European Review for Medical and Pharmacological Sciences 2016; 20: 3373-3377

Clinical significance of up-regulated lncRNA NEAT1 in prognosis of ovarian cancer

Z.-J. CHEN, Z. ZHANG, B.-B. XIE, H.-Y. ZHANG

Department of Gynaecology, Linyi People's Hospital, Linyi, Shandong, China

Abstract. – OBJECTIVE: The purpose of the present study was to examine the expression levels of lncRNA NEAT1 in fresh ovarian cancer (OC) tissue and matched adjacent normal tissue specimens, and investigate the clinical significance of NEAT1 in OC.

PATIENTS AND METHODS: NEAT1 levels in OC tissues and matched adjacent normal tissue specimens were tested by qRT-PCR. Then, statistical analysis was performed to explore the associations of NEAT1 expression with the clinical features and the prognosis of OC.

RESULTS: NEAT1 is upregulated in ovarian cancer tissues compared with the corresponding adjacent non-neoplastic tissues. The expression level of NEAT1 was positively correlated with FIGO stage ($p = 0.004$), tumor grade ($p = 0.009$) and distant metastasis ($p = 0.000$). Also, the Kaplan-Meier survival curves revealed that high NEAT1 expression was associated with poor prognosis in OC patients. Multivariate Cox regression analysis showed that NEAT1 expression level ($p < 0.001$) was an independent factor in predicting the overall survival of OC patients.

CONCLUSIONS: These results suggest that NEAT1 has a potential to function as a clinical biomarker for predicting disease invasiveness and prognosis of patients with OC.

Key Words
LncRNAs, NEAT1, Ovarian cancer, Prognosis.

Introduction

Ovarian cancer (OC) is one of the most common tumors of the female reproductive system, and the mortality rate of this disease ranks first among gynecological malignant tumors[1,2]. Despite advances in surgery and chemotherapy, the overall survival of OC patients remains unsatisfactory, with a five-year survival rate of only 30%[3]. One of the most important reasons that lead to the poor outcome of OC patients is most cases diagnostic at an advanced stage. Therefore, the identification of novel diagnostic and prognostic biomarkers, and potential therapeutic targets is crucial for improving the prognosis of OC patients.

Long non-coding RNAs (lncRNAs), a member of the non-coding RNAs family, are greater than 200 nucleotides in length and do not code for proteins, but lncRNAs can regulate gene expression in transcriptional or post-transcriptional level[4-6]. In recent years, always more evidences showed that the expression levels of some lncRNAs were associated with recurrence, metastasis, and prognosis of cancers[7,8]. Gupta et al[9] showed that lncRNA HOTAIR expression was increased in primary breast tumors, and HOTAIR expression level in breast tumors was a powerful predictor of eventual metastasis and death. Zhang et al[10] determined that upregulation of long noncoding RNA TUG1 promotes osteosarcoma cell growth and inhibits apoptosis. Qiu et al[11] found that overexpression of long non-coding RNA HOTAIR predicts poor patient prognosis and promotes tumor metastasis in epithelial ovarian cancer. Unfortunately, the emerging functional role of lncRNA NEAT1 in OC remains largely unknown.

In the present study, we examined the expression level of NEAT1 in OC tissues by using qRT-PCR. Next, we analyzed the relationships between NEAT1 expression and clinicopathological features. Our research revealed that NEAT1 involved in the progression of RCC.

Patients and Methods

Patients and tissue samples

All tumor and matched normal ovarian surface tissue samples were obtained from 149 OC patients who underwent surgery at Linyi People's Hospital in January 2006 and December 2011. None of the patients recruited in this study had

Corresponding Author: Zhen Zhang, MD; e-mail: zhasd321@163.com

undergone preoperative chemotherapy or radiotherapy. All patients had complete 5-year follow-up. After surgical resection, the specimens were put immediately into liquid N2 for 10 min and then into a -70°C ultra-freezer. The clinicopathological features of the patients are summarized in Table I. This work was approved by the Research Ethics Committee of Linyi People's Hospital. Written informed consent was obtained from all of the patients.

