Int J Clin Exp Med 2015;8(7):11824-11830
www.ijcem.com /ISSN:1940-5901/IJCEM0010751

Original Article

Upregulated lncRNA-UCA1 contributes to progression of lung cancer and is closely related to clinical diagnosis as a predictive biomarker in plasma

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Received May 27, 2015; Accepted July 13, 2015; Epub July 15, 2015; Published July 30, 2015

Abstract: Objective: Long non-coding RNAs (lncRNAs) have been shown to play an important regulatory roles in cancer biology, and the lncRNA-UCA1 is upregulated in several cancers such as bladder cancer, breast cancer and colorectal cancer, however, the contributions of UCA1 to non-small cell lung cancer (NSCLC) remain largely unknown. Methods: Expression levels of lncRNA-UCA1 in tumor tissues and plasma from NSCLC patients was evaluated by quantitative real-time PCR, and its association with overall survival of patients was analyzed by statistical analysis. Moreover, the UCA1 expression correlation between tumor tissues and plasma was demonstrated by linear regression analysis. Results: the results showed that the expression of UCA1 in NSCLC tissues was obviously higher than that observed in pair-matched adjacent nontumourous tissues, ($P < 0.001$). The agarose gel electrophoretogram of RT-PCR products further confirmed that UCA1 was increased in NSCLC tissues. To assess the correlation of UCA1 expression with Clinicopathological data, we found that the expression level of UCA1 was associate with histological grade ($P < 0.001$) and lymph node metastasis ($P < 0.001$). Intriguingly, the expression of UCA1 was significantly increased in plasma from NSCLC patients. The UCA1 expression measurements obtained from plasma and tumor tissues were strongly correlated in 60 patient samples ($r = 0.881$). By receiver operating characteristic curve (ROC) analysis, plasma UCA1 provided the highly diagnostic performance for detection of NSCLC (the area under the ROC curve (AUC), 0.886; $P < 0.001$). In conclusion, the current results indicated that Plasma UCA1 could serve as a potential biomarker for diagnosis of NSCLC. UCA1 as a biomarker in clinical application might significantly improve the efficacy of human NSCLC screening.

Keywords: Non-small cell lung cancer, long non-coding RNA, UCA1, tumor biomarker

Introduction

Lung cancer (LC), including small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC), is the most common cause of global cancer-related mortality with an approximate 5-year survival rate of 16.6% [1, 2]. However, the existence of multiple known carcinogens and varying genetic backgrounds makes it difficult to determine which factors are most important in the development of LC [3-5]. Therefore, the underlying pathogenic mechanism and the more accurate predictive biomarkers are essential to be exploited.

Eukaryotic genomes encode numerous long non coding RNAs (LncRNAs), which is defined as endogenous cellular RNAs with length longer than 200 nucleotides, but lack open reading frames of significant length [6]. Within 4 years, the number of identified lncRNA genes increase more than 8000 [7]. Although the function of most lncRNAs is still unknown, their increasing numbers and the accumulating evidence for their involvement in many biologic processes provide compelling arguments in support of the dysregulation of lncRNAs has been correlated to cancer development, invasion and metastasis in the malignant cell [7-9]. For example, upregulated lncRNAs ANRIL [10], AK001796 [11], BCYRN1 [3] and HNF1A-AS1 [1] are proved to induce cell migration and tumor metastasis of LC. In contrast, an lncRNA named HMlin-

Table 1. Correlation clinicopathological factors and UCA1 expression levels in NSCLC patients

Variable	Number of patients	UCA1-Low	UCA1-High	*P* value
Gender				0.745
Male	37	15	22	
Female	23	9	14	
Age (years)				0.362
< 60	39	16	23	
≥ 60	21	8	13	
Tumor size (cm)				
< 3	35	15	20	0.637
≥ 3	25	9	16	
Histological				0.001
I	28	17	11	
II-III	32	7	25	
Lymph nodes metastasis				0.001
Absence (A)	26	16	10	
Presence (P)	34	8	26	

