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Correlation between expression levels of lncRNA UCA1 and miR-18a with prognosis of hepatocellular cancer

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Abstract. – OBJECTIVE: To uncover the prognostic potentials of long non-coding RNA (lncRNA) UCA1 and miR-18a in hepatocellular cancer (HCC).

PATIENTS AND METHODS: Expression levels of UCA1 and microRNA-18a (miR-18a) in HCC tissues and adjacent normal ones harvested from 55 HCC patients were determined by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). Clinical data of HCC patients were recorded, including pathological grading, tumor staging, intrahepatic metastasis, serum level of α-fetoprotein (AFP), tumor size, tumor number, recurrence, etc. Based on the median levels of UCA1 and miR-18a, enrolled HCC patients were classified into high-level and low-level group. Potential correlation between expression levels of UCA1 and miR-18a with survival of HCC patients was analyzed. The 4-year follow-up data of HCC patients were collected for analyzing factors that may influence prognosis in HCC patients by the Cox regression model.

RESULTS: UCA1 was upregulated and miR-18a was downregulated in HCC tissues. HCC patients with stage III-IV, tumor size ≥5 cm or multiple tumors expressed high level of UCA1. Besides, HCC patients with stage I-II, non-intrahepatic metastasis or primarily diagnosed expressed a relatively low level of miR-18a. High-level UCA1 and low-level miR-18a predicted worse prognosis in HCC patients. Cox regression analysis revealed that tumor node metastasis (TNM) staging, intrahepatic metastases, postoperative recurrences, and UCA1 were risk factors for HCC, while miR-18a was the protective factor.

CONCLUSIONS: LncRNA UCA1 is upregulated and miR-18a is downregulated in HCC tissues. High-level UCA1 and low-level miR-18a are closely linked to poor prognosis in HCC.

Key Words:
HCC, UCA1, MiR-18a, Prognosis.

Introduction

Globally, hepatocellular cancer (HCC) is a highly prevalent malignancy that threats human health and lives[1]. Diagnosis and intervention as early as possible remarkably prolong the survival of HCC patients. Nevertheless, the detective rate of HCC in the early stage is relatively low[2]. With the advances achieved in medical technologies, therapeutic approaches for HCC have made great strides. Currently, surgical procedures, liver transplantation, and percutaneous intrahepatic treatment are major approaches for primary HCC treatment. Hepatectomy, however, is still the preferred therapy for HCC throughout the world even though its indications are limited[3,4]. High rates of postoperative recurrence and mortality seriously restrict the clinical outcomes of HCC patients[5]. It is necessary to uncover potential mechanisms underlying the recurrent and metastatic HCC. Meanwhile, effective tumor markers for HCC are urgently required.

Long non-coding RNAs (lncRNAs) participate in the occurrence and progression of human diseases as transcription regulators[6,7]. LncRNA UCA1 is a tumor-related RNA, serving as a proto-oncogene in many types of malignancies. UCA1 exerts a certain potential for tumor diagnosis and treatment by mediating tumor cell behaviors[8]. Relevant studies[9,10] have demonstrated

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the critical functions of UCA1 in tumorigenesis, tumor progression, and prognosis.

MicroRNAs (miRNAs) degrade or inhibit translation of target mRNAs by binding 3′-untranslated region (3′-UTR) of mRNAs. MiR-18a is one of the six mature miRNAs produced by the miR-17-92 cluster[11]. Unlike other members in the miR-17-92 family, miR-18a is able to arrest cell cycle progression in G1 phase and inhibits proliferation in pancreatic cancer stem cells[12]. Zheng et al[13] pointed out that overexpression of miR-18a markedly suppresses *in vitro* proliferation and tumor growth of gastric cancer. MiR-18a is closely linked to drug-resistance in HCC[14].

LncRNA-miRNA interaction is able to influence the occurrence and progression of tumor diseases[15]. It is reported that lncRNA UCA1 exerts an anti-desensitizing effect on trastuzumab in breast cancer cells by downregulating miR-18a level[16]. This work mainly explored the correlation between UCA1 and miR-18a levels with the prognosis of HCC patients.