RNA isolation and quantitative r eal-time PCR (qRT-PCR)

Total RNA was extracted from OC tissues using Trizol reagent (Invitrogen, San Diego, CA, USA) according to the manufacturer's protocol. The isolated total RNA was reversing transcribed using PrimeScirpt RT Master Mix (Takara, Pudong, Shanghai, China) according to the manufacture's protocol. The primers for NEAT1 and reference gene GAPDH were as following: NEAT1 Forward-5'-TGGCTAGCTCAGGGCTTCAG-3', NEAT1 Reverse-5'-TCTCCTTGCCAAGCTTCCTTC-3'; GAPDH Forward-5'-TGAACGGGAAGCTCACTGG-3', GAPDH Reverse-5'-TCCACCACCCTGTTGCTGTA-3'. The expression level of NEAT-1 was measured by quantitative real-time PCR (qRT-PCR), which was performed using the Applied Biosystems 7900HT (Invitrogen, San Diego, CA, USA) with 1.0 μl of cDNA and SYBR Green Real-time PCR Master Mix (Takara, Pudong, Shanghai, China). The relative expression of NEAT1 was calculated and normalized using the $2^{-\Delta\Delta Ct}$ method about GAPDH.

Statistical Analysis

All statistical analyses were performed using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). Data are expressed as the mean ± SD from at least three separate experiments. Differences between groups were analyzed using Student's *t*-test or chi-square test analysis. Kaplan-Meier and Cox regression analyses were utilized to determine the association between lncRNA NEAT1 and overall survival as well as the prognosis of OC. *p*-values less than 0.05 were considered statistically significant.

Results

lncRNA NEAT1 is Significantly Up-regulated in OC Tissues

qRT-PCR was used to measure NEAT1 expression levels in a total of 149 patients with ccRCC. Data showed that the expression of NEAT1 in OC tissues was higher than in adjacent non-tumor tissues ($p < 0.01$, as shown in Figure 1).

Correlation between NEAT1 Expression and Clinicopathologic Features in Patients with OC

We next assessed the association between NEAT1 expression and clinicopathologic features of OC patients. NEAT1 expression in OC tissues was categorized as low or high according to the median level. As shown in Table I, the results showed that NEAT1 expression was significantly associated with poor clinicopathologic features including FIGO stage (*p*

Table I. Associations between lncRNA NEAT1 expression and clinicopathological characteristics in ovarian cancer.

Characteristics	Group	Number	lncRNA NEAT1		*p*-value
			High	Low	
Age (years)	< 55	66	35	31	0.464
	≥ 55	83	39	44	
Histological subtypes	Serous	112	55	57	0872
	Endometrioid	13	7	6	
	Mucinous	9	4	5	
	Clear cell	9	6	3	
	Others	6	2	4	
FIGO stage	I + II	53	18	35	0.004
	III + IV	96	56	40	
Grade	G1	60	22	38	0.009
	G2 + G3	89	52	37	
Distant metastasis	Yes	56	45	11	0.000
	No	93	29	64	
Tumor size (cm)	≤ 10	85	41	44	0.688
	> 10	64	33	31	

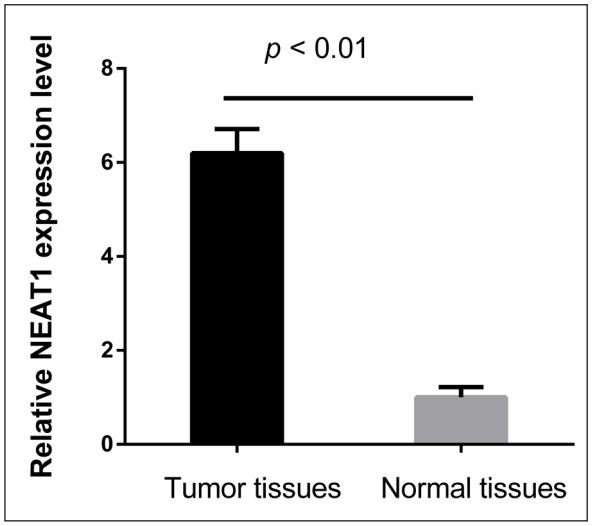

Figure 1. LncRNA NEAT1 expression levels assessed by qRT-PCR in ovarian cancer tissue and adjacent non-tumor tissues.

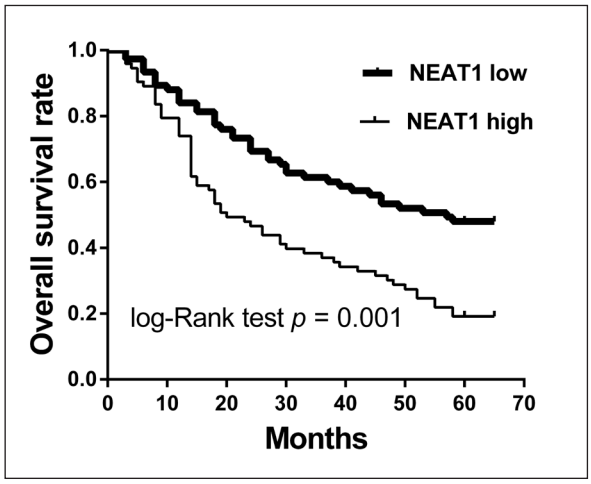

Figure 2. Kaplan-Meier curves for survival time in patients with ovarian cancers divided according to NEAT1 expression: significantly shorter survival times for patients with high NEAT1 expression than for those with low NEAT1 expression ($p = 0.001$).

