

# Upregulation of long non-coding RNA HNF1A-AS1 is associated with poor prognosis in urothelial carcinoma of the bladder

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**Abstract.** – **OBJECTIVE:** Long noncoding RNAs (lncRNAs) play important roles in the pathogenesis of bladder cancer. A recent study reported that lncRNA HNF1A-AS1 (HNF1A-AS1) was upregulated in urothelial carcinoma of the bladder (UCB) and served as a tumor promoter. However, the clinical significance of HNF1A-AS1 in the prognosis of patients with UCB was poorly understood. This work was designed to investigate the relationship between HNF1A-AS1 expression level and the prognosis of UCB.

**PATIENTS AND METHODS:** Relative expression levels of HNF1A-AS1 in UCB tissues were determined by qRT-PCR. Then, the associations between HNF1A-AS1 expression and clinical pathological parameters were further evaluated. Survival and Cox proportional-hazards regression analyses were performed to determine the correlation between HNF1A-AS1 expression levels and prognosis in the patients.

**RESULTS:** Data showed that the majority of UCB tissues showed higher HNF1A-AS1 levels than the corresponding normal tissues controls ( $p < 0.01$ ). Statistical assay revealed that high HNF1A-AS1 expression was significantly correlated with histological grade ( $p = 0.008$ ), tumor stage T ( $p = 0.003$ ) and lymph nodes metastasis ( $p = 0.007$ ). In addition, the overall survival time of patients with high HNF1A-AS1 expression was significantly shorter compared to those with low HNF1A-AS1 expression. Furthermore, multivariate analysis confirmed that relative HNF1A-AS1 expression was an independent predictor of overall survival in patients with UCB.

**CONCLUSIONS:** HNF1A-AS1 expression was upregulated in UCB, and it may be a useful prognostic biomarker for patients with UCB.

*Key Words:*

lncRNA, HNF1A-AS1, Urothelial carcinoma of the bladder, Prognosis.

## Introduction

Bladder cancer is the most common malignant tumor of urinary system, causing about 429,800

new cases and 165000 deaths worldwide<sup>1</sup>. Urothelial carcinoma of the bladder (UCB) is the most common histological subtype of bladder cancer<sup>2</sup>. Bladder cancer can be classified into two types: muscle-invasive tumor (20-30%) and non-muscle invasive tumor (70-80%)<sup>3</sup>. Up to date, surgical resection is the primary and most effective treatment for patients with bladder cancer<sup>4</sup>. However, the outcome of muscle-invasive tumor is still poor due to a relatively high rate of local recurrence and metastasis<sup>5</sup>. A major challenge for improving clinical outcomes is to screen novel biomarkers that can be used to detect UCB at an early stage and predict the prognosis of this cancer.

Long non-coding RNAs (lncRNAs) are a class of noncoding RNAs > 200 nucleotides, with limited protein-coding potential<sup>6</sup>. They can directly and imperfectly modulate gene expression in transcriptional or post-translational levels<sup>7</sup>. lncRNAs have been reported to be implicated in the regulation of various cellular processes, including proliferation, differentiation, cell death, and cell mobility<sup>8,9</sup>. Some highly expressed lncRNAs could serve as oncogenes, whereas low-expressed lncRNAs could function as tumor suppressors<sup>10-12</sup>. Recent evidence<sup>13,14</sup> has shown that the global alteration of lncRNAs expression functions as a diagnosis and prognosis signature. These findings suggest that lncRNAs could be used as putative biomarkers to classify tumors.

lncRNA HNF1A antisense RNA 1 (HNF1A-AS1), located on chromosome 12q24.31, has been reported to be abnormally expressed in several tumors. Previous studies<sup>15,16</sup> indicated that HNF1A-AS1 was a tumor suppressive lncRNA in gastric cancer and pancreatic cancers. However, the oncogenic role of HNF1A-AS1 was confirmed in other cancer types, such as lung cancer and osteosarcoma<sup>17,18</sup>. Zhan et al<sup>19</sup> firstly reported that HNF1A-AS1 was highly expressed in bladder

cancer and served as a tumor promoter. However, the clinical significance of HNF1A-AS1 in patients with UCB remains largely unclear.

## Patients and Methods

### Patients and Tissue Samples

A total of 191 pairs of UCB tumor and matched, non-tumor tissues were collected at the Department of Urology, Weifang People's Hospital between July 2009 and December 2012. These tissues were immediately frozen in liquid nitrogen, and stored at liquid nitrogen until use. All the tumor cases were confirmed by histological examination. None of the patients received any biotherapy or chemotherapy treatment before recruitment to this study. The characteristics of the UCB patients are displayed in Table I. Overall survival is defined as the time elapsed from the surgery to the death of the patients with UCB. The study protocol was approved by Weifang People's Hospital Ethical Committee. Informed consent was obtained from all patients.