cRNA717 is downregulated and associated with tumor progression in human NSCLC [2]. In accordance to the literature, Urothelial carcinoma associated 1 (UCA1), the entire sequence consists of three exons with 1.4 kb in length, is an lncRNA originally identified in bladder transitional cell carcinoma [12] and found highly expressed in some carcinomas of the hepatocellular carcinoma [13], esophageal squamous cell carcinoma [14], ovarian cancer [15] and colorectal cancer [16], etc., suggesting that UCA1 may serve as a biomarker for the diagnosis of these cancers. Moreover, upregulated UCA1 contributes to progression of hepatocellular carcinoma through inhibition of miR-216b and activation of FGFR1/ERK signaling pathway [13]. In bladder cancer cell, UCA1 can enhance cell proliferation and metastasis through PI3K, Wnt or Akt signaling pathway [17-19]. In addition, microRNA-1 plays a tumor suppressive role via downregulating UCA1 in bladder cancer [20]. These results indicate that UCA1 plays an important role in the occurrence and development progress of malignant tumors. Nevertheless, there is no relevant report about the interaction between UCA1 and the progression of LC. Thus, the role of UCA1 in LC and its underlying mechanism remain to be determined.

In the present study, we performed a hierarchical cluster analysis of the differentially expressed lncRNA in the tumor tissues of LC patients to identify the role of UCA1 in the development progress of LC. Moreover, it was also examined in serum, and its potential use as tumor marker for LC detection was evaluated.

Materials and methods

Patients and specimens

Sixth NSCLC tissues and matched adjacent non-tumor tissues were collected from Shanghai Chest Hospital and Shanghai First People's Hospital, Shanghai Jiaotong University (Shanghai, China) between Jan 2012 and June 2014. All patients recruited in this study were not subjected to preoperative radiotherapy or chemotherapy and diagnosed with NSCLC based on histopathological evaluation. Clinicopathological characteristics analysis were shown in **Table 1**. All collected tissue samples were immediately stored at liquid nitrogen until use. Human samples were obtained with written informed consent from all patients. The study was approved by the Ethics Committee of the Shanghai Chest Hospital and Shanghai First People's Hospital, Shanghai Jiaotong University, China.

Real-time PCR

Total RNA was extracted by Trizol reagent (Invitrogen, Carlsbad, CA, USA). Reaction mixture (20 μl) containing 2 μg of total RNA was reversely transcribed to cDNA by using PrimeScript RT-polymerase (Takara, Dalian, China). Quantitative PCR was performed on the cDNA using specific primers (Sangon, Shanghai, China) for UCA1. The first strand cDNAs served as the template for the regular polymerase chain reaction (PCR) performed using a DNA Engine (ABI 9700). The cycling conditions were 30 s polymerase activation at 95°C followed by 40 cycles at 95°C for 5 s and 60°C for 30 s. PCR with the following primers: UCA1, Forward 5'-CTCTCCATTGGGTTCAC-3' and Reverse 5'-GCGGCAGGTCTTAAGAGATGAG-3'; GAPDH, Forward 5'-ACAGGGGAGGTGATAGCATT-3' and Reverse 5'-GACCAAAAGCCTTCATACATCTC-

