Int J Clin Exp Pathol 2016;9(4):4241-4246
www.ijcep.com /ISSN:1936-2625/IJCEP0012296

Original Article

Tissue expression level of lncRNA UCA1 is a prognostic biomarker for colorectal cancer

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Received July 2, 2015; Accepted August 20, 2015; Epub April 1, 2016; Published April 15, 2016

Abstract: Background: The aberrant expression of urothelial carcinoma-associated 1 (UCA1) was reported in gastric cancer, esophageal squamous cell carcinoma, melanoma, breast cancer, tongue squamous cell carcinomas, as well as colorectal cancer (CRC). In the present study, we investigated the clinical significance and prognostic value of UCA1 in CRC. Methods: A total of 121 fresh cancer tissue samples were obtained from Binzhou Medical University Hospital between April 2009 and December 2014. The expression levels of UCA1 were examined by quantitative real-time PCR. Kaplan-Meier method was used to estimate the survival rate, and differences in survival of subgroups of the study were compared by log-rank test. Multivariate analysis was performed to estimate the association between clinical and genetic features and overall survival using Cox proportional hazard models. Results: We found that UCA1 expression was significantly higher in CRC tissues compared with adjacent normal tissues (P<0.001). UCA1 expression was significantly associated with TNM stage (P=0.006), lymph node metastasis (P=0.012), distant metastasis (P=0.037) and tumor differentiation (P<0.001). Kaplan-Meier analysis indicated that patients with higher expression levels of UCA1 had significantly shorter overall survival than those with lower expression levels (P=0.012). Furthermore, the multivariate Cox regression model demonstrated that UCA1 expression (P=0.027) was an independent prognostic factors for CRC. Conclusions: Our results indicated that UCA1 might be an important indicator of poor survival rate and an independent prognostic factor for CRC. More high quality studies are needed to confirm our finding in the future.

Keywords: Colorectal cancer, UCA1, expression level, prognosis

Introduction

Colorectal cancer (CRC) is the third most common cancer and the fourth most common cause of cancer deaths worldwide, with over 1.2 million new cancer cases and 608,700 cancer deaths estimated to have occurred in 2008 [1]. Current treatments for CRC include surgery, radiotherapy, chemotherapy and targeted therapy, but the five-year survival rate is still not high, especially in patients with advanced CRC [2]. Therefore, a better understanding of the oncogenic activities and molecular markers underlying CRC as well as the identification of new therapeutic targets for the treatment of this disease, is urgently needed.

Long non-coding RNAs (lncRNAs) are non-coding transcripts ranging from 200 to 100,000 nucleotides in length [3, 4]. lncRNA makes up the biggest class of ncRNAs, with ~58,000 human lncRNA genes annotated thus far [5]. Recent studies have demonstrated that lncRNAs play important roles in carcinogenesis and cancer metastasis and aberrant expression of lncRNAs has been identified in CRC [6-8].

Urothelial carcinoma-associated 1 (UCA1) is an lncRNA originally identified in bladder transitional cell carcinoma, which is belonging to the human endogenous retrovirus H (HERV-H) family [9]. The aberrant expression of UCA1 was reported in gastric cancer, esophageal squamous cell carcinoma, melanoma, breast cancer, tongue squamous cell carcinomas, as well as CRC [10]. Previously, Han et al. found that UCA1 levels were markedly increased in CRC tissues and cells compared to controls. Furthermore, UCA1 was found to influence the proliferation, apoptosis and cell cycle progression of CRC cells [11]. In the present study, we

Table 1. Relationship between UCA1 expression and clinicopathologic parameters of CRC patients

Variables	Cases (n)	UCA1 expression High (n=61)	UCA1 expression Low (n=60)	*P* value
Age (years)				
<50	44	26	18	0.187
≥50	77	35	42	
Gender				
Male	65	35	30	0.468
Female	56	26	30	
Location				
Colon	79	38	41	0.568
Rectum	42	23	19	
Depth of tumor				
T1 and T2	66	31	35	0.467
T3 and T4	55	30	25	
Lymphatic metastasis				
Yes	31	22	9	0.012
No	90	39	51	
Distant metastasis				
Yes	9	8	1	0.037
No	112	53	59	
TNM stage				
I+II	82	34	48	0.006
III+IV	39	27	12	
Histologic grade				
Well and moderately	76	27	49	<0.001
Poorly	45	34	11	

