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Expression of lncRNA UCA1 in ovarian cancer and its clinical significance

Y.R. Qiu[1], M.Y. Zhao[2], L. Sun[3], B.C. Yang[1], K.W. Hei[1], X. Du[4], Y.M. Li[1]

[1] Department of Medical Microbiology, School of Basic Medical Sciences, Tianjin Medical University, Tianjin
[2] Department of Obstetrics & Gynecology, Yantai City Zhifu district Marternal and Child health Hospital, Shandong
[3] Department of Obstetrics & Gynecology, Second Affiliated Hospital, Tianjin Medical University, Tianjin
[4] Department of Obstetrics & Gynecology, General Hospital, Tianjin Medical University, Tianjin (China)

Summary

Objectives: To explore the expression and clinical significance of lncRNA-UCA1 in ovarian cancer. *Materials and Methods:* The expression of lncRNA-UCA1 in 26 ovarian cancer tissue and 16 normal and benign ovarian tissue were detected using qRT-PCR method, and the correlation of expression level with clinicopathological features were analyzed. *Results:* Higher lncRNA-UCA1 expression level were detected in ovarian cancer tissue than those in normal ovarian tissue ($p < 0.05$). There were significant correlations between higher expression of lncRNA-UCA1 with tumor staging ($p = 0.000$), histological grades ($p = 0.000$), peritoneal effusion ($p = 0.001$), and lymph node metastasis ($p = 0.000$), but not with age. *Conclusion:* lncRNA-UCA1 may play a vital role in the metastasis of ovarian cancer and it is expected to be a potential novel biomarker and therapeutic target of ovarian cancer.

Key words: lncRNA; UCA1; Ovarian cancer; MACC1; Metastasis.

Introduction

Ovarian cancer is a lethal gynecologic malignancy with 225,500 estimated new cases and 140,200 deaths every year [1-3]. Owing to its vague and non-specific symptoms, some 70% of women initially present with advanced malignancy (Stage III or IV) and their five-year overall survival rate could be as low as 25% [1,4,5]. While, it is in stark contrast to patients identified and treated with Stage I ovarian cancer, who have greater than 90% five-year overall survival rate [6-8]. Screening methods for ovarian cancer have been well developed, including pelvic examination, transvaginal ultrasonography (TVS), and single-threshold serum cancer antigen (CA-125), but their specificity and sensitivity are still not high [9, 10]. That surgical removal of the ovaries with pathological biopsy is considered to be definitive diagnosis of ovarian cancer, which may result in harm through unnecessary follow-up testing and surgery for women without ovarian cancer [9]. Therefore, effective diagnostic biomarkers [11] and novel therapeutic approaches are still urgently required for ovarian cancer.

Recently, many studies have highlighted the roles of a group of long (>200 nt) non-coding RNAs (lncRNAs). LncRNAs are a novel class of mRNA-like transcripts with no protein-coding capacity in carcinogenesis. They are pervasively transcribed in the genome and involved in a spectrum of biological processes, such as cell proliferation, differentiation, and apoptosis [12-15]. Although lncRNAs have little or no protein-coding capacity, many studies have shown that they can regulate protein-coding gene expression at epigenetic, transcriptional, post-transcriptional, and other levels. Moreover, they participate in chromatin modification, X chromosome inactivation, and genomic imprinting [16, 17]. The expression of lncRNAs during tumorigenesis and progression can be either downregulated or upregulated compared with corresponding normal tissues, demonstrating either oncogenic or tumor suppressive functions [18]. Those findings indicate that aberrant expression of lncRNAs might be a substantial contributor in cancer development and could be used as potential novel biomarkers and targets for cancer treatment [19-23].

Urothelial carcinoma associated 1 (UCA1) belongs to the human endogenous retrovirus H (HERV-H) family [24]. The transcript levels of UCA1 were significantly elevated in tongue squamous cell carcinoma (TSCC) [25], bladder cancer [26], urothelial cancer [27], and ovarian cancer cells [28]. Ectopic expression of UCA1 could promote cell proliferation, motility, and invasion. Also UCA1 increase drug resistant ability of bladder cancer cells [26, 29, 30]. However the expression of UCA1 in ovarian cancer patients and the relationship between UCA1 and ovarian cancer has not been reported yet.