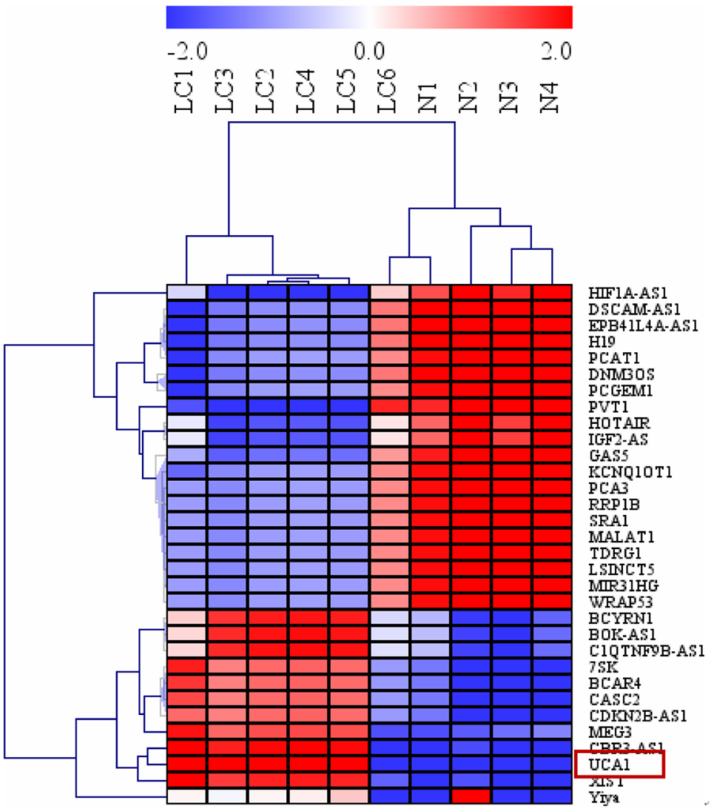

Figure 1. Microarray and hierarchical cluster analysis of the differentially expressed long non-coding RNAs (LncRNA) in tumor tissues of NSCLC patients and corresponding nontumourous tissues. The figure is drawn by MeV software (version 4.2.6). Differentially expressed LncRNAs chosen from lncRNA and disease database. Correlation similarity matrix and average linkage algorithms are used in the cluster analysis. Each row represents an individual LncRNA, and each column represents a sample. The dendrogram at the left side and the top displays similarity of expression among LncRNAs and samples individually. The color legend at the top represents the level of mRNA expression, with red indicating high expression levels and blue indicating low expression levels.

3'. Glyceraldehyde-phosphate dehydrogenase (GAPDH) as an internal control was used to normalize the data to determine the relative expression of the target genes. The reaction conditions were set according to the kit instructions. After completion of the reaction, the amplification curve and melting curve were analyzed. Gene expression values are represented using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

All statistical analyses were performed using SPSS version 18.0 software. Data were analyzed using independent two-tailed t test. Categorical data were analyzed using the two-side chi-square test. Overall survival was estimated by using Kaplan-Meier method, and univariate analysis was conducted by log-rank test. The Cox proportional hazards model was used in the multivariate analysis. Values of $P < 0.05$ were considered statistically significant.

Results

Microarray and hierarchical cluster analysis

Firstly, the lncRNA expression profiles and hierarchical cluster analysis were performed in 4 NSCLC tissues and paired corresponding nontumourous tissues. Fold change greater than 2 and *P* value less than 0.05 between tumor tissues and adjacent normal tissues were set as the criteria in filtering differently expressed lncRNAs. After the removal of redundant and unannotated sequences, 20 lncRNAs were found to be significantly down-regulated and 12 lncRNAs to be significantly up-regulated in the NSCLC tissues by qRT-PCR, and we finally focused on UCA1 in our study (**Figure 1**).

UCA1 was upregulated and associated with NSCLC progression

UCA1 plays a key role in the proliferation and apoptosis of tumor cells in vitro and in vivo, which may contribute to the pathogenesis of various kinds of cancers [13]. To further validated the interaction between the NSCLC and UCA1, the real-time PCR analysis was performed to determine the expression level of UCA1 in 60 pairs of human NSCLC tissues and corresponding nontumourous specimens. The results showed that the expression of UCA1 in NSCLC tissues was obviously higher than that observed in pair-matched adjacent nontumourous tissues, ($P < 0.001$, **Figure 2A**). The agarose gel electrophoretogram of RT-PCR products further confirmed that UCA1 was increased in NSCLC tissues as compared to adjacent nontumourous tissues (**Figure 2B**). To assess the correlation of UCA1 expression with Clinicopathological data, the expression levels of UCA1 in tumor tissues