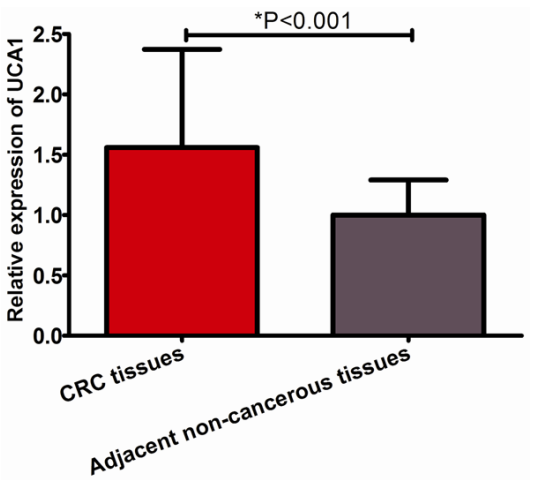

Figure 1. Expression levels of UCA1 in CRC tissues and adjacent non-cancerous tissues by RT-qPCR.

investigated the clinical significance and prognostic value of UCA1 in CRC.

Materials and methods

Patients and samples

The present study was conducted with the approval of the Ethical and Scientific Committees of Binzhou Medical University Hospital. Through the surgery consent form, patients were informed that the resected specimens would be kept by our hospital and might be used for scientific research, and that their privacy would be maintained. A total of 121 fresh cancer tissue samples were obtained from the Department of Colorectal Surgery, Binzhou Medical University Hospital in China between April 2009 and December 2014. All specimens were immediately frozen in tubes containing RNAlater preservation liquid after removal and stored at -80°C until RNA extraction. Clinical data were collected, including gender, age, tumor size, tumor location, serum carcinoembryonic antigen (CEA) level, tumor differentiation, tumor invasion depth, lymph node metastasis, and TNM stage, which was determined according to the 7th TNM classification of malignant tumors. None of the patients received radiotherapy, chemotherapy, or immunotherapy prior to simply surgery. The detailed clinicopathological characteristics of the recruited patients are summarised in **Table 1**.

RNA extraction and qRT-PCR analyses

Total RNA was isolated from tissues using TRIZOL reagent according to the manufacturer's protocol (Invitrogen). The 10 ul RT reactions were performed using the GoScript reverse transcription (RT) system (Promega, Madison, WI, USA) following the manufacturer's instructions. RT-PCR was performed using the 7500 real-time PCR system (Applied Biosystems, Hayward, CA, USA). The PCR primers for UCA1 or GAPDH were as follows: UCA1 forward, 5'-ACGCTAAC TGGCACCTTGTT-3' and reverse, 5'-TGGGGATTACTGGGGTAGGG-3'; GAPDH forward, 5'-AGCCACATCGCTCAGACAC-3' and reverse, 5'-GCCCAATACGACCAAATCC-3'. The expression level of candidate gene was internally normalized against that of the GAPDH. The relative quantitative value was expressed by the $2^{-\Delta\Delta Ct}$ method.