In the present study, the authors examined the role of UCA1 in ovarian cancer by a retrospective analysis of 26 patients' ovarian cancer specimens and clinicopathological parameters, hoping to reveal the potential efficacy of UCA1 as a biomarker and therapeutic target in ovarian cancer.

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Materials and Methods

Tissue samples and cell lines

A total of 26 patients diagnosed with International Federation of Gynecology and Obstetrics (FIGO). Stage I to IV ovarian cancer tissues and 16 normal ovarian tissues were collected from 2012 through 2013 at General Affiliated Hospital to Tianjin Medical University (Tianjin, China). Tissue samples were rinsed in saline and immediately frozen with liquid nitrogen for storage at -80˚C. These ovarian cancer patients were the subjects of various histopathological parameter reviews. All cases underwent pathological confirmation: there were 13 serous cystadenocarcinoma, seven mucinous cystadenocarcinoma, three clear cell tumour, and three dysgerminoma. Furthermore all cases had not undergone chemo- or radiotherapy before surgery. The normal ovarian tissues were obtained from oophorectomy of the patients with benign uterine diseases, such as uterine fibroids. Informed consent to use the samples for diagnostic and research purposes were obtained according to the procedures established at this institution. Clinicopathological variables including age, histological grade, FIGO Stage, ascites, and distant metastasis were abstracted from the medical records of each patient. The human ovarian cancer cell lines SKOV3 and OVCAR3 cells were cultured in DMEM, supplemented with 10% fetal bovine serum, 100 U/ml of penicillin, and 100 μg/ml of streptomycin, in a humidified atmosphere of 5% CO_2 at 37˚C.

RNA extraction and cDNA synthesis

Total RNA was isolated from frozen tissue samples and ovarian cancer cells using TRIzol reagent according to the manufacturer's instruction. Briefly, RNA pellet was dissolved in RNase-free water and stored at −80˚C till further use. The samples were quantified and assessed for quality with a nano-drop bioanalyzer. The purity of total RNA was examined by the absorbance ratio at 260 to 280 nm. Then cDNA synthesis was carried out using a quantitative reverse transcription reagent as given instructions. One microgram of total RNA was reverse-transcribed in 20 μL of reaction volume using reverse transcriptase.

Quantitative real-time PCR analysis

Quantitative real-time polymerase chain reaction (qRT-PCR) was carried out using the sequence detection system at 95˚C for 15 seconds followed by 45 cycles at 95˚C for five seconds and at 60˚C for 30 seconds. Analysis was performed with SYBR green supermix using standard protocols. The primers for UCA1 were upstream 5'-CTTCTGCATAGGATCTGCAATCAG-3' and downstream 5'-TTTTGTCCCCATTTTCCATCATACG-3'. The primers for 18s rRNA were upstream 5'-GTAACCCGTTGAACCCCATT-3' and downstream 5'-CCATCCAATCGGTAGTAGCG-3'. The expression of 18s rRNA was used to normalized that of UCA1. Each assay was done in triplicate, the average was calculated, and the relative expression level of UCA1 were expressed as $2^{-\Delta\Delta Ct}$. Ct was defined as the cycle at which fluorescence was determined to be significantly above background [31].

Statistical analysis

Statistical analysis was performed with SPSS 17.0. Data were presented as means ± standard deviation or the median and interquartile range (IQR). The X^2 test or Fisher exact probability test were used to compare clinicopathological features of the ovarian cancer tissues and normal ovarian tissues with UCA1 expression. All statistical tests were two-sided and a p-value of <0.05 was considered statistically significant.

