European Review for Medical and Pharmacological Sciences 2016; 20: 3987-3991

Expression of lncRNA HULC in cervical cancer and its correlation with tumor progression and patient survival

Y.-F. WANG[1], S. ZHANG[2], X.-Q. LI[3], Y. WANG[1]

[1]Department of Gynaecology and Obstetrics, Henan Provincial People's Hospital and Zhengzhou University People's Hospital, Zhengzhou, China
[2]Autobio Diagnostics Co. Ltd., Zhengzhou, China
[3]Department of Oncology, Henan Provincial People's Hospital & Zhengzhou University People's Hospital, Zhengzhou, China

Abstract. – OBJECTIVE: To investigate Long non-coding RNA HULC (HULC) expression in cervical cancer and to evaluate its clinical significance.

PATIENTS AND METHODS: HULC expression in 244 pairs of human cervical cancer and adjacent normal tissues was detected by real-time quantitative RT-PCR assay. The correlation between HULC and clinicopathological factors was also evaluated. The clinical and prognostic significance of HULC expression was analyzed statistically by Kaplan-Meier estimate and Cox regression model.

RESULTS: HULC expression was high in cervical cancer ($p < 0.01$). Also, the high HULC expression was significantly associated with the FIGO stage, lymph node metastasis and depth of cervical invasion ($p < 0.05$). Kaplan-Meier analysis showed that cervical cancer pa¬tients with high expression of HULC possessed poorer outcome than those with low expression of HULC ($p < 0.001$). Based on the univariate and multivariate analysis, the elevated expression of the HULC protein was a significant predictor of poor prognosis for cervical cancer patients ($p = 0.001$).

CONCLUSIONS: Our data firstly showed that the expression of HULC was upregulated in cervical cancer, and associated with overall survival, indicating that HULC may serve as a predictive biomarker for the prognosis of cervical cancer.

Key Words:
LncRNA HULC, Overall survival, Cervical cancer.

Introduction

Cervical cancer is one of the most common malignant cancers in woman worldwide, with an estimated global incidence of 529,800 new cases and 275,100 deaths per year[1,2]. In developing countries, the incident case of cervical cancer accounts for more than 80% of world incident case[3]. Despite marked efforts in developing multimodal treatments, the clinical outcome of cervical cancer patients remains unfavorable. Many studies have shown that tumor size, lymph node metastasis and lymph-blood vessel invasion are independent prognostic factors for survival of cervical cancer patients[4]. However, these factors may not accurately estimate prognosis because of heterogeneity in the patient population. Thus, it is highly necessary to explore novel biomarkers for the early identification of a more effective, clinical therapeutic strategy against cervical cancer.

Long non-coding RNAs (lncRNAs) are a type of non-coding RNAs which own a length of more than 200 nucleotides and are located in nuclear or cytoplasmic fractions[5,6]. Accumulating evidences showed that lncRNAs participated in cancer cell biological processes, such as cell growth, cell metastasis, and cell differentiation[7,8]. Meanwhile, lncRNAs have been shown to be an aberrant expression in cancer tissue considered as oncogenes or tumor suppressors[9-11]. Unfortunately, the emerging functional role of lncRNAs in cervical cancer remains largely unknown.

In the present report, we aimed to investigate the expression of HULC in cervical cancer and further explore the clinical significance of HULC in cervical cancer. We firstly explored the expression level of HULC in cervical cancer tissues using qRT-PCR. Next, we investigated whether HULC expression was associated with the outcome of cervical cancer patients.

Corresponding Author: Yue Wang, MD; e-mail: wyf901@126.com

Patients and Methods

Patients and Tissue Samples

A total of 244 paired of primary cervical cancer tissues and the adjacent normal cervical epithelial tissues from cervical carcinoma patients were collected from February 2007 to December 2013 at Department of Gynaecology and Obstetrics in Henan Provincial People's Hospital. All the cancer tissues were confirmed by pathological diagnosis after surgery. All patients had provided consent in the sense that their tumor samples could be used for investigational purposes. The tissues were immediately frozen in liquid nitrogen and stored at -80°C until use. The date of death and the date of relapse were used to calculate estimate overall survival (OS). Written informed consent was obtained from all patients, and research protocols were approved by the Clinical Research Ethics Committee of Henan Provincial People's Hospital