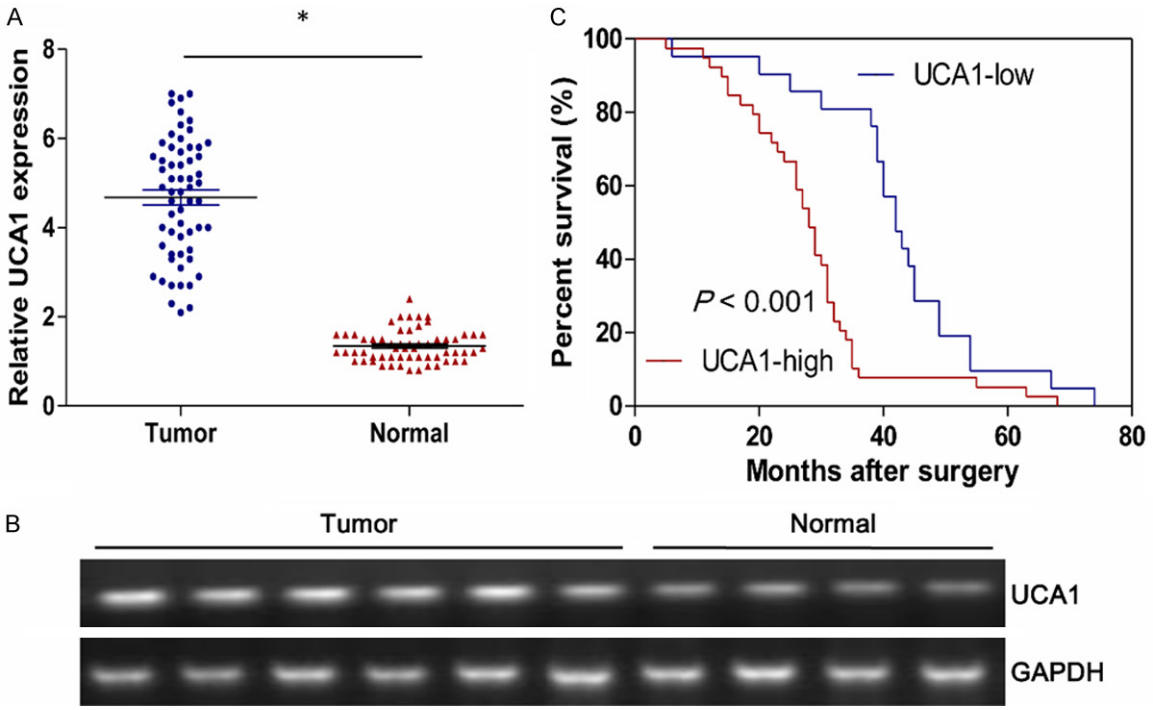

Figure 2. UCA1 was upregulated and associated with NSCLC progression. UCA1 expression was examined by real-time PCR and normalized to GAPDH expression in 60 pairs of NSCLC tissues compared with adjacent nontumourous tissues (A). Semiquantitative RT-PCR analysis of UCA1 expression from 5 patients with NSCLC (B). Kaplan-Meier survival curve and log-rank test were used to evaluate whether UCA1 expression level was associated with overall survival rate. Patients were segregated into UCA1-high group and UCA1-low according to the median of UCA1 expression in NSCLC (C). Values were expressed as mean ± SEM, $^{*}P < 0.05$ versus nontumourous group.

Table 2. Univariate and multivariate regression analyses of parameters associated with prognosis of NSCLC patients

Characteristics	Subset	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Gender	Male/Female	1.136 (0.742-1.896)	0.673		
Age	< 60/≥ 60	1.256 (0.873-2.045)	0.459		
Tumor size (cm)	< 3/≥ 3	1.227 (0.835-1.983)	0.471		
Histological	I/II-III	2.892 (1.645-5.069)	0.001	2.143 (1.152-3.569)	0.017
Lymph nodes metastasis	A/P	3.527 (1.942-6.241)	0.001	2.425 (1.372-4.225)	0.002
UCA1	High/Low	2.679 (1.538-4.927)	0.001	1.936 (1.062-3.258)	0.029

were categorized as low or high. As shown in **Table 1**, the expression level of UCA1 was associated with histological grade ($P < 0.001$) and lymph node metastasis ($P < 0.001$). However, there was no significant correlation between UCA1 and other clinicopathological parameters, such as gender, age or tumor size ($P > 0.05$). As shown in **Figure 2C**, patients with high UCA1 expression had a significantly poorer prognosis than those with low expression patients ($P < 0.001$). Univariate and multivariate Cox proportional hazards analyses showed that UCA1, as well as histological and metastasis, were identified to be independent prognostic factors for survival in NSCLC patients (**Table 2**). In general, these results suggested that the upregulation of UCA1 might be involved in development, progression and prognosis of the majority of human NSCLC.

