Overexpression of long noncoding RNA PTPRG-AS1 is associated with poor prognosis in epithelial ovarian cancer

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SUMMARY

OBJECTIVE: Long noncoding RNAs (lncRNAs) have been shown to play a critical role in tumor progression. Abnormal expression of LncRNA PTPRG antisense RNA 1 (PTPRG-AS1) has been reported in several tumors. Hence, we aimed to determine the expression and clinical significance of PTPRG-AS1 in epithelial ovarian cancer (EOC) patients.

METHODS: The expressions of PTPRG-AS1 were assessed in 184 pairs of EOC tumor specimens and adjacent normal tissues. The levels of target lncRNAs and GAPDH were examined using standard SYBR-Green methods. The relationships between the expressions of PTPRG-AS1 and the clinicopathological features were analyzed using the chi-square test. Multivariate analysis using the Cox proportional hazards model was performed to assess the prognostic value of PTPRG-AS1 in EOC patients.

RESULTS: We confirmed that the expressions of PTPRG-AS1 were distinctly higher in the EOC tissue compared with the adjacent non-tumor specimens ($p < 0.01$). Higher levels of PTPRG-AS1 in EOC patients were associated with advanced FIGO stage ($p = 0.005$), grade ($p = 0.006$), and distant metastasis ($p = 0.005$). Survival analyses revealed that patients with high expressions of PTPRG-AS1 had a distinctly decreased overall survival ($p = 0.0029$) and disease-free survival ($p = 0.0009$) compared with those with low expressions of PTPRG-AS1. Multivariate assays indicated that PTPRG-AS1 expression was an independent prognostic factor for both overall survival and disease-free survival in EOC (Both $p < 0.05$).

CONCLUSIONS: Our study suggests that PTPRG-AS1 may serve as a novel prognostic biomarker for EOC patients.

KEYWORDS: RNA, long noncoding. Carcinoma, ovarian epithelial. Prognosis.

INTRODUCTION

Ovarian cancer is a leading cause of death among gynecological tumors worldwide[1]. Epithelial ovarian cancer (EOC) is a major subtype of ovarian cancer, accounting for ~85% cases[2]. Although the quality of life of many EOC patients has been distinctly improved with the advancement of treatment strategies in recent years, the five-year survival rate of advanced-stage EOC is unsatisfactory (below 25 %)[3,4]. The poor prognosis of EOC patients is mainly due to the frequent recurrence and metastasis and various drug-resistance[5]. Thus, the identification of reliable biomarkers for EOC is urgently required for the improvement of EOC treatments.

Long noncoding RNAs (lncRNAs), > 200 nucleotides in length, are a newly identified type of by-products of genetic transcription with limited protein-coding function due to the lack of open reading frame[6]. Growing studies have demonstrated the potential regulators of lncRNAs in the modulation of genes by several mechanisms, such as transcriptional and post-transcriptional levels[7]. Given the positive influence of lncRNAs on tumor-related genes, it is no surprise that lncRNAs may be involved in the progression of cancers[8,9]. In recent years, more and more dysregulated lncRNAs in various tumors have been identified using high throughput sequencing, followed by further demonstration using RT-PCR experiments[10,11]. Then, many functional experiments revealed that lncRNAs can modulate tumor cellular behaviors by acting as tumor promoters or anti-oncogenes[12,13]. The frequent dysregulation of lncRNAs and their important function in tumor progression highlighted the great potential of lncRNAs as novel biomarkers[14,15].

LncRNA PTPRG antisense RNA 1 (PTPRG-AS1), a newly identified lncRNA, was firstly functionally elucidated in nasopharyngeal carcinoma by Yi et al.[16]. Previously, the expression of PTPRG-AS1 was also reported in lung and breast cancer[17,18]. However, their function in the above two tumors remained to be explored. Up to date, whether PTPRG-AS1 was abnormally expressed in EOC has not been confirmed. In this study, for the first time, we provided evidence that PTPRG-AS1 was highly expressed in EOC tissues and has the potential to act as a novel prognostic biomarker for EOC patients.