RNA Isolation and Quantitative RT-PCR

Total RNA was extracted from all tissue samples with TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. 1 g RNA was reverse transcribed in a final volume of 20 l using random primers under standard conditions for the PrimeScript RT reagent Kit (NanoDrop Technologies, Wilmington, DE, USA). Real-time PCR analyses were performed with SYBR Green (Takara, Dalian, Liaoning, China). GAPDH was measured as an internal control for paired tumor and normal tissues. The primer sequences of HULC were as follows: (forward primer, 5'-ATCTGCAAGCCAGGAAGAGTC-3', and reverse primer,

5'-CTTGCTTGATGCTTTGGTCTGT-3'). The relative expression levels of HULC were calculated using the 2^{-Ct} method

Statistical Analysis

The significance of differences between groups was estimated by Student's *t*-test, ^{2} test or Wilcoxon test, as appropriate. Overall survival was obtained using the Kaplan-Meier method. Independent prognostic factors were assessed in the univariate and multivariate analysis using the Cox proportional-hazards regression model. All of the statistical analyses were performed using SPSS for Windows v.16.0 (SPSS Inc., Chicago, IL, USA). $p < 0.05$ was considered to indicate a statistically significant difference.

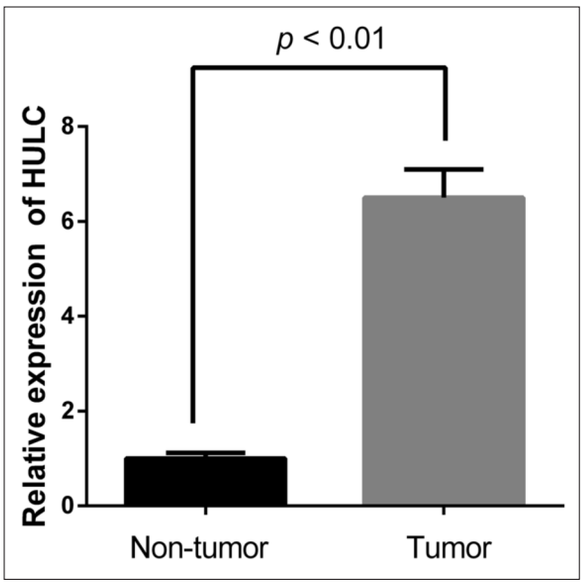

Figure 1. Expression of HULC is increased in cervical cancer tissues compared with non-cancerous tissues through qRT-PCR ($p < 0.01$).

Results

HULC Expression is Elevated in Cervical Cancer Tissues

Using qRT-PCR, HULC gene expression was assessed in 244 ovarian cancer tissue samples and 65 normal ovarian tissue specimens. In contrast to normal cervical tissues, the expression of HULC was increased in most cervical cancer tissues($p < 0.01$).

Correlation Between HULC and ClinicoPathological Features in Cervical Cancer Patients

To further explore the role of HULC in determining the clinical significance of cervical cancer, we analyzed the association between HULC expression and clinicopathological features in 244 cervical cancer patients. The median HULC expression level was used as a cutoff value to divide all 244 patients into two groups: high HULC expression group (n = 120) and low HULC expression group (n = 124). The results were summarized in Table I. High HULC expression was negatively associated with FIGO stage ($p = 0.001$), Lymph node metastasis ($p = 0.000$), and Depth of cervical invasion ($p = 0.000$) of patients with cervical cancer. Nevertheless, no significant correlation was found between HULC expression and other clinicopathological characteristics.

Table I. Clinicopathological characteristics of cervical cancer patients.

Variables	No. of cases (n = 244)	HULC expression	*p*-value
Age (years)			0.883
< 50	113	58	55
≥ 50	131	66	65
Tumor size (cm)			0.256
< 4	113	53	60
≥ 4	131	71	60
Histological grades			0.720
Well/moderate	135	70	65
Poor	109	54	55
FIGO stage			0.001
Ib-IIa	117	73	44
IIb-IIIa	127	51	76
Lymph node metastasis			0.000
No	133	82	51
Yes	111	42	69
Depth of cervical invasion			0.000
< 2/3	119	78	41
≥ 2/3	125	46	79

Association of HULC Expression with Clinical Prognosis

Kaplan-Meier analyses were performed to investigate the correlation of HULC expression with overall survival of cervical cancer patients. Our results showed that patients with high level of HULC expression had significantly shorter overall survival time (Figure 2, $p < 0.001$) than patients with low level of HULC expression. Univariate analysis demonstrated that FIGO stage ($p = 0.012$), Lymph nodes metastasis ($p < 0.002$), Depth of cervical invasion ($p = 0.009$), and HULC expression ($p = 0.006$) were significantly associated with overall survival of cervical cancer patients (Table II). Multivariate analysis was conducted using the Cox proportional hazards model confirmed that The expression of HULC emerged as an independent and significant factor associated with poor five-year survival rates ($p = 0.001$, shown in Table II).