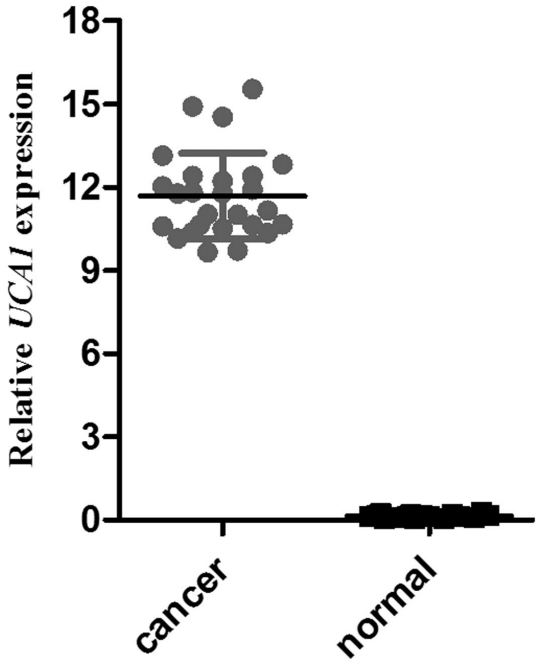

Figure 1. — UCA1 expresssion in ovarian cancer tissues and in normal ovarian tissues.

Results

UCA1 expression in ovarian cancers tissues and normal ovarian tissues

UCA1 expressions were significantly higher in ovarian cancers that those in normal ovarian tissues; the relative expression levels were 11.68 ± 1.54 in ovarian cancer tissues and 0.14 ± 0.07 in normal ovarian tissues (t = -29.868, p = 0.000, Figure 1).

Correlation of UCA1 expression with clinicopathological features in ovarian cancers

Significant associations were found between the *UCA1* mRNA expression levels and clinicopathological features as shown in Table 1. The UCA1 expression was found to be significantly correlated with the FIGO Stage (t = 3.083, p = 0.005), the histological grade(t=5.309, p=0.000), peritoneal effusion condition(t = -3.653, p = 0.001), lymphatic metastasis (t = -4.726, p = 0.000), but not with age (t = -1.047, p = 0.306). These results indicated UCA1 may be involved in invasion and metastasis of ovarian cancer.

Expression of UCA1 in ovarian cell lines.

With the hypothesis that UCA1 is involved in the metastatic progression of ovarian cancer, and given that UCA1 expression levels were associated with lymph node metastasis and a poor prognosis of ovarian cancer patients, the authors then detected the expression of *UCA1* in ovarian cancer cell lines. Total RNA were ex-

Table 1. — *Correlation of UCA1 expression in ovarian cancer with clinical features.*

	No. of patient	UCA1 ($\bar{x} \pm S$)	*p* value	*t*
Age (years)			0.306	-1.047
< 55	10	11.28±1.67		
≥ 55	16	11.93±1.45		
FIGO Stage			0.005*	3.083
I/II	17	12.26±1.53		
III/IV	9	10.58±0.77		
Histological grade			0.000*	5.309
G1	7	13.50±1.47		
G2/G3	19	11.01±0.89		
Peritoneal effusion			0.001*	-3.653
No	12	12.65±1.61		
Yes	14	10.84±0.86		
Lymphatic metastasis			0.000*	-4.726
No	9	10.92±0.81		
Yes	17	13.12±1.59		

* Statistically significance ($p < 0.05$).

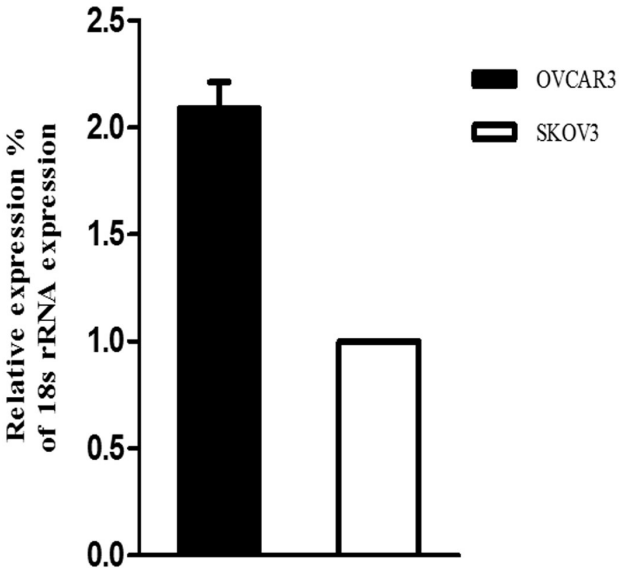

Figure 2. — UCA1 expression in SKOV3 and OVCAR3 cells.

tracted from two ovarian cancer cell lines with different metastatic potential, SKOV3 cells with low invasive ability, and OVCAR3 cells with relatively highly invasive ability. QRT-PCR results showed that UCA1 expressions were detectable in both cell lines and higher expression levels of *UCA1* could be detected in OVCAR3 cells than that in SKOV3 cells (t = -12.530, p = 0.006, Figure 2), which also give the evidence that UCA1 is involved in ovarian cancer metastasis.

