97: 1205-1210.

[28] Okochi-Takada E, Nakazawa K, Wakabayashi M, Mori A, Ichimura S, Yasugi T and Ushijima T. Silencing of the UCHL1 gene in human colorectal and ovarian cancers. Int J Cancer 2006; 119: 1338-1344.

[29] Kagara I, Enokida H, Kawakami K, Matsuda R, Toki K, Nishimura H, Chiyomaru T, Tatarano S, Itesako T, Kawamoto K, Nishiyama K, Seki N and Nakagawa M. CpG hypermethylation of the UCHL1 gene promoter is associated with pathogenesis and poor prognosis in renal cell carcinoma. J Urol 2008; 180: 343-351.

[30] Yu J, Tao Q, Cheung KF, Jin H, Poon FF, Wang X, Li H, Cheng YY, Rocken C, Ebert MP, Chan AT and Sung JJ. Epigenetic identification of ubiquitin carboxyl-terminal hydrolase L1 as a functional tumor suppressor and biomarker for hepatocellular carcinoma and other digestive tumors. Hepatology 2008; 48: 508-518.

[31] Yan WJ, Guo MZ and Yang YS. [The role of hypermethylation in promoter region of ubiquitin carboxyl-terminal hydrolase L1 in human esophageal cancer]. Zhonghua Nei Ke Za Zhi 2012; 51: 390-393.

[32] Jin C, Yu W, Lou X, Zhou F, Han X, Zhao N and Lin B. UCHL1 Is a Putative Tumor Suppressor in Ovarian Cancer Cells and Contributes to Cisplatin Resistance. J Cancer 2013; 4: 662-670.

[33] Tezel E, Hibi K, Nagasaka T and Nakao A. PGP9.5 as a prognostic factor in pancreatic cancer. Clin Cancer Res 2000; 6: 4764-4767.

[34] Takase T, Hibi K, Yamazaki T, Nakayama H, Taguchi M, Kasai Y, Ito K, Akiyama S, Nagasaka T and Nakao A. PGP9.5 overexpression in esophageal squamous cell carcinoma. Hepatogastroenterology 2003; 50: 1278-1280.

[35] Kung JT, Colognori D and Lee JT. Long noncoding RNAs: past, present, and future. Genetics 2013; 193: 651-669.