### Quantitative Reverse Transcription PCR (qRT-PCR)

Total RNA was extracted from UCB tissues with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The first strand cDNA was compounded using a Transcript RT kit (Tiangen Biotech., Pudong, Shanghai, China). Real-time RT-PCR was performed to detect the expression of HNF1A-AS1 using One-Step SYBR PrimeScript RT-PCR Kit (TaKaRa, Otsu, Shiga, Japan). The results of HNF1A-AS1 values were normalized to GAPDH. The primers were purchased from GeneCopoeia (Donghu, Wuhan, China). Relative quantification of RNA expression was calculated by using the  $2^{-\Delta\Delta CT}$  method.

### Statistical Analysis

All statistical analyses were performed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA) or the GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla CA, USA) software packages. The differences were analyzed using two-sided Student's *t*-test or  $\chi^2$ -test. The Kaplan-Meier survival analysis was performed to determine the survival curves, and the differences were identified using the log-rank test. Cox regression was used for univariate and multivariate analysis. *p*-value < 0.05 was considered statistically significant, and all tests were two-sided.

## Results

### HNF1A-AS1 is Upregulated in UCB Tissues

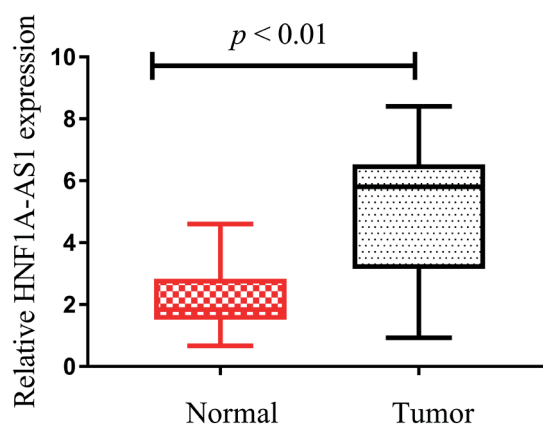
Firstly, we detected the relative expression levels of HNF1A-AS1 in 191 UCB tissues and their corresponding adjacent normal tissues by qRT-PCR. As shown in Figure 1, HNF1A-AS1 was upregulated in UCB tissues compared to non-tumorous tissues ( $p < 0.01$ ). The data suggested that high HNF1A-AS1 expression may contribute to the development of UCB.

### Relationship Between HNF1A-AS1 and Clinicopathological Characteristics of UCB patients

For statistical analysis, HNF1A-AS expression was divided into high and low expression groups. Then, associations between HNF1A-AS expression and various clinicopathological features of UCB patients were evaluated statistically. We found that high expression of HNF1A-AS was significantly correlated with histological grade ( $p = 0.008$ ), tumor stage T ( $p = 0.003$ ) and lymph nodes metastasis ( $p = 0.007$ ) (Table I). In contrast, there was no association between HNF1A-AS expression and other clinical factors, such as gender, age, tumor size and multiplicity (all  $p > 0.05$ ).

### HNF1A-AS Expression is a Prognostic Biomarker in Patients with UCB

Overall survival curves were plotted according to HNF1A-AS expression level using the Kaplan-Meier method. As shown in Figure 2, we found that patients with higher levels of HNF1A-AS had significantly



**Figure 1.** The expression of HNF1A-AS1 in tissues samples was significantly higher in UCB tissues than in adjacent bladder tissues ( $p < 0.01$ ).

**Table I.** Association of HNF1A-AS1 expression with clinicopathological features of bladder cancer.

Variables	Cases (N)	HNF1A-AS1 expression		p-value
		Low (N = 93)	High (N = 98)	
Gender				0.669
Male	120	57	63	
Female	71	36	35	
Age (years)				0.368
< 60	74	33	41	
≥ 60	117	60	57	
Tumor size (cm)				0.246
< 3	111	58	53	
≥ 3	80	35	45	
Multiplicity				0.745
Single	121	60	61	
Multiple	70	33	37	
Histological grade				0.008
L	94	55	39	
H	97	38	59	
Tumor stage T				0.003
Ta, T1	104	61	43	
T2-T4	87	32	55	
Lymph nodes metastasis				0.007
No	119	67	52	
Yes	72	26	46	

