长非编码RNA GAS5在卵巢癌患者中的低表达及其临床意义

李 婕[1]，李银广[1]，陈爱月[1]，李珠玉[2]，侯文汇[2]，游泽山[2]
（中山大学附属第一医院 1. 东院妇产科 广东 广州 510700；2. 妇产科 广东 广州 510080）

摘 要：【目的】探讨长链非编码RNA GAS5在卵巢癌中的表达水平及其临床意义。【方法】收集卵巢癌患者的瘤体组织标本，通过实时荧光定量PCR检测GAS5在卵巢癌组织中的表达水平。采用相关性分析，分析其表达水平与临床病理特征之间的关系、及其与疾病预后之间的关系。【结果】在卵巢癌瘤体组织中GAS5表达显著下降（$P = 0.0004$）；GAS5的表达与瘤体体积呈负相关（d < 5 cm *vs.* d > 5 cm，$P < 0.000\ 1$）；GAS5的表达与肿瘤期别呈负相关（Ⅰ~Ⅱ grades *vs.* Ⅲ~Ⅳ grades，$P = 0.0086$）。采用Kaplan-Meier分析表明，GAS5表达水平越低患者预后和生存越差。【结论】GAS5在卵巢癌中的表达情况与临床病理特征以及预后相关，可作为潜在的卵巢癌检测标记物。

关键词：长非编码RNA GAS5；卵巢癌；临床意义
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Decreased Expression of Long Non-Coding RNA GAS5 in Ovarian Cancer Patients and Its Clinical Significance

LI Jie[1], LI Yin-guang[1], CHEN Ai-yue[1], LI Zhu-yu[2], HOU Wen-hui[2], YOU Ze-shan[2]
(1. Department of Gynecology, The Eastern Hospital of the First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510700, China; 2. Department of Gynecology, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou 510080, China)
Corresponding to: YOU Ze-shan, E-mail: youzeshan888@21cn.com

Abstract: 【Objective】 Dysregulated long noncoding RNAs (lncRNAs) have been found involved in human diseases, including cancers. Long non-coding RNA growth arrest-specific 5 (GAS5) was reported to be dysregulated in different types of cancers. However, the role of GAS5 in ovarian cancer remains elusive. 【Methods】 In the present study, the expression of GAS5 was detected in 108 ovarian cancer tissues and compared adjacent normal tissues by quantitative real-time PCR (qRT-PCR). 【Results】 The results showed that the expression levels of lncRNA GAS5 were significantly decreased in cancer tissues ($P = 0.0004$), and it was negatively correlated with tumor size (< 5 cm *vs.* > 5 cm, $P < 0.0001$), invasion depth (T1-T2 *vs.* T3-T4, $P = 0.0021$), and tumor grade (Ⅰ~Ⅱ grades *vs* Ⅲ~Ⅳ grades, $P = 0.0086$) in ovarian cancer patients. Kaplan-Meier analysis demonstrated that decreased lncRNA GAS5 expression contributed to poor disease-free survival and overall survival. 【Conclusion】 In conclusion, our study suggested that decreased lncRNA GAS5 expression may beidentified as a potential poor prognostic biomarker in ovarian cancer.

Key word: long non-coding RNA GAS5; ovarian cancer; clinical significance
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Ovarian cancer is a high lethal gynecologic malignancy, which represents more than 3% of human cancers among women, and the fifth leading cause of death among the female population[1]. In the last two decades, although advances intherapeutic modalities ofovarian cancer had been greatly improved, the mortality of ovarian cancer has remained virtually unimproved[1]. In order to improve the disease free and

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作者简介：李婕，硕士，研究方向：Lnc RNA与卵巢肿瘤发病的关系，E-mail：jannielee1997@163.com。游泽山，通信作者，教授，主任医师，研究方向：妇科恶性肿瘤，E-mail：youzeshan888@21cn.com。