Discussion

The prognosis for cervical cancer remains poor despite recent advances in the diagnosis of this cancer, and the 5-year overall survival rate of cervical cancer is a dismal 28%[12,13]. Accurate prediction of the prognosis for the individual patient with cervical cancer is of great importance. However, many prognostic biomarkers are not satisfactory. Thus, it is urgently necessary to identify reliable molecular markers to precisely predict tumor response to the treatment and the clinical outcomes of cervical cancer patients.

In recent years, roles of lncRNAs in cervical cancer have attracted much interest from urological researchers and are becoming a hot spot in cervical cancer research. Wu et al[14] showed that lncRNA CCAT2 expression is significantly higher in cervical cancer tissue than in adjacent normal tissues. Furthermore, functional experiments demonstrated LncRNA CCAT2 promoted the proliferation and survival of cervical cancer cells. Yang et al[15] found that lncRNA MALAT1 was increased in cervical cancer and associated with

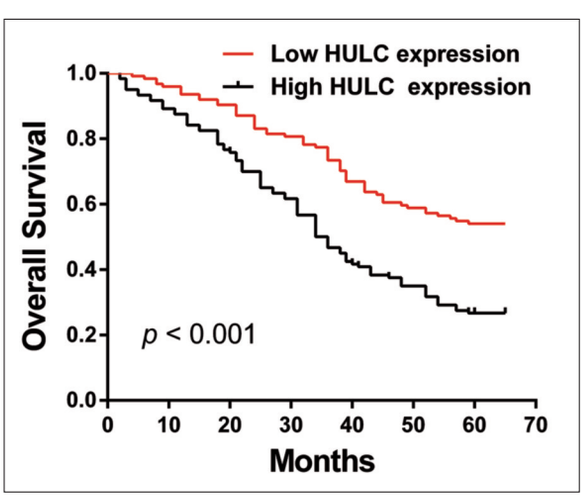

Figure 2. High HULC predicts a poor prognosis in cervical cancer patients ($p < 0.001$).

Table II. Prognostic factors in Cox proportional hazards model.

Variable	Univariate analysis			Multivariate analysis		
	Risk ratio	95% CI	*p*-value	Risk ratio	95% CI	*p*-value
Age (years) ≥ 50 *vs*. < 50	2.361	0.673-3.893	0.356			
Tumor size ≥ 4 *vs*. < 4	2.933	0.451-4.664	0.317			
Histological grades	3.149	0.518-4.348	0.093			
Well/moderate *vs*. poor	5.215	1.831-7.774	0.012	4.147	1.362-6.223	0.009
FIGO stage IIb~IIIa vs. Ib-IIa						
Lymph nodes metastasis Yes *vs*. No	5.773	2.028-8.831	0.002	5.149	1.873-7.741	0.008
Depth of cervical invasion < 2/3 *vs*. ≥ 2/3	3.129	1.773-7.451	0.009	2.348	1.632-6.338	0.004
HULC high *vs*. low	3.125	2.117-8.219	0.006	2.559	1.323-7.041	0.001

advanced clinical features. Furthermore, patients with higher expression of lncRNA MALAT1 had a significantly poor prognosis. Jiang et al[16] reported that lncRNA LET was not only up-regulated but could promote the progress of cervical cancer and impact prognosis of the cancer patients. Kim et al[17] showed that over-expression of HOTAIR might promote cervical cancer progression by inducing cell migration and invasion through the upregulation of VEGF, MM P-9 and expression of EMT-related genes. The above studies revealed that lncRNAs played an important role in the progression of cervical cancer.

The lncRNA HULC which is located on chromosome 6p24.3 and is conserved in primates is a newly confirmed lncRNA. It was firstly identified to be strongly overexpressed noncoding transcripts in human HCC[18]. Peng et al[19] revealed that HULC was up-regulated in human pancreatic cancer tissues and can be considered an independent prognostic factor. Sun et al[20] reported that the expression level of HULC in osteosarcoma was up-regulated. Furthermore, functional experiments demonstrated Down-regulated HULC inhibited cell proliferation, migration, and invasion of osteosarcoma cells. Those results revealed that HULC severed as an oncogene in cancer. In the present study, We found that HULC expression in cervical cancer tissues was significantly higher than that in matched normal adjacent tissues. Our data showed that high-level HULC was closely associated with poor overall survival of cervical cancer patients. Univariate and multivariate analyses indicated that HULC was an independent predictor of shorter overall survival of cervical cancer patients.