= 0.004), tumor grade ($p = 0.009$) and distant metastasis ($p = 0.000$). In contrast, there was no association between NEAT1 expression and other clinical factors, such as age and tumor size ($p > 0.05$).

Correlation between NEAT1 Expression and Prognosis of OC Patients

Then, we evaluated the prognostic significance of NEAT1 expression in patients with OC. Using Kaplan-Meier method and log-rank test, we found that the overall survival rate was significantly shorter in patients with high NEAT1 expression compared to those with low NEAT1 expression ($p = 0.001$, Figure 2). Univariate analysis identified tumor grade, distant metastasis, FIGO stage, and high expression of NEAT1 as prognostic factors. Furthermore, multivariate analysis confirmed that NEAT1 expression was an independent risk factor for OS ($p < 0.001$; Table II) in OC patients.

Table II. Univariate and multivariate analyses for overall survival by Cox regression model.

Variable	Univariate analysis			Multivariate analysis		
	Risk ratio	95 % CI	p-value	Risk ratio	95 % CI	p-value
Age (years)						
≥ 55 vs. < 55	1.461	0.554-2.569	0.582			
Histologic grade	1.633	0.791-2.451	0.619			
Serous vs. (Endometrioid + Mucinous + Clear cell + Others)						
Tumor size (cm)	1.216	0.765-2.418	0.715			
≤ 10 vs. > 10						
Tumor grade						
G1 vs. G2 + G3	3.336	1.591-5.458	0.012	3.149	1.417-7.766	0.001
Distant metastasis						
Yes vs. No	4.231	1.688-7.221	0.003	3.515	1.592-6.449	< 0.001
FIGO stage						
I + II vs. III + IV	2.558	1.231-5.477	0.006	2.443	1.398-6.129	0.002
lncRNA NEAT1						
High vs. low	3.443	1.459-7.257	0.005	3.039	1.342-5.784	< 0.001

Discussion

Ovarian cancer is the seventh most common cancer and the eighth most common cause of death from cancer in women[12]. Although a combination of chemotherapy and surgery would improve survival, OC that is associated with malignant metastasis and migration, has an unusually poor prognosis[13]. Thus, discovering more molecular markers associated with the relapse and prognosis of OC is essential. LncRNAs, a group of transcripts without protein coding potential, have gained massive attention in recent years for potentially new and crucial player in gene regulation[14]. In recent years, always more evidences revealed the contribution of lncRNAs as having oncogenic or tumor suppressor roles in tumorigenesis[15]. An investigative focusing on these LncRNAs and their functions in the pathogenesis of OC is warranted.

NEAT1, a nuclear-restricted long non-coding RNA, encodes two isoforms: 3700-nucleotide (nt) NEAT1_1 and 23,000-nt NEAT1_2[16]. Recently, many studies revealed that NEAT1 play an important role in the progression of the tumor. Chen et al[17] found that the expression of NEAT1 was up-regulated in esophageal squamous cell carcinoma tissues and enhanced expression of NEAT1 stimulated the proliferation of esophageal squamous cell carcinoma cells. Guo et al[18] showed that NEAT1 might promote tumorigenesis and metastasis in hepatocellular carcinoma. Chai et al[19] found that NEAT1 was up-regulated in OC patients and cell lines, and NEAT1, whose expression was collaboratively controlled by HuR and miR-124-3p, could regulate ovarian carcinogenesis. All those findings revealed that NEAT1 served as a tumor promoter in OC.

We found that NEAT1 expression in OC tissues was significantly higher than that in matched normal adjacent tissues. We next analyzed the association between the NEAT1 expression and various clinicopathologic factors of the OC patients. We also found that increased NEAT1 expression in OC tissues was significantly correlated with aggressive clinicopathologic features. Kaplan-Meier analysis showed that glioma patients with high NEAT1 expression level had distinctly shorter OS. Moreover, Cox regression analysis proved that NEAT1 could be an independent prognostic indicator for NSCLC patients.