long-term survival of women with ovarian cancer, intensive research efforts have been made towards the detection and treatment of ovarian cancer for the last 10 years, butthe improvement seems modest[2]. Therefore, there is still urgent need for researchers to investigate the pathogenesis of ovarian cancer and provide some promising new therapy targets for ovarian cancer patients. It's well known that multiple alterations in tumor suppressing genes and oncogenes are involved in the carcinogenesis ovarian cancer[3-4]. Recent studies on epigenetics have helped us better understand the carcinogenesis of ovarian cancer, which also provide some potential therapy targets for ovarian cancer[5-6]. Large number of basic research clinical experiments suggested the critical roles of epigenetics in the development and progression of ovarian cancer[4-6]. Epigenetics mainly including histone modifications, DNA methylation, and non-coding RNAs, and long noncoding RNAs (lncRNAs) are developed as newly epigenetic biomarker[7]. LncRNAs are a class of RNAs that with more than 200 nt in length with limited or no protein-coding capacity[8]. However, lncRNAs play critical roles in regulating gene expression and other biological functions, including human cancers[9-11]. LncRNA HOTAIR (Hox transcript antisense intergenicRNA) is a well known oncogenic lncRNA involved in tumor pathogenesis. LncRNAHOTAIR has been consistently increased and identified as a strong prognosis biomarker of patient with different human cancers[12-13]. LncRNA GAS5 (Growth Arrest-Specific Transcript 5) previously was consistently decreased and identified as a tumor-suppressor in stomach cancer[14], breast cancer[15] and gastric cancer[16], though its functional significance has not yet been established. In our present study, we found that lncRNA GAS5 was decreased in human ovarian cancer[17]. However, the clinical significance of lncRNA GAS5 in ovarian cancer is still known poorly. We thus conducted this study to examine the expression of lncRNA GAS5 in ovarian cancer patients and assess its clinical significance by analyzing the relationships of lncRNA GAS5 with clinicopathological characteristics of ovarian cancer patients.

1 Materials and methods

1.1 Clinical ovarian cancer samples

A total of 108 ovarian cancer tissues and matched adjacent normal tissues were obtained from patients who had underwent surgery at the First Affiliated Hospital of Sun Yat-sen University between 2008 and 2013, and were diagnosed with ovarian cancer based on histopathological evaluation according to the FIGO (International Federation of Obstetrics and Gynecology) criteria. Clinical pathology information was available for all samples (Table 1). No local or systemic treatment was conducted in these patients before the operation. All tissues were collected and immediately frozen in liquid nitrogen, and stored at -80 °C until RNA extraction. This study was approved by the Research Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University and written consent forms were obtained from all recruited patients.

1.2 RNA extraction and quantitative real-time PCR (qRT-PCR) analysis

The expression level of lncRNA GAS5 in cancer tissues was detected by qRT-PCR. Total RNA was extracted from tissues using TRIzol reagent (Invitrogen Inc., USA) following the manufacturer's instructions. Next, a total of 2 μg RNA for each sample was reverse transcribed into cDNA by using a Reverse Transcription Kit (TaKaRa, Dalian, China). Quantitative real-time PCR analyses were performed with SYBR green Real-time Master Mix (TOYOBO, Japan), and GAPDH was used as a normalizing control. The primers for lncRNA GAS5 were 5'-CTTCTGGG CTCAAGTGATCCT-3' (forward) and 5'-TTGTGC-CATGAGACTCCATCAG-3' (reverse). The primers for GAPDH were 5'-ACACCCACTCCTCCACCTTT-3' (forward) and 5'-TTACTCCTTGGAGGCCATGT-3' (reverse). The qRT-PCR and data collection were performed on Applied Biosystems 7500 Sequence Detection system (ABI, USA) following the program as 95 ℃ for 10 min, followed by 40 repeated cycles at 95 ℃ for 10 s, and 60 ℃ for one minute. The relative

expression of lncRNA GAS5 was calculated and normalized to GAPDH using the $2^{-\Delta\Delta Ct}$ method.

1.3 Statistical analysis

For quantitative data, data were expressed as mean ± SD. Statistical analysis was performed using the SPSS 20.0 (SPSS Inc, USA). Pared student *t*-test was used to analyze the difference in the expression of lncRNA GAS5 between tumor tissues and normal tissues. Chi-square test or logistic regression analysis was then performed to assess the relationships of lncRNA GAS5 expression with clinicopathological characteristics in ovarian cancer patients. Survival analysis was performed using the Kaplan-Meier method. A *P*-value of < 0.05 was considered statistically significant.

2 Results

2.1 GAS5 is down-expressed in human ovarian cancer tissues

To investigate the expression level of lncRNA GAS5 in human ovarian cancer tissues, qRT-PCR was used to detect the expression of GAS5 in 108 paired ovarian cancer samples and matched adjacent normal tissues. As shown in Fig.1, lncNRA GAS5 expression level was significantly decreased in ovarian cancer tissues compared with corresponding adjacent, histological normal tissues (1.84 ± 0.87 *vs* 1.48 ± 0.84) (P = 0.0004). In tumor specimens, the expression level of GAS5 was lower than that of normal tissues, with the median ratio of 1.54 compared with normal counterparts.