Patients and Methods

Baseline Characteristics of HCC Patients

A total of 55 HCC patients undergoing surgery or tumor biopsy in The Fifth Affiliated Hospital of Guangxi Medical University from April 2014 to April 2016, all were enrolled, including 31 male and 24 female HCC patients with 39-69 years (50.7±7. 31 years). Their clinical data was completely recorded. None of them received preoperative chemotherapy, radiotherapy, and immunotherapy. They were postoperatively diagnosed as HCC. Paracancerous tissues were harvested 5 cm away from the border of HCC tissues and pathologically diagnosed as normal liver tissues. Tissue samples were immediately placed in liquid nitrogen during the surgery and preserved at –80°C. According to the Edmondson grading, 20 cases were stage I-II and 35 were stage III-IV. Based on tumor node metastasis (TNM) staging, 14 cases were stage I-II, and 41 were stage III-IV. There were 14 cases of primary HCC and 41 of recurrent cases. Patients and their families in this study have been fully informed. This investigation was approved by the Ethics Committee of The Fifth Affiliated Hospital of Guangxi Medical University.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Total RNA and small RNA were extracted using the TRIzol method and Star Spin Small-RNA Kit (Invitrogen, Carlsbad, CA, USA), respectively. They were reversely transcribed into complementary deoxyribose nucleic acid (cDNA) using the LnRcute LncRNAs cDNA kit and miRNA First Strand cDNA, respectively. SYBR Green method (TaKaRa, Otsu, Shiga, Japan) was applied for determining relative levels of UCA1 [glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the internal reference] and miR-18a (U6 as the internal reference). Primer sequences were listed as follows: UCA1, forward: 5′-CTCTCCATTGGGTTCACCATTC-3′, reverse: 5′-GCGGCAGGTCTTAAGAGATGAG-3′; MiR-18a, forward: 5′-GATAGCAGCACAGAAATATTGGC-3′, reverse: 5′-GTGCAGGGTCCGAGG-3′; GAPDH, forward: 5′-TGAAGGTCGGAGTCAACGG-3′, reverse: 5′-CCTGGAAGATGGTGATGCG-3′; U6, forward: 5′-GATTAGAACCGTCGGTAACGGAA-3′, reverse: 5′-AGCGATCTCGTTGGCCTTTCTACC-3′.

Postoperative Follow-Up

From April 2014 to April 2016, all enrolled HCC patients were postoperatively followed up by telephone or visit once every three months in the first year, and every six months since after. Survival statues, including the cause of death and the date of death were recorded. All patients were successfully followed up. During the period of follow-up, the deaths were counted by censored data, and the survivors were followed up for 4 years.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 (IBM, Armonk, NY, USA) was used for data analysis. Measurement data were expressed as mean ± standard deviation ($\bar{x}\pm SD$) and analyzed by the independent *t*-test. Counting data were analyzed by the χ^2-test. Pearson correlation analysis was conducted to compare the relationship between the two genes. Kaplan-Meier method was introduced for survival analysis, followed by Log-rank test for comparing differences between two curves. To validate factors influencing survival in HCC, multivariate Cox regression was conducted. $p<0.05$ considered the difference was statistically significant.

Results

UCA1 Was Upregulated and MiR-18a Was Downregulated in HCC Tissues

Expression levels of UCA1 and miR-18a in 55 matched HCC and paracancerous tissues were de-

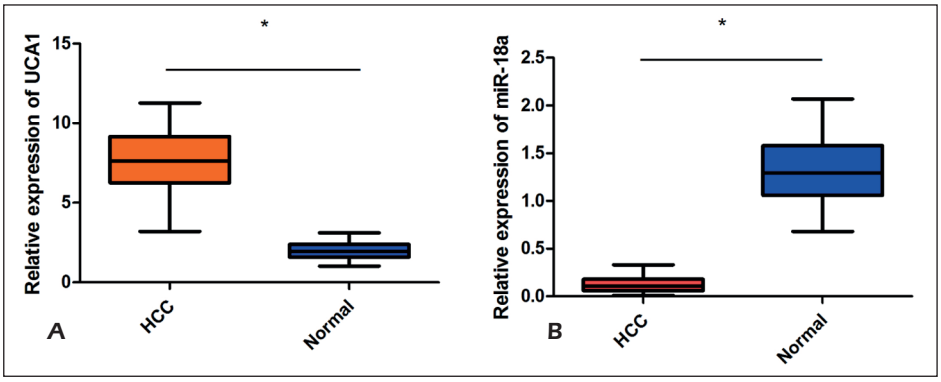

Figure 1. UCA1 was upregulated and miR-18a was downregulated in HCC tissues. **A-B**, UCA1 (**A**) and miR-18a (**B**) levels in HCC tissues and paracancerous tissues.

termined. Compared with adjacent normal tissues, UCA1 was upregulated (Figure 1A) and miR-18a was downregulated (Figure 1B) in HCC tissues.