METHODS
Patients and Specimens

EOC tissues (184 cases) and matched non-tumor specimens from patients who underwent operations between July 2011 and June 2014 were obtained from Changzhi Medical College. Post-operative EOC samples were verified via pathological diagnosis by three experienced pathologists. None of the patients had received chemotherapy and radiotherapy prior to the collection of specimens. All specimens were frozen immediately in liquid nitrogen and stored at -80 °C for the application of RT-PCR. The clinical data of 184 patients are presented in Table I. Clinical specimens were applied for experiments after obtaining informed consent from all patients. Our research protocols were approved by the Ethics Committee of our hospital.

TABLE 1. RELATIONSHIP BETWEEN PTPRG-AS1 EXPRESSION AND CLINICOPATHOLOGICAL VARIABLES IN EOC PATIENTS.

Variable	Number	PTPRG-AS1 expression		p value
		High	Low	
Age (y)				NS
<50	94	41	53	
≥50	90	50	40	
Tumor size (cm)				NS
≤ 8	109	49	60	
> 8	75	42	33	
Ascites				NS
<100	104	47	57	
≥100	80	44	36	
FIGO stage				0.005
I + II	112	46	66	
III + IV	72	45	27	
Grade				0.006
G1	119	50	69	
G2 + G3	65	41	24	
Distant metastasis				0.005
Yes	127	54	73	
No	57	37	20	

RNA isolation and real-time RT-PCR

Total RNA was extracted from EOC tissues and matched normal specimens by Trizol reagent (Invitrogen, Carlsbad, CA, USA). 400 ng RNAs were collected and then converted into cDNA using the PrimeScript™ RT reagent Kit (Takara, Dalian, Niaoning, China). The levels of target lncRNAs and GAPDH were examined using standard SYBR-Green methods on ABI-7300Plus PCR System (Applied Biosystems, Foster City, CA, USA). The Opticon Monitor 2 software was used for the assays of the PCR data. Human GAPDH was used as an endogenous control. All primers were purchased from Gema (Pudong, Shanghai, China) and the sequences are were as follows: PTPRG-AS1 sense 5'-AAGCCAAGCAGTCAGAAGC-3'; PTPRG-AS1 antisense 5'-CAATGACCCCTTCATTGAC-3'; GAPDH sense 5'-GACAAGCTTCCCGTTCTCA-3'. The levels of PTPRG-AS1 were quantified by examining Ct values and normalized by the use of the $2^{-\Delta\Delta ct}$ methods.

Statistical analysis

All statistical analyses were performed using the SPSS 17.0 software package (SPSS Inc., Chicago, IL, USA). The differences in PTPRG-AS1 expressions between the two groups were examined by applying the two-sided Student's t-tests. Chi-square tests were

performed to determine the clinical significance of PTPRG-AS1 on clinical characteristics. The survival curve was calculated using Kaplan-Meier methods. The univariate and multivariate assays were performed in a Cox's regression model. Differences were considered statistically significant when $p < 0.05$.

RESULTS

Upregulated PTPRG-AS1 was observed in EOC tissues

Previously, PTPRG-AS1 levels had been confirmed to be overexpressed in breast cancer and nasopharyngeal carcinoma. Hence, we also performed RRT-PCR to