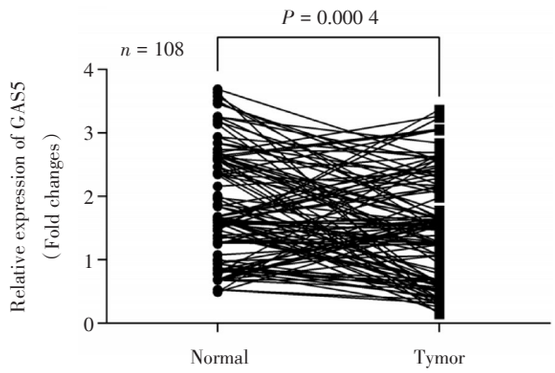

Fig.1 The expression of GAS5 in 108 paired ovarian cancer samples and matched adjacent normal tissues

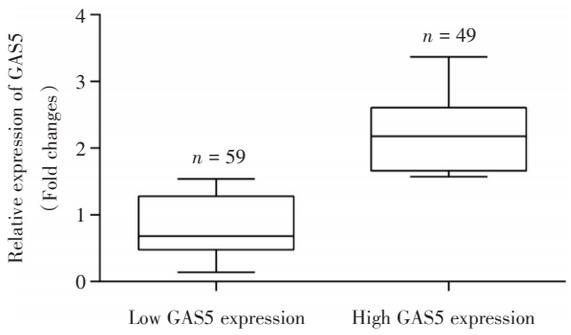

Fig.2 Compared high GAS5 expression group with low GAS5 expression group. High GAS5 expression group (*n* = 49, GAS5 expression ratio ⩾ median ratio). Low GAS5 expression group (*n* = 59, GAS5 expression ratio ⩽ median ratio))

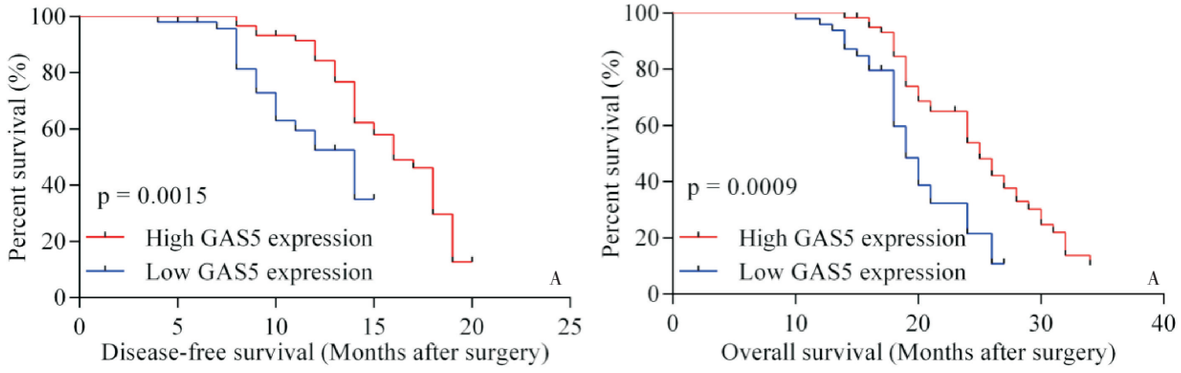

Fig.3 Association of GAS5 expression with patients' survival

Disease-free survival (DFS) and overall survival (OS) curves were plotted according to GAS5 expression level by the Kaplan-Meier analysis and log-rank test.

Table 1　Clinicopathological characteristics and GAS5 expression in 108 patient samples of ovarian cancer

Clinical parameter	Number of cases(%)
Age(years)	
< 55	46(42.59)
> 55	62(57.41)
Size	
> 5 cm	61(56.48)
< 5 cm	47(43.52)
Histologic differentiation	
Well	15(13.89)
Moderately	39(36.11)
Poorly	46(42.59)
Undifferentiatedly	8(7.41)
Invasion depth	
T1-T2	53(49.07)
T3-T4	55(50.93)
FIGO Stages	
Ⅰ~Ⅱ	49(45.37)
Ⅲ~Ⅳ	59(54.63)
Lymphatic metastasis	
Yes	42(38.89)
No	66(61.11)
Distant metastasis	
Yes	46(42.59)
No	62(57.41)
Expression of GAS5	
Low expression	59(54.63)
High expression	49(45.37)

Table 2　The relationship between GAS5 expression and clinicopathological factors in ovarian cancer patients (n = 108)