**Table II.** Univariate and multivariate analysis of overall survival in bladder cancer patients.

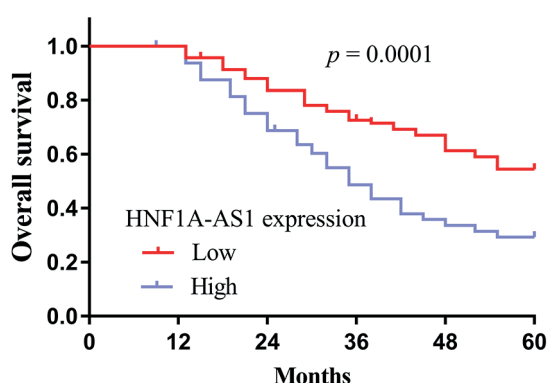
Variables	HR	95% CI	p
Univariate analysis			
Gender (Male vs. Female)	1.325	0.774-1.836	0.328
Age (<60 vs. ≥ 60)	0.893	0.577-1.639	0.217
Tumor size (< 3 vs. ≥ 3)	1.138	0.844-1.945	0.136
Multiplicity (Single vs. Multiple)	1.213	0.829-2.123	0.122
Histological grade (H vs. L)	2.213	1.542-3.994	0.006
Tumor stage (T2-T4 vs. Ta, T1)	3.542	1.775-6.482	0.002
Lymph nodes metastasis (Yes vs. No)	2.679	1.332-3.832	0.007
HNF1A-AS1 expression (High vs. Low)	3.231	1.554-5.598	0.001
Multivariate analysis			
Histological grade (H vs. L)	1.766	1.127-3.044	0.008
Tumor stage (T2-T4 vs. Ta, T1)	2.782	1.423-5.213	0.006
Lymph nodes metastasis (Yes vs. No)	2.012	1.114-2.894	0.009
HNF1A-AS1 expression (High vs. Low)	2.783	1.213-4.155	0.005

poorer survival than those with lower expression of this lncRNA in patients. The 5-year overall survival rate in the low expression group was 54.4%, compared with 29.3% in the high expression group ( $p = 0.001$ ). Subsequently, we performed univariate and multivariate analysis using the Cox regression model. We observed that the tumor stage, lymph nodes metastasis and HNF1A-AS1 expression were significantly associated with the overall survival of UCB patients (Table II). Further multivariate analysis confirmed that the level of HNF1A-AS1 expression

was independent prognostic factors for patients with UCB (Table II,  $p = 0.005$ ).

## Discussion

As the most common malignant tumor of urinary system, bladder cancer has become crushing burden for Chinese medical management<sup>20</sup>. Approaches to improve diagnosis or prognosis in patients with bladder cancer may help to establish tailored



**Figure 2.** Prognostic value of HNF1A-AS1 for UCB patients. Kaplan-Meier survival curves for UCB patients according to HNF1A-AS1 expression for overall survival.

therapy<sup>21</sup>. Although several clinicopathological features have been the standard for judging the clinical prognosis of UCB patients, clinical outcomes vary significantly between patients and can be difficult to predict<sup>22,23</sup>. Therefore, it is still urgent to identify novel and reliable prognostic markers.

Emerging evidence supports that lncRNAs play a critical role in progression of tumor. Similar with other lncRNAs, the expression patterns and functions of HNF1A-AS1 may be both different in various types of tumor. For instance, Liu et al<sup>24</sup> found that HNF1A-AS1 was up-regulated in hepatocellular carcinoma and promoted cell proliferation, invasion and EMT through sponging hsa-miR-30b-5p. Wang et al<sup>25</sup> also confirmed HNF1A-AS1 as a tumor promoter by repressing NKD1 and P21 expression. Cai et al<sup>26</sup> showed that high HNF1A-AS1 expression may be an independent poor prognostic factor and HNF1A-AS1 can suppress migration and invasion by reducing the EMT program in osteosarcoma cells. Furthermore, HNF1A-AS1 was reported to promote lung cancer proliferation and metastasis, both *in vitro* and *in vivo*. In addition, high HNF1A-AS1 was associated with poor prognosis in patient with lung cancer<sup>17,27</sup>. On the other hand, HNF1A-AS1 was found to be lowly expressed in gastric cancer and its low expression was associated with advanced clinicopathological features<sup>15</sup>. In 2017 Zhan et al<sup>19</sup> reported that HNF1A-AS1 promoted proliferation and suppresses apoptosis of bladder cancer cells by sponging miR-30b-5p. However, there were no reports about prognostic significance of HNF1A-AS1 expression in human UCB.

We explored the clinical significance of HNF1A-AS1 in UCB patients. The data indicated that

the expression of HNF1A-AS1 was up regulated in UCB tissues compared with normal tissues. In addition, we found that HNF1A-AS1 was positively associated with histological grade, tumor stage T and lymph nodes metastasis in UCB patients. Furthermore, Kaplan-Meier analysis indicated that patients with high HNF1A-AS1 expression lived shorter than those with low expression. It is more important that we also proved that HNF1A-AS1 expression was an independent predictor for overall survival. To date, the research about biological function of HNF1A-AS1 in UCB is very small. The underlying molecular mechanism of HNF1A-AS1 in UCB is needed to be elucidated. Our investigation may provide a valuable clue.

## Conclusions

We firstly showed that the expression profile of HNF1A-AS1, an oncogenic lncRNA, might serve as a prognostic marker in patients with UCB.

## Conflict of Interest

The Authors declare that they have no conflict of interest.

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