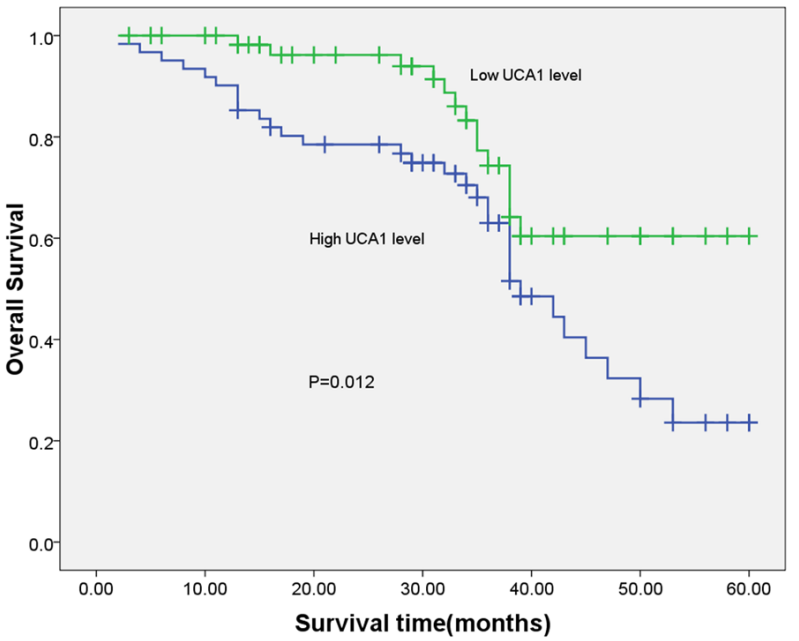

Figure 2. Kaplan-Meier overall survival curves of CRC patients according to the levels of UCA1 expression.

Table 2. Multivariate analysis of clinicopathological factors for overall survival in CRC

Variable	HR	95% CI	*P* value
Age	0.927	0.726-2.192	0.383
Gender	0.671	0.227-1.781	0.725
Location	1.281	0.372-2.774	0.469
Depth of tumor	2.011	0.821-4.555	0.117
Lymphatic metastasis	2.934	1.283-9.454	0.017
Distant metastasis	3.203	2.102-19.553	0.002
TNM stage	2.839	1.923-14.585	0.007
Histologic grade	2.375	1.035-8.927	0.034
UCA1 expression level	2.039	1.382-9.091	0.027

CI= confidence interval, HR= Hazard ratio.

Statistical analysis

The statistical analyses were performed using the SPSS version 18.0 (SPSS Inc, IL, USA). Comparisons of continuous data between two groups were performed using an independent t-test, and categorical data were analysed using the chi-square test or Fisher's exact test. Kaplan-Meier method was used to estimate the survival rate, and differences in survival of subgroups of the study were compared by log-rank test. Multivariate analysis was performed to estimate the association between clinical and genetic features and overall survival using Cox proportional hazard models. A *P* value <0.05 was considered significant.

Results

UCA1 expression in CRC tissues and adjacent normal tissues

The expression levels of UCA1 in 121 cancerous and noncancerous tissues were examined by quantitative real-time PCR. Using GAPDH as the normalization control, we found that UCA1 expression was significantly higher in CRC tissues compared with adjacent normal tissues (P<0.001, shown in **Figure 1**). For better understanding of the clinical relevance of UCA1 expression in CRC, the 121 CRC cases were classified into UCA1 high-expression group (n=61) and UCA1 low-expression group (n=60), according to the median expression level of UCA1 in all CRC samples.

Relationship between UCA1 expression and the clinicopathological features of CRC patients

We next determined whether UCA1 expression levels were associated with specific clinicopathological characteristics of CRC. Patient characteristics with respect to UCA1 expression were shown in **Table 1**. We found that UCA1 expression was significantly associated with TNM stage (P=0.006), lymph node metastasis (P=0.012), distant metastasis (P=0.037) and tumor differentiation (P<0.001). No association was found between UCA1 expression and age, sex, tumor location, as well as depth of tumor (all P>0.05). These data suggested that UCA1 overexpression was associated with the clinical progression and development of CRC.

Association between UCA1 expression and survival in CRC patients

To assess the correlation between UCA1 expression and CRC prognosis, the expression

levels of UCA1 in tumor tissues were categorized as low or high relative to the median level. Kaplan-Meier analysis indicated that patients with higher expression levels of UCA1 had significantly shorter overall survival than those with lower expression levels in 121 CRC patients (P=0.012, shown in **Figure 2**). Furthermore, the multivariate Cox regression model demonstrated that UCA1 expression (P=0.027), lymphatic metastasis (P=0.017), distant metastasis (P=0.002), TNM stage (P=0.007), and histologic grade (P=0.034) were independent prognostic factors for CRC (shown in **Table 2**).

Discussion

In china, CRC is the third most common cancer by annual incidence and the fifth leading cause of cancer-related death [12], with an upward trend in incidence rate in recent decades. Given that there are typically no specific symptoms in the early stage of CRC, most patients are diagnosed in an advanced stage. Current treatments for CRC include surgery, radiotherapy, chemotherapy and targeted therapy, but the five-year survival rate is still not high, especially in patients with advanced CRC. Early diagnosis and prognostic evaluation of CRC are crucial for timely and appropriate treatment. Thus, an urgent need exists to develop new screening tools and identify biomarkers for CRC.