Clinical parameter	GAS5	
	Low	High
Age(years)[1)]		
< 55	28	18
> 55	31	31
Size[2)]		
> 5 cm	21	40
< 5 cm	38	9
Histologic differentiation[3)]		
Well	8	7
Moderately	17	22
Poorly	28	18
Undifferentiatedly	6	2
Invasion depth[4)]		
T1-T2	21	32
T3-T4	38	17
FIGO Stages[5)]		
Ⅰ~Ⅱ	20	29
Ⅲ~Ⅳ	39	20
Lymphatic metastasis[6)]		
Yes	25	21
No	34	28
Distant metastasis[7)]		
Yes	32	27
No	27	22

Chi-squared test P-value: [1)] P = 0.2619; [2)] P < 0.0001; [3)] P = 0.2626; [4)] P = 0.0021; [5)] P = 0.0086; [6)] P = 0.9596; [7)] P = 0.9284

2.2　The relationship between GAS5 expression and clinicopathological factors in ovarian cancer patients

The clinical pathology findings of 108 ovarian cancer patients are shown in Table 1. The 108 ovarian cancer patients were classified into two groups base on the median ratio of relative GAS5 expression (1.54) in tumor tissues. High GAS5 expression group (n = 49, GAS5 expression ratio≥median ratio). Low GAS5 expression group (n = 59, GAS5 expression ratio ≤ median ratio) (Fig.2). Clinicopathologic factors compared between the two groups are shown in Table 2. The low-GAS5 group was correlated with larger tumor size (P < 0.0001), deeper depth of invasion (P = 0.0021), and higher FIGO stage (P = 0.0086) than the high-GAS5 group. However, GAS5 expression level was not associated with other parameters such as age (P = 0.2619), Histologic differentiation (P = 0.2626), lymphatic metastasis (P = 0.9596), and distant metastasis (P = 0.9284) (Table 2).

2.3　Association of GAS5 expression with patients' survival

In order to conform whether GAS5 expression level correlated with outcome of ovarian cancer patients after ovariectomy. Disease-free survival (DFS) and overall survival (OS) curves were plotted according to GAS5 expression level by the Kaplan-Meier analysis and log-rank test, respectively, and the results were presented in Fig.3A and B. Remarkably, patients with low GAS5 expression level had poorer disease-free survival (P = 0.0015) and overall survival (P = 0.0009). For the OS, 3 years of overall ac-

cumulative survival rates of ovarian cancer patients with high level and low level of GAS5 expression were 74.58% and 46.94%, separately. Low GAS5 expression indicated a shorter overall survival time of ovarian cancer patients (median OS: 19 months) compared with high GAS5 expression patients (median OS: 25 months). Moreover, the 3-year disease-free survival rates for high GAS5 expression and low GAS5 expression were 61.02% and 36.73%, respectively. The median survival time for high GAS5 expression is 16 months, while is 14 months for low GAS5 expression.

3 Discussion

An increasingly studies found that lncRNAs played important roles in fundamental cellular developments and involved in major pathologies, including human cancer[9]. Recently, many studies have reported that lncRNA expression was closely associated with tumorinegesis and prognosis[4-5,14]. LncRNAs may regulate certain tumorigenic processes in ovarian cancer such as cellular growth[18], cell apoptosis[19] and cancer metastasis[20], advocating the usage of lncRNAs as novel biomarkers and therapeutic targets for ovarian cancer. In addition, some lncRNAs, such as lncRNAN-BAT-1[21], Meg3[22], RP11-190D6.2 [23], have been characterized as tumor suppressors in ovarian cancer.

long non-coding RNA growth arrest-specific 5 (GAS5) was originally isolated from a screen for potential tumor suppressors in 1988 which is increased in growth-arrested cells [24]. Previously studies had shown lncRNA GAS5 was unconventionally regulated in prostate cancer, breast cancer, and other human cancers[14,25-27]. Low expression of GAS5 is an adverse prognostic factor for survival in breast cancer and neck squamous cell carcinoma (HNSCC)[27-28]. In addition, overexpression of GAS5 suppressed various cancer cell proliferation and induced cell death[14,25-27]. These data demonstrate that GAS5 is a potential tumor-suppressor.

At present, several long non-coding RNAs have been found to be directly involved in ovarian cancer initiation and development[22-23,29-30]. LncRNA Meg3 is decreased in human ovarian cancer tissues, and upregulated expression of Meg3 interacts with ATG3 to induce autophagic flux which results in suppression of tumorigenesis and progression of human ovarian cancer[22]. LncRNA RP11-190D6.2 was decreased in human ovarian cancer tissues and its functional mechanism is closely associated with cell proliferation, migration and invasion by regulating the WWOX gene expression[23]. Zhang et al found that lncRNA