Correlation Between Expression Levels of UCA1 and MiR-18a in HCC

Pearson correlation analysis uncovered a negative correlation between expression levels of UCA1 and miR-18a in HCC tissues (r=-0.823, p<0.001, Figure 2).

Correlation Between UCA1 and MiR-18a with Clinical Data of HCC Patients

Based on the median level of UCA1 (7.75±1.89) in 55 HCC patients, they were classified into high-level (n=36) and low-level (n=19) group. As the data revealed, the ratios of HCC patients with stage III-IV, tumor size ≥5 cm or multiple tumors were markedly higher in high-level group relative to those in low-level group (Table I). Similarly, HCC patients were classified into high-level (n=26) and low-level (n=29) group according to the median level of miR-18a (0.13±0.075). The ratios of HCC patients with stage I-II, non-intrahepatic metastasis or primarily diagnosed were higher in high-level group than those in low-level group (Table I).

Regulatory Effects of UCA1 and MiR-18a on Survival of HCC Patients

We conducted 4-year follow-up for HCC patients postoperatively. By depicting survival curves, it is shown that overall survival was shorter in HCC patients expressing high-level UCA1 (HR=5.758, p=0.0164, Figure 3A). Conversely, overall survival was better in HCC patients expressing high-level miR-18a (HR=8.051, p=0.0045, Figure 3B). The above data demonstrated that UCA1 was unfavorable and miR-18a was favorable to the prognosis in HCC patients.

Factors Influencing Survival in HCC

To further validate factors influencing survival in HCC, the multivariate Cox regression was conducted. The results showed that the mortality risk of TNM stage III-IV was 2.474 times that of stage I-II; mortality risk of intrahepatic metastasis was 1.673 times that of non-metastasis; mortality risk of recurrent HCC was 2.671 times that of primary HCC; mortality risk of high-level UCA1 was 3.711 times that of low-level UCA1; and mortality risk of high-level miR-18a was 0.525 times that of low-level miR-18a (Table II). It is suggested that TNM staging, intrahepatic metastases, postoperative recurrences, and UCA1 were risk factors for HCC, while miR-18a was the protective factor.

Discussion

In recent years, early diagnosis and therapeutic technologies for HCC have been advanced, including molecular target therapy, gene therapy, and immunotherapy[17]. Nevertheless, the overall survival of HCC is relatively low even though

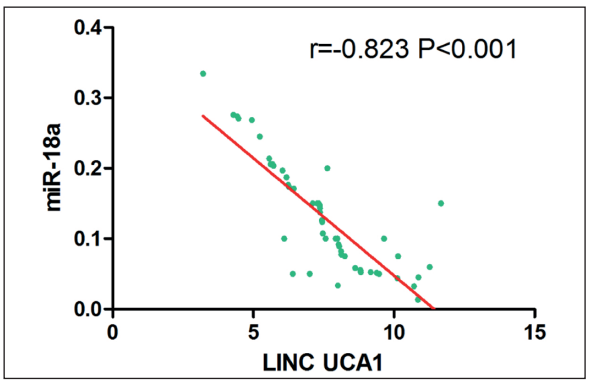

Figure 2. A negative correlation between expression levels of UCA1 and miR-18a in HCC tissues (r=-0.823, p<0.001).

Table I. Correlation between UCA1 and miR-18a with clinical data of HCC patients.

Variable	n	UCA1		p	MiR-18a		p
		High level (n=36)	Low level (n=19)		High level (n=26)	Low level (n=29)	
Sex							
Male	31	20	11	0.868	17	14	0.201
Female	24	16	8		9	15	
Age							
<55	26	16	10	0.562	10	13	0.633
≥55	29	20	9		16	16	
Edmondson grade							
I-II	20	10	10	0.068	12	8	0.153
III-IV	35	26	9		14	21	
TNM stage							
I-II	14	3	11	<0.001	18	8	0.002
III-IV	41	33	8		8	21	
Intrahepatic transfer							
Yes	26	16	10	0.563	8	18	0.020
No	29	20	9		18	11	
AFP							
<20 ng/mL	28	12	6	0.895	13	15	0.898
≥20 ng/mL	27	24	13		13	14	
Tumor size							
<5 cm	23	11	12	0.020	12	8	0.153
≥5 cm	32	25	7		14	21	
Number of tumors							
Single	24	10	14	0.001	14	10	0.148
Multiple	31	26	5		12	19	
Postoperative recurrence							
Yes	41	32	9	0.001	3	11	0.025
No	14	4	10		23	18	

AFP=α-fetoprotein

anti-tumor treatments for HCC are improved[18,19]. It is necessary to uncover effective and sensitive hallmarks for diagnosis and prognosis of HCC.