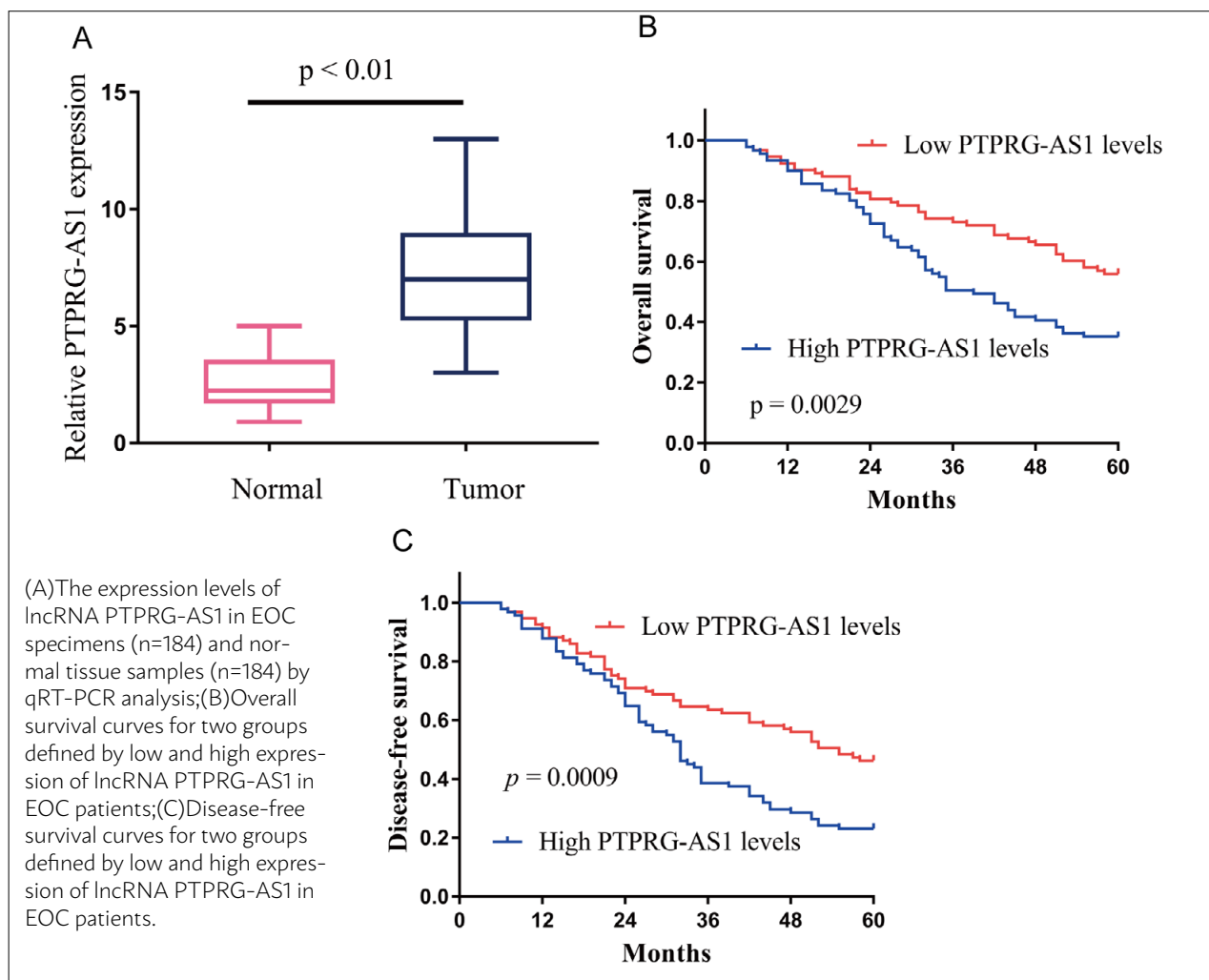**FIGURE 1.** CORRELATIONS BETWEEN PTPRG-AS1 EXPRESSIONS AND CLINICAL OUTCOME OF EOC PATIENTS

(A)The expression levels of lncRNA PTPRG-AS1 in EOC specimens (n=184) and normal tissue samples (n=184) by qRT-PCR analysis;(B)Overall survival curves for two groups defined by low and high expression of lncRNA PTPRG-AS1 in EOC patients;(C)Disease-free survival curves for two groups defined by low and high expression of lncRNA PTPRG-AS1 in EOC patients.