Conclusions

We demonstrated that NEAT1 expression was significantly upregulated in OC, and the high expression of NEAT1 was associated with worse prognosis. Our results suggested that increased NEAT1 expression could be a valuable marker of tumor progression and for prognosis of OC. Further research is needed to clarify the exact mechanism of NEAT1 in OC.

Conflict of Interests

The authors declare no conflicts of interest.

References

1) Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.
2) Yan HC, Fang LS, Xu J, Qiu YY, Lin XM, Huang HX, Han QY. The identification of the biological characteristics of human ovarian cancer stem cells. Eur Rev Med Pharmacol Sci 2014; 18: 3497-3503.
3) Rustin G, van der Burg M, Griffin C, Qian W, Swart AM. Early versus delayed treatment of relapsed ovarian cancer. Lancet 2011; 377: 380-381.
4) Tsai MC, Manor O, Wan Y, Mosammaparast N, Wang JK, Lan F, Shi Y, Segal E, Chang HY. Long noncoding RNA as modular scaffold of histone modification complexes. Science 2010; 329: 689-693.
5) Tsai MC, Spitale RC, Chang HY. Long intergenic noncoding RNAs: new links in cancer progression. Cancer Res 2011; 71: 3-7.
6) Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, Brugmann SA, Goodnough LH, Helms JA, Farnham PJ, Segal E, Chang HY. Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. Cell 2007; 129: 1311-1323.
7) Qiu MT, Hu JW, Yin R, Xu L. Long noncoding RNA: an emerging paradigm of cancer research. Tumour Biol 2013; 34: 613-620.
8) Zhang JH, Wei HW, Yang HG. Long noncoding RNA SNHG15, a potential prognostic biomarker for hepatocellular carcinoma. Eur Rev Med Pharmacol Sci 2016; 20: 1720-1724.
9) Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, Tsai MC, Hung T, Argani P, Rinn JL, Wang Y, Brzoska P, Kong B, Li R, West RB, van de Vijver MJ, Sukumar S, Chang HY. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature 2010; 464: 1071-1076.
10) Zhang Q, Geng PL, Yin P, Wang XL, Jia JP, Yao J. Down-regulation of long non-coding RNA TUG1 inhibits osteosarcoma cell proliferation and promotes apoptosis. Asian Pac J Cancer Prev 2013; 14: 2311-2315.

11) Qiu JJ, Lin YY, Ye LC, Ding JX, Feng WW, Jin HY, Zhang Y, Li Q, Hua KQ. Overexpression of long non-coding RNA HOTAIR predicts poor patient prognosis and promotes tumor metastasis in epithelial ovarian cancer. Gynecol Oncol 2014; 134: 121-128.
12) Zhang J, Yin XJ, Xu CJ, Ning YX, Chen M, Zhang H, Chen SF, Yao LQ. The histone deacetylase SIRT6 inhibits ovarian cancer cell proliferation via down-regulation of Notch 3 expression. Eur Rev Med Pharmacol Sci 2015; 19: 818-824.
13) Knutson KL, Karyampudi L, Lamichhane P, Preston C. Targeted immune therapy of ovarian cancer. Cancer Metastasis Rev 2015; 34: 53-74.
14) Yang G, Lu X, Yuan L. LncRNA: a link between RNA and cancer. Biochim Biophys Acta 2014; 1839: 1097-1109.
15) Spizzo R, Almeida MI, Colombatti A, Calin GA. Long non-coding RNAs and cancer: a new frontier of translational research? Oncogene 2012; 31: 4577-4587.
16) Naganuma T, Hirose T. Paraspeckle formation during the biogenesis of long non-coding RNAs. RNA Biol 2013; 10: 456-461.
17) Chen X, Kong J, Ma Z, Gao S, Feng X. Up regulation of the long non-coding RNA NEAT1 promotes esophageal squamous cell carcinoma cell progression and correlates with poor prognosis. Am J Cancer Res 2015; 5: 2808-2815.
18) Guo S, Chen W, Luo Y, Ren F, Zhong T, Rong M, Dang Y, Feng Z, Chen G. Clinical implication of long non-coding RNA NEAT1 expression in hepatocellular carcinoma patients. Int J Clin Exp Pathol 2015; 8: 5395-5402
19) Chai Y, Liu J, Zhan.g Z, Liu L. HuR-regulated lncRNA NEAT1 stability in tumorigenesis and progression of ovarian cancer. Cancer Med 2016; 5: 1588-1598.