Unlike the smaller noncoding micoRNAs, the functions of the majority of lncRNAs are not fully clear. However, with the improvement of technology and research in transcriptome profiles, increasing evidence shows that some lncRNAs, which can regulate gene expression at transcriptional, post-transcriptional, and epigenetic levels by interacting with DNA, RNA, and protein, play important roles in serial steps of cancer development. These lncRNAs are involved in both oncogenic and tumor-suppressive pathways [13]. Epigenetic studies have shown that lncRNA can predict cancer outcomes and further identify those patients who should require more aggressive treatments [14]. The aberrant expression patterns of lncRNAs can also be used to diagnose cancer or reflect disease prognosis and serve as predictors of patient outcomes [15-17].

UCA1 is an lncRNA originally identified in bladder transitional cell carcinoma. The entire sequence consists of three exons with 1.4 kb in length. As it is highly expressed in bladder transitional cell carcinoma, it was suggested to serve as a biomarker for the diagnosis of bladder cancer [18]. Zheng et al. found that that UCA1 expression was remarkably increased in gastric cancer tissues and cell lines compared with that in the normal control. Clinicopathologic analysis revealed that high UCA1 expression correlated with worse differentiation, tumor size, invasion depth and TNM stage in gastric cancer. Kaplan-Meier analysis showed that increased UCA1 expression contributed to poor overall survival (P=0.017) and disease-free survival (P=0.024) of patients. A multivariate survival analysis also indicated that UCA1 could be an independent prognostic marker. The levels of UCA1 in gastric juice from gastric patients were significantly higher than those from normal subjects (P=0.016). Moreover, validation analysis showed that UCA1 levels were robust in differentiating gastric cancer patients from control subjects [area under the curve (AUC) =0.721; 95% confidence interval (CI) =0.655-0.788, P<0.01]. These results suggested that UCA1 might serve as a promising biomarker for early detection and prognosis prediction of gastric cancer [19]. In the study by Srivastava et al., UCA1 expression was found to be significantly higher in the bladder cancer group as compared to the controls (P<0.001). UCA1 can be used as a noninvasive diagnostic biomarker in the early diagnosis of primary urinary bladder cancer [20]. Li et al. found that the relative level of UCA1 was significantly higher in ESCC tissues compared to the adjacent non-tumor tissues. The ESCC patients with higher UCA1 expression had an advanced clinical stage and a poorer prognosis than those with lower expression. In vitro assays, their data indicated that downregulation of UCA1 decrease cell proliferation, migration, and invasion ability. Therefore, lncRNA UCA1 might be considered as a novel molecule involved in ESCC progression, which provides a potential prognostic biomarker and therapeutic target [21].

Previously, Han et al. found that UCA1 levels were markedly increased in CRC tissues and cells compared to controls. Furthermore, UCA1 was found to influence the proliferation, apoptosis and cell cycle progression of CRC cells [11]. In the present study, we investigated the clinical significance and prognostic value of UCA1 in CRC. We found that UCA1 expression

was significantly higher in CRC tissues compared with adjacent normal tissues. We next determined whether UCA1 expression levels were associated with specific clinicopathological characteristics of CRC. UCA1 expression was found to be significantly associated with TNM stage, lymph node metastasis, distant metastasis and tumor differentiation. These data suggested that UCA1 overexpression was associated with the clinical progression and development of CRC. Kaplan-Meier analysis indicated that patients with higher expression levels of UCA1 had significantly shorter overall survival than those with lower expression levels in CRC patients. Furthermore, the multivariate Cox regression model demonstrated that UCA1 expression, lymphatic metastasis, distant metastasis, TNM stage, and histologic grade were independent prognostic factors for CRC. In conclusion, our results indicated that high UCA1 expression level might be an important indicator of poor survival and an independent prognostic factor for CRC. More high quality studies are needed to confirm our finding in the future.

Disclosure of conflict of interest

None.

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