TABLE 2. MULTIVARIATE ANALYSES FOR OVERALL SURVIVAL AND DISEASE-FREE SURVIVAL BY COX REGRESSION MODEL.

Variable	Overall survival			Disease-free survival		
	HR	95% CI	p	HR	95% CI	p
Age	0.724	0.436-1.885	0.273	0.982	0.587-1.992	0.318
Tumor size	0.562	0.328-1.852	0.437	0.882	0.572-2.127	0.114
Ascites	0.852	0.562-1.775	0.218	0.774	0.627-2.014	0.128
FIGO stage	3.276	1.237-4.886	0.012	3.352	1.375-5.012	0.015
Grade	3.015	1.382-4.662	0.018	3.229	1.429-5.212	0.013
Distant metastasis	3.427	1.428-5.337	0.003	3.672	1.528-5.663	0.001
PTPRG-AS1 expression	2.896	1.283-4.952	0.009	3.018	1.365-5.328	0.005

examine whether the expression levels of PTPRG-AS1 were abnormal in EOC tissues. As presented in Figure 1A, the higher levels of PTPRG-AS1 were observed in EOC specimens compared to matched normal specimens ($p < 0.01$). Thus, the expression trend of PTPRG-AS1 in EOC tissues was in line with that in the two tumors above, suggesting its possible functional effects in EOC patients.

Association between clinicopathological characteristics and PTPRG-AS1 expressions in EOC patients

Having shown the distinct up-regulation of PTPRG-AS1 in EOC, we further explored its possible influence in the clinical progress of EOC. Using the median PTPRG-AS1 levels of all EOC samples, all 184 samples were classified into a low-expressing PTPRG-AS1 group and a high-expressing PTPRG-AS1 group. Then, the chi-square test was performed, and the results revealed that high levels of PTPRG-AS1 in EOC patients displayed advanced FIGO stage ($p = 0.005$), grade ($p = 0.006$) and distant metastasis ($p = 0.005$). However, no distinct differences were observed between PTPRG-AS1 levels and other factors ($p > 0.05$).

Correlations between PTPRG-AS1 expressions and clinical outcome of EOC patients

Then, we further examined whether PTPRG-AS1 had prognostic value for the prediction of overall survival and disease-free survival of EOC patients. Our group collected five-year survival data from 184 EOC patients and then performed statistical assays using Kaplan-Meier methods. As shown in Figure 1B, patients with high levels of PTPRG-AS1 had shorter overall survival than low-level groups ($p = 0.0029$). In addition, a similar influence of high PTPRG-AS1 on disease-free survival was also observed ($p = 0.0009$, Figure1C). Then, we performed multivariate assays for the prognostic determination of several clinical factors, finding that PTPRG-AS1 levels, FIGO stage, grade, and distant metastasis were independent prognostic indicators for both overall survival and disease-free survival ($p < 0.05$, Table II).

DISCUSSION

EOC, a major concern for women's health worldwide, is correlated with a high mortality rate[19]. The lack of satisfactory therapeutic outcomes encouraged a big scientific effort for the discovery of new approaches for early screening, prediction of clinical outcome, and cancer monitoring[20,21]. In recent years, many possible biomarkers were identified. However, low sensitivity and specificity of these markers limited their clinical application[22]. Recently, as novel gene modulators, lncRNAs may be used as novel biomarkers due to their involvement in tumor progression and the development of chip sequencing[23,24].