Discussion

The present results are the first to report the upregulation of *UCA1* expression in ovarian cancer tissues and to analyze cancer patients' relative clinicopathological parameters. The authors showed that UCA1 was overexpressed in ovarian cancer tissues compared to those in the normal tissues. Also UCA1 overexpression was associated with a lower histopathological subtype and cancer metastasis.

In the last few years, lncRNAs have been strongly supported to their diagnostic value and therapeutic potential for cancer. Ectopic expression of UCA1 could influence cell growth and promote invasion of the bladder cancer cells [26, 30], and promote metastatic but not proliferation ability of TSCC cells [25]. Liu *et al.* [28] firstly reported that UCA1 deregulated in high metastatic potential of ovarian cancer cells by microarray analysis. However expression status and clinical significance of UCA1 in ovarian cancer remains to be clarified. The present authors analyzed the detailed expression pattern of UCA1 in human ovarian cancer tissues. Their results showed that UCA1 expressions were significantly higher in ovarian cancer tissues than those in normal tissues, which was consistent with previous findings in other cancer types. Furthermore, significant associations had been found between UCA1 gene expression and clinicopathological features, including clinical stages, histological grades, peritoneal effusion, and lymph node metastasis. Therefore, the present results suggested an important role of *UCA1* mRNA expression in ovarian tumorigenesis and metastasis.

Functionally, UCA1 regulates cell cycle through CREB via PI3K-Akt dependent pathway in bladder cancer [29]. The UCA1 promoter activity could be regulated by Ets-2 since there were Ets-2 binding sites between nucleotides -385 and -380 in the upstream of transcription start site of the UCA1 gene by EMSA and ChIP analysis [32]. The role of UCA1 is demonstrated to be involved in cisplatin resistance of bladder cancer cell by enhancing the expression of Wnt6 [33]. SRSF protein kinase 1 and apoptosis pathway protein may be involved in the effect of UCA1 in SKOV3 cells. Expression of UCA1 RNA in SKOV3 cells enhanced the cell migration, invasion, and cisplatin resistance [34]. In the present study, the authors evaluated the positive expression of UCA1 and metastasis features in ovarian cancer, but the precise roles and molecular mechanisms of UCA1 in ovarian cancer remain to be further studied in the future.

For ovarian cancer, despite the rapid advancement in proteomic and genomic technology, most of its biomarkers are not very satisfactory and not suitable for asymptomatic populations [35], which substantially contributes to the high mortality rate. The present study showed that

UCA1 might be involved in ovarian cancer proliferation and metastasis. The classification of patients according to UCA1 expression levels provides a valuable tool with which to identify ovarian cancer patients with poor prognoses. The present findings might also extend our knowledge of the biological progression of ovarian cancer and could provide a new therapeutic target for it.

Conclusion

In conclusion, this study provided the first evidence regarding the upregulation of UCA1 mRNA expression in ovarian cancer and revealed its close association with clinical stage, histological grade, peritoneal effusion, and lymph node metastasis. It is worthy of further investigations as this may give new insights into ovarian cancer tumorgenesis, thus working as a novel biomarker and could hold promise for the design of new therapeutic strategies. Attention should be paid to these results which were interpreted within the context of the small patient number, and the inherent limitation selection bias raised a non-randomized design. The present authors recommend that more patients data should be collected, and survival data, including overall and disease-free survival, could be obtained to confirm the conclusion. Further study will be needed to elucidate the underlying mechanism of UCA1 in ovarian cancer.

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Corresponding Author:
Y.M. LI, M.D.
Department of Medical Microbiology
School of Basic Medical Sciences
Tianjin Medical University
No. 22 Qixiangtai Road, Heping District
300070 Tianjin (China)
e-mail: liym@tmu.edu.cn