LncRNA UCA1 was initially discovered in bladder transitional cell carcinoma[20]. UCA1 is found to be upregulated in many types of tumor diseases, and its level is related to tumor severity and prognosis[21]. Hu et al[22] analyzed lncRNA microarray and pointed out that UCA1 is highly expressed in HCC tissues relative to paracancerous ones. Consistently,

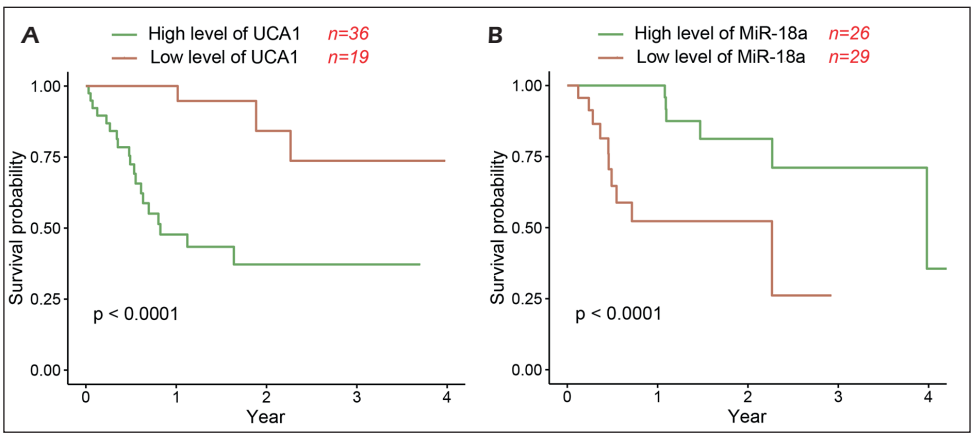

Figure 3. Regulatory effects of UCA1 and miR-18a on survival of HCC patients. **A-B**, Kaplan-Meier curves in HCC patients expressing high or low levels of UCA1 (**A**) or miR-18a (**B**).

Table II. Cox regression analysis on factors influencing survival in HCC.

Variable	HR (95%CI)	p
TNM staging	2.474 (1.309-3.946)	0.028
Intrahepatic metastases	1.673 (1.128-3.263)	0.032
Postoperative recurrence	2.671 (1.784-4.183)	0.008
UCA1	3.711 (1.523-5.178)	<0.001
miR-18a	0.525 (0.297-0.931)	0.035

TNM staging compared with those in stage I-II; Intrahepatic metastases compared with those non-metastases; Postoperative recurrence compared with those with primary HCC; UCA1 compared with those in low-level group; MiR-18a compared with those in low-level group. HR=hazard ratios, CI=confidence interval.

our findings uncovered the upregulated UCA1 in HCC tissues. Furthermore, UCA1 level was closely linked to TNM staging, tumor size, tumor number, and postoperative recurrence in HCC patients.

Some studies[23,24] have uncovered a novel lncRNA-miRNA-mRNA regulatory loop in tumor diseases. In HCC, UCA1 accelerates tumor cell invasion and metastasis by sponging miR-216b. The regulatory loop UCA1/miR-203/Snail2 stimulates EMT and inhibits proliferation and growth of metastases in HCC[25]. In this paper, we found that miR-18a was downregulated in HCC tissues, and its level was negatively correlated to UCA1. In addition, miR-18a was also related with TNM staging, intrahepatic metastasis, and postoperative recurrence in HCC patients. Through survival analysis and Cox regression analysis, prognostic potentials of UCA1 and miR-18a in HCC have been identified. Hence, UCA1 and miR-18a may be utilized as tumor hallmarks for diagnosis, treatment, and prognosis of HCC.

Conclusions

We detected that LncRNA UCA1 is upregulated and miR-18a is downregulated in HCC tissues. High-level UCA1 and low-level miR-18a are closely linked to poor prognosis in HCC.

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Conflict of Interests

The authors declare that they have no conflict of interests.

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