Correlation between plasma UCA1 and tumor tissues UCA1

To test whether there was a relationship between plasma and tumor tissues UCA level, which was measured in EDTA-plasma samples

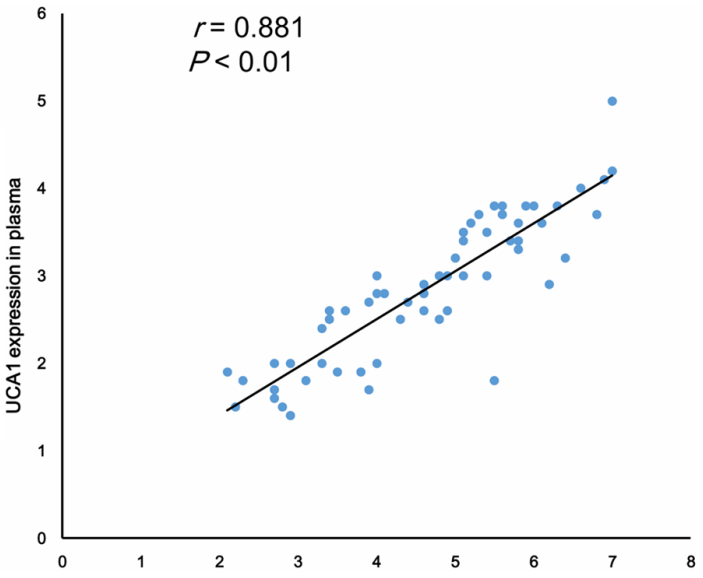

Figure 3. Compared UCA1 levels of plasma and tumor tissues in NSCLC patients. $R \geq 0.8$ means highly correlation between plasma and tumor tissue UCA1 expression levels.

and tumor tissues from the same individuals. As shown in **Figure 3**, measurements obtained from plasma and tumor tissues were strongly correlated for UCA1 in 60 patient samples (r = 0.881, **Figure 3**).

Receiver operating characteristic (ROC) curve analysis of PAX1 in tumor tissues and plasma

To investigate the characteristics of UCA1 as potential tumor markers of NSCLC, ROC curve and the area under the ROC curves (AUC) were performed on data from all subjects, including 60 NSCLC patients and 60 healthy donors. The ROC curves illustrated strong separation between the tumor tissues and control group, with an AUC of 0.912 (95% CI: 0.864-0.961; P < 0.001) for UCA1 (**Figure 4A**). Moreover, the ROC curves indicated that there was strong separation between the plasma and control group, with an AUC of 0.886 (95% CI: 0.827-0.945; P < 0.001) for UCA1 (**Figure 4B**). Therefore, UCA1 provided the highly diagnostic power for the detection of NSCLC, suggesting that plasma UCA1 could serve as a promising tumor marker for NSCLC diagnosis.

Discussion

Recent genome-wide studies have indicated that the mammalian genome is abundantly transcribed, and that at least 80% of this transcription is exclusively associated with lncRNAs [21]. Emerging data strongly implicate lncRNAs in the basal regulation of protein-coding genes, which are central to normal development and oncogenesis, at both the transcriptional and the posttranscriptional levels [21]. Mounting evidence has showed that lncRNA play a central role in the regulation of cell development, differentiation, proliferation and apoptosis [1, 13]. So identification of tumor associated lncRNAs is critical for understanding the roles of lncRNAs in tumorigenesis and may be important for novel therapeutic targets and improve the clinical strategies of cancer patients.In recent years, more and more evidences revealed the contribution of UCA1 as having oncogenic roles in tumorigenesis [13, 14, 16, 19]. Therefore, we tried to investigate the role of lncRNA-UCA1 in the development of NSCLC.

In this study, we demonstrated that the increase in UCA1 expression was confirmed by microarray assays and agarose gel electrophoretogram of RT-PCR products in NSCLC tissues compared to adjacent normal tissues. Previous studies suggested that UCA1 was significantly increased in tongue squamous cell carcinoma tissues [22], as well as hepatocellular carcinoma [13] and esophageal squamous cell carcinoma tissues [14], and was correlated with lymph node metastasis. Our results showed that the expression level of UCA1 was associated with clinical stage and lymph node metastasis of NSCLC patients. However, lncRNA-UCA1 expression was not correlated with age, gender and tumor size. Intriguingly, when the correlation between plasma and tumor tissues in UCA1 expression was analyzed as a continuous variable, we found a highly positive correlation between plasma and tumor tissues from NSCLC patients. In addition, UCA1 overexpression was associated with poor survival rates and could be an independent prognostic factor in patients with NSCLC. Taken together, these findings supported our previous hypothesis that lncRNA-UCA1 might play an important role in development and progression of NSCLC.