Conclusions

To the best of our knowledge, this is the first report demonstrating an association between HULC expression and clinical relevance in cervical cancer. Our results suggest that HULC may represent a novel prognostic indicator and a target for gene therapy in cervical cancer.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieu¬lent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.
2) Siegel RL, Miller KD, Jemal A. Cancer sta¬tistics, 2015. CA Cancer J Clin 2015; 65: 5-29.
3) de Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B, Tous S, Felix A, Bravo LE, Shin HR. Human papillomavi¬rus genotype attribution in invasive cervical cancer: a retrospective cross-sectional world¬wide study. Lancet Oncol 2010; 11: 1048-1056.
4) Moore DH. Cervical cancer. Obstet Gynecol 2006; 107: 1152-1161.
5) Kornienko AE, Guenzl PM, Barlow DP, Pauler FM. Gene regulation by the act of long non-coding RNA transcription. BMC Biol 2013; 11: 59.
6) Brosnan CA, Voinnet O. The long and the short of noncoding RNAs. Curr Opin Cell Biol 2009; 21: 416-425.
7) Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. Nature Reviews Genetics 2009; 10: 155-159.

8) Wang KC, Chang HY. Molecular mechanisms of long noncoding RNAs. Mol Cell 2011; 43: 904-914.

9) Tuo YL, Li XM, Luo J. Long noncoding RNA UCA1 modulates breast cancer cell growth and apoptosis through decreasing tumor suppressive miR-143. Eur Rev Med Pharmacol Sci 2015; 19: 3403-3411.

10) Cheng WS, Tao H, Hu EP, Liu S, Cai HR, Tao XL, Zhang L, Mao JJ, Yan DL. Both genes and lncRNAs can be used as biomarkers of prostate cancer by using high throughput sequencing data. Eur Rev Med Pharmacol Sci 2014; 18: 3504-3510.

11) Yang M, Zhai X, Xia B, Wang Y, Lou G. Long non-coding RNA CCHE1 promotes cervical cancer cell proliferation via upregulating PCNA. Tumour Biol 2015; 36: 7615-7622.

12) Dasari S, Wudayagiri R and Valluru L. Cervical cancer: biomarkers for diagnosis and treatment. Clin Chim Acta 2015; 445: 7-11.

13) Martin-Hirsch PL1, Wood NJ. Cervical cancer. BMJ Clin Evid 2011; 27: 2011.

14) Wu L, Jin L, Zhang W, Zhang L. Roles of Long Non-Coding RNA CCAT2 in Cervical Cancer Cell Growth and Apoptosis. Med Sci Monit 2016; 22: 875-879.

15) Yang L, Bai HS, Deng Y, Fan L. High MALAT1 expression predicts a poor prognosis of cervical cancer and promotes cancer cell growth and invasion. Eur Rev Med Pharmacol Sci 2015; 19: 3187-3193.

16) Jiang S, Wang HL, Yang J. Low expression of long non-coding RNA LET inhibits carcinogenesis of cervical cancer. Int J Clin Exp Pathol 2015; 8: 806-811.

17) Kim HJ, Lee DW, Yim GW, Nam EJ, Kim S, Kim SW, Kim YT. Long non-coding RNA HOTAIR is associated with human cervical cancer progression. Int J Oncol 2015; 46: 521-530.

18) Liu Y1, Pan S, Liu L, Zhai X, Liu J, Wen J, Zhang Y, Chen J, Shen H, Hu Z. A genetic variant in long non-coding RNA HULC contributes to risk of HBV-related hepatocellular carcinoma in a Chinese population. PLoS One 2012; 7: e35145.

19) Peng W, Gao W, Feng J. Long noncoding RNA HULC is a novel biomarker of poor prognosis in patients with pancreatic cancer. Med Oncol 2014; 31: 346.

20) Sun XH, Yang LB, Geng XL, Wang R, Zhang ZC. Increased expression of lncRNA HULC indicates a poor prognosis and promotes cell metastasis in osteosarcoma. Int J Clin Exp Pathol 2015; 8: 2994-3000.