Aberrant expressions of several lncRNAs have been demonstrated to be involved in the modulation of the recurrence, invasion, and clinical outcome of EOC[25]. For instance, Liu et al.[26] reported that lncRNA NEAT1, a positive regulator involved in many tumors, was overexpressed in ovarian cancer and promoted the metastasis of tumor cells via the modulation of miRNA-382-3p/ROCK1 axis. Li et al.[27] showed that lncRNA H19 whose upregulation was a common event in various tumors was a distinct upregulated lncRNA in EOC. Further assays revealed that lncRNA H19 served as a tumor promoter in EOC progression due to its knockdown suppressing TGF-β-induced EMT pathway by regulating miRNA-370-3p. These findings supported the fact that lncRNAs acted as important regulators in the development of EOC. Thus, the possibilities of lncRNAs used as novel biomarkers encouraged us to further identify novel functional lncRNAs in EOC.

Recently, a newly identified lncRNA, PTPRG-AS1, was reported to be highly expressed in several tumors. Besides, its oncogenic roles in nasopharyngeal carcinoma were also confirmed using loss-of-function assays. In this study, we firstly provided clinical evidence that PTPRG-AS1 was distinctly overexpressed in human EOC tissues. The expression trend of PTPRG-AS1 in EOC tissues was consistent with that in lung cancer and breast cancer. Then, we analyzed the clinical value of PTPRG-AS1 in EOC patients and found that PTPRG-AS1 expression predicted distant metastasis, suggesting that the levels of PTPRG-AS1 may influence the tumor progression of EOC. Furthermore, Kaplan-Meier analysis suggested that the patients with high PTPRG-AS1 expression had a poor clinical outcome. Finally, the multivariate analysis suggested that PTPRG-AS1 expression provided an independent prognostic biomarker for the prediction of both overall survival and disease-free survival. However, the precise roles of

PTPRG-AS1 on the progression of EOC remain to be elucidated, and further studies using cell and animal models are necessary.

CONCLUSION

Our results showed that PTPRG-AS1 may be useful for evaluating the clinical outcome and may provide a new treatment target for patients with EOC.

Conflict of Interest

The authors declare that they have no conflict of interests.

Author's Contribution

All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

RESUMO

OBJETIVO: Sabe-se que RNAs longos não codificantes (lncRNAs) desempenham um papel crítico na progressão tumoral. A expressão anormal do RNA 1 anti-senso LncRNA PTPRG (PTPRG-AS1) já foi relatada em diversos tumores. Assim, buscamos determinar a expressão e significância clínica do PTPRG-AS1 em pacientes com câncer de ovário epitelial (COE).

METODOLOGIA: As expressões do PTPRG-AS1 foram avaliadas em 184 pares de amostras tumorais de COE e tecidos normais adjacentes. Os níveis de lncRNAs e GAPDH alvo foram examinados usando o método padrão de SYBR Green. As relações entre as expressões do PTPRG-AS1 e as características clínico-patológicas foram analisadas através do teste qui-quadrado. Uma análise multivariada utilizando o modelo de riscos proporcionais de Cox foi realizada para avaliar o valor prognóstico do PTPRG-AS1 em pacientes com COE.

RESULTADOS: Constatou-se que as expressões do PTPRG-AS1 foram nitidamente maiores nos tecidos de COE em relação aos espécimes adjacentes não tumorosos (p<0,01). Níveis mais elevados do PTPRG-AS1 em pacientes com COE foram associados a um estágio avançado de FIGO (p = 0,005), grau (p = 0,006) e metástases à distância (p = 0,005). As análises de sobrevida revelaram que pacientes com expressões elevadas do PTPRG-AS1 tiveram uma diminuição significativa da sobrevida global (p = 0,0029) e da sobrevida livre de doença (p = 0,0009) em relação àqueles com baixas expressões do PTPRG-AS1. As análises multivariadas indicaram que a expressão do PTPRG-AS1 foi um fator de prognóstico independente tanto para a sobrevida global quanto para a sobrevida livre de doença em pacientes com EOC (p < 0,05).

CONCLUSÃO: Nosso estudo sugere que o PTPRG-AS1 pode ser um novo biomarcador prognóstico para pacientes com COE.

PALAVRAS-CHAVE: RNA longo não codificante. Carcinoma epitelial do ovário. Prognóstico.

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