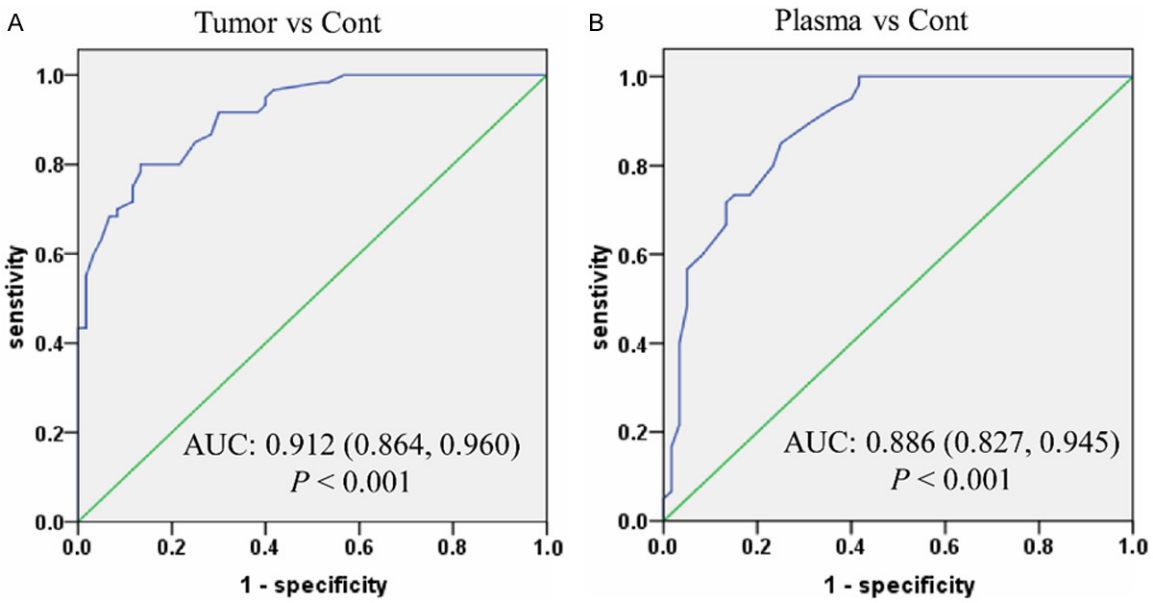

Figure 4. The ROC curve of UCA expression for distinguishes NSCLC. The area under the ROC curve (AUC) was calculated for the diagnosis of tumor tissues vs nontumourous control (A) and plasma vs nontumourous control (B).

In order to investigate the prognostic role of UCA1 on NSCLC, we performed Kaplan-Meier analysis of overall survival. The results showed that high UCA1 expression in NSCLC patients had a tendency to be worse overall survival in comparison to patients with low UCA1 expression, which suggested that UCA1 expression was a prognostic marker for patients with NSCLC. To further evaluate the prognostic value of UCA1 in NSCLC, we performed Cox proportional hazards model. Results proved that increased UCA1 expression was an independent marker of poor overall survival of NSCLC patients. Our study also compared UCA1 levels of plasma and tumor tissues in NSCLC patients, the results of which represented strong consistency. There were no significant differences in UCA1 levels between tumor tissues and plasma, which prompted that the quantitative detection of the levels of UCA1 of plasma could well reflect that of the tumor tissues.

This was the first report to demonstrate the functional significance of UCA1 expression in human NSCLC, and our results indicated that the overexpression of UCA1, as an oncogene, promoted NSCLC malignant progression, and that could be predicted by detecting the level in plasma. Moreover, our results demonstrated that plasma UCA1 level was highly correlated with tumor tissue. UCA1 as a biomarker in clinical application might significantly improve the efficacy of human NSCLC screening. Thus, UCA1 held great promise as a novel diagnostic and prognostic marker and therapeutic target for NSCLC.

Disclosure of conflict of interest

None.

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