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Increased expression of LncRNA BANCR and its prognostic significance in human epithelial ovarian cancer

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Summary

Purpose: Long non-coding RNAs (lncRNAs) have been proved to play important roles in the tumorigenesis and development of human epithelial ovarian cancer (EOC). The aim of the present study was to investigate the expression and clinical value of BRAF-activated non-coding RNA (BANCR) in EOC patients. *Materials and Methods:* BANCR expression was detected in 84 EOC and 36 normal ovarian epithelial tissue samples. Association between BANCR levels and clinicopathological factors and patient prognosis was also analyzed. *Results:* BANCR expression was increased in EOC compared with normal ovarian epithelial tissues. Moreover, high expression of BANCR was closely correlated with advanced FIGO stage, higher serum CA125 expression level, and lymph node metastasis. Multivariate regression analysis identified BANCR overexpression as an independent unfavorable prognostic factor in EOC patients. *Conclusions:* These findings suggested that BANCR may act as a tumor promoter in EOC and would be a novel diagnostic and prognostic marker for this disease.

Key words: Long non-coding RNA; BANCR; Ovarian cancer; Prognosis.

Introduction

Epithelial ovarian cancer (EOC) is the most lethal gynecological worldwide [1]. The incidence of EOC is gradually rising because of the increasing number of women who postpone childbearing [2]. More than 70 % of EOC patients are diagnosed at advanced stage, due to vague initial symptoms and the lack of effective screening strategies [3]. Despite recent advances in surgery and chemotherapy, the clinical outcome of EOC patients remains poor, with a five-year survival rate of only 30% [4]. The molecular mechanisms underlying EOC initiation and progression are still obscure, and it is urgent to distinguish reliable biomarkers of EOC for its early detection and effective therapy.

Long non-coding RNA (lncRNA), > 200 nucleotides in length, is a type of non-coding RNA molecular that can regulate gene expression in transcriptional or post-transcriptional level [5, 6]. Recent studies have shown that lncRNAs participate in a large number of cellular processes, such as cell proliferation, differentiation, apoptosis, and cell cycle progression [7]. Emerging evidence indicates that lncRNAs play important roles in the biology of human cancers, which may provide a new but promising way to deal with cancer [8]. Dysregulated lncRNA expression and its involvement in EOC carcinogenesis and development have also been demonstrated [9-11].

BRAF-activated non-coding RNA (BANCR), a 693-bp lncRNA, was originally identified in melanoma cells by Flockhart *et al*. [12]. Subsequently, aberrant lncRNA BANCR expression has been confirmed in papillary thyroid carcinoma [13], retinoblastoma [14], lung cancer [15, 16], gastric cancer [17], and colorectal cancer [18]. In these tumors, BANCR regulated cell proliferation, migration, and invasion, and may serve as a potential oncogene or a candidate tumor suppressor. However, the significance of lncRNA BANCR in EOC is still unclear. In the present study, the authors examined the expression of BANCR in EOC and normal ovarian tissues. Then, the clinicopathological and prognostic significance of BANCR expression in human EOC were statistically analyzed.

Materials and Methods

Patients and clinical specimens

Eighty-four fresh surgical tissue samples of EOC and 36 normal ovarian epithelial tissue samples were collected at The Fourth People's Hospital of Shenzhen, China between February 2007 and October 2009. All samples were frozen immediately in liquid nitrogen and stored at −80 °C until analysed. Patients with two or more different malignancies were excluded. None of the patients had received preoperative radiotherapy, chemotherapy, or hormonal therapy. Normal ovarian epithelial tissue samples were obtained from participants diagnosed with uterine fibroids scheduled to undergo hysterectomy and oophorectomy. Clinical and pathological data of the EOC patients are shown in Table 1. Tumor stage was classified according to the criteria of the International Federation of Gynecologists and Obstetricians (FIGO). Follow-up data were available for all patients. Overall survival (OS) was

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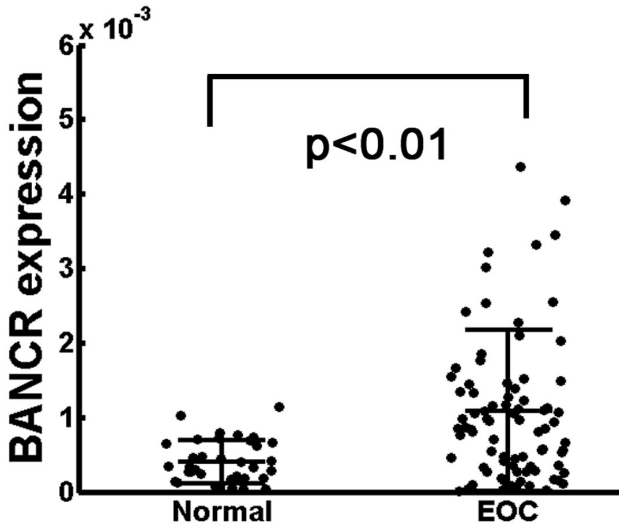

Figure 1. — Relative BANCR expression levels in epithelial ovarian cancer tissues and normal ovarian surface epithelial tissues ($p < 0.01$, Student's *t*-test).

defined as the amount of time from the day of primary surgery to the date of death or the end of follow-up (for living patients). The ethical committees of the present hospital approved this study, and informed consent was obtained from all patients.

RNA extraction, reverse transcription, and qRT-PCR

Total RNA was extracted using the Trizol reagent according to the manufacturer's instructions. RNA was reverse transcribed into cDNA using the Prime-Script one step RT-PCR kit. BANCR expression levels were measured with qRT-PCR using an ABI7500 system and the SYBR Green PCR Master Mix. GAPDH was used as an internal control. The primer sequences for BANCR were 5'-ACAGGACTCCATGGCAAACG-3' (forward) and 5'-ATGAAGAAAGCCTGGTGCAGT-3' (reverse). Each assay was performed in triplicate, and relative BANCR expression was normalized to GAPDH using the 2^{-DCt} method.

Statistics

All statistical analyses were performed using the SPSS 17.0 software package. Continuous data were analyzed with an independent *t*-test, and the Chi-square test was applied to examine the relationship between BANCR expression and clinicopathologic characteristics. Survival curves were constructed with the Kaplan-Meier method and compared by log-rank tests. The significance of survival variables was evaluated using a multivariate Cox proportional hazards regression analysis. A $p < 0.05$ was considered statistically significant.

Results

BANCR expression is increased in EOC tissues

BANCR expression levels in 84 epithelial ovarian tumors and in 36 samples of normal ovarian surface epithelial tissues were measured by qRT-PCR. As in Figure 1, the results showed that BANCR levels were significantly higher in EOC tumors than in normal tissues ($p < 0.001$).

Table 1. — *Correlation between BANCR expression and different clinicopathological features in patients with epithelial ovarian cancer.*

	High BANCR expression (n, %)	Low BANCR expression (n, %)	*p*-value
Age (years)			
≥ 50	30 (54.5%)	25 (45.5%)	
< 50	12 (41.4%)	17 (58.6%)	0.359
Histological type			
Serous	18 (56.2%)	14 (43.8%)	
Others	24 (46.2%)	28 (53.8%)	0.501
Histological grade			
G1	22 (53.7%)	19 (46.3%)	
G2	10 (45.5%)	12 (54.5%)	0.799
G3	10 (47.6%)	11 (52.4%)	
FIGO stage			
I/II	11 (28.9%)	27 (71.1%)	0.001
III/VI	31 (67.4%)	15 (32.6%)	
Serum CA 125 level (U/L)			
$< 5.0 \times 10^5$	13 (33.3%)	26 (66.7%)	0.008
$\geq 5.0 \times 10^5$	29 (64.4%)	16 (35.6%)	
Ascites			
No	17 (43.6%)	22 (56.4%)	0.382
Yes	25 (55.6%)	20 (44.4%)	
Lymph node involvement			
No	10 (30.3%)	23 (69.7%)	0.009
Yes	32 (62.7%)	19 (37.3%)	

Association between clinicopathological characteristics and BANCR expression in EOC patients

The authors further analyzed the association between the expression of BANCR and clinicopathological characteristics of EOC. EOC samples were classified into the low expression group (n = 42) and the high expression group (n = 42) according to the median expression level of all EOC samples. The association between clinicopathological characteristics and BANCR expression is summarized in Table 1. The authors found that high BANCR expression levels were closely correlated with advanced FIGO stage ($p = 0.001$), higher serum CA125 expression level ($p = 0.008$), and the occurrence of lymph node metastasis ($p = 0.009$), but not with other clinicopathological variables, such as the age of the patient, histological grade, the histological type of the tumor, and ascites.

BANCR expression is conversely associated with overall survival in EOC patients

In order to identify the prognostic value of BANCR expression, the authors measured the correlation between BANCR levels and OS of EOC patients through Kaplan–Meier analysis and log-rank test. Figure 2 shows that patients in low BANCR expression group had better OS than those in high BANCR expression group ($p < 0.001$). Aside from BANCR expression, univariate Cox proportional hazard regression analysis revealed that histological grade ($p =$

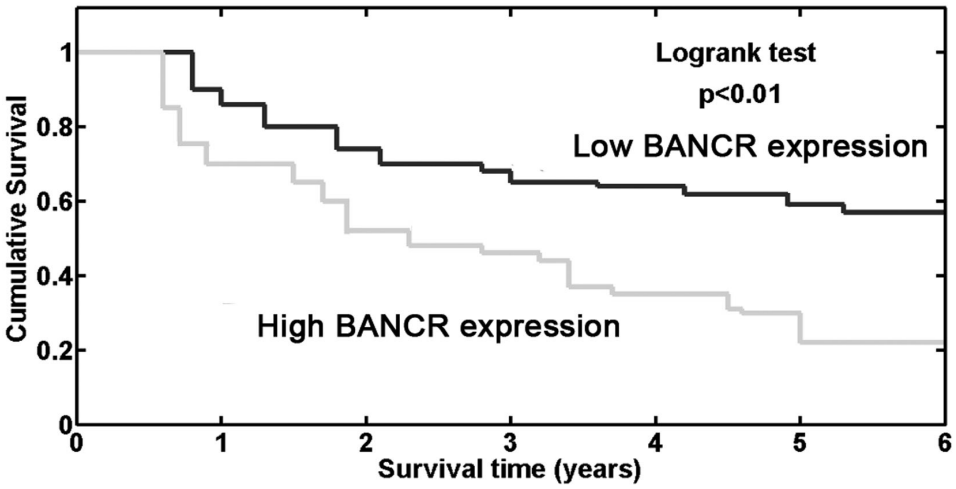

Figure 2. — Overall survival curves for two groups defined by low and high expression of lncRNA BANCR in patients with epithelial ovarian cancer. High BANCR expression levels were significantly associated with poor outcome ($p < 0.001$, log-rank test).

Table 2. — *Univariate and multivariate analysis of overall survival in 84 patients with epithelial ovarian cancer.*

Variables	Univariate analysis		Multivariate analysis	
	RR	P-value	RR	*p*-value
Age (years) (≥50/<50)	0.94	0.225	1.44	0.095
Histological type (serous/non-serious)	1.41	0.136	0.98	0.272
Histological grade (G1/G2+G3)	2.73	0.008	1.49	0.078
FIGO stage (III+IV/I+II)	3.09	0.006	3.04	0.003
Ascites (yes/no)	0.85	0.448	1.53	0.072
Serum CA 125 ($\geq 5.0 \times 10^5$/$<5.0 \times 10^5$)	1.33	0.142	1.16	0.214
Lymph node involvement (yes/no)	2.14	0.012	2.54	0.016
Expression of BANCR (low/high)	3.78	<0.001	2.92	0.008

0.008, RR = 2.73), FIGO stage ($p = 0.006$, RR = 3.09), and lymph node status ($p = 0.012$, RR = 2.14) were also predictive factors for prognosis. Multivariate Cox proportional hazard regression analysis confirmed that high BANCR expression ($p = 0.008$, RR = 2.92) was an unfavorable prognostic factor independent of other clinicopathological factors, including FIGO stage ($p = 0.003$, RR = 3.04) and lymph node metastasis ($p = 0.016$, RR = 2.54; Table 2).

Discussion

The relationship between lncRNAs and tumors has currently become one of the focuses of cancer studies. Abnormal expression of several lncRNAs have been reported in EOC [9, 10, 19-22]. Qiu *et al*. found that lncRNA HOTAIR was overexpressed in ovarian cancer and that its overexpression was correlated with aggressive clinicopathological features and poor survival [9]. Downregulation of HOTAIR would reduce ovarian cancer cell proliferation and promote cell apoptosis in vitro, and inhibit tumor growth in vivo. Gao *et al*. indicated that lncRNA HOST2 promoted tumor cell migration, invasion, and proliferation in EOC through a mechanism involving microRNA let-7b, could be a potent tumor suppressor [11]. In the present study, the authors confirmed increased BANCR expression in EOC tumor samples and its correlation with advanced tumor stage, lymph node involvement, higher serum CA125 level, and shorter OS. Multivariate Cox hazard regression analysis identified high BANCR expression as an indicator of unfavorable prognosis independent of other clinicopathological factors. To the present authors' knowledge, this is the first study to analyze the expression and clinical significance of BANCR in EOC.

BANCR, a recently found lncRNA on chromosome 9, has been proven to be involved in tumorigenesis and progression of several types of human malignancies. BANCR levels in human malignant melanoma tissues increased with advanced tumor stages and knockdown of BANCR suppressed melanoma cell proliferation and migration through MAPK pathway [12, 23]. BANCR overexpression correlated with tumor stage and lymph node metastasis in colorectal cancer and contributed to cancer cell migration through inducing epithelial-mesenchymal transition. In gastric cancer, BANCR expression was increased in tumor tissues compared with paired adjacent normal tissues. High BANCR levels were positively associated with clinical stage, tumor depth, lymph node and distant metastasis, and poor prognosis [17]. In retinoblastoma, BANCR regulated cell proliferation, migration, and invasion in vitro, and overexpressed BANCR expression linked with tumor size, choroidal invasion, and optic nerve invasion [14].

In contrast to the tumor-promotive properties mentioned above, there were few studies indicating that BANCR serves as a potential tumor suppressor gene. Jiang *et al*. reported that BANCR was obviously downregulated in small cell lung cancer tissues, and that suppression of BANCR markedly promoted cancer cell proliferation and migration [16]. In non-small cell lung cancer, reduced BANCR expression was associated with larger tumor size, lymph node

metastasis, advanced TNM stage, and shorter overall survival. Ectopic expression of BANCR impaired cell viability and invasion, leading to the inhibition of metastasis in vitro and in vivo [15]. Altogether, the role of lncRNA BANCR in human malignancies may be multifaceted, depending on the involved tissue.

In conclusion, the authors found that lncRNA BANCR expression was significantly increased in human EOC tissues and correlated with malignant status. This study also showed that high BANCR levels confered poor prognosis in EOC patients. These findings suggested that BANCR may act as a tumor promoter in EOC and would be a novel diagnostic and prognostic marker for this disease. Due to the limited sample size in this study, more studies would be needed to further verify the clinical significance of BANCR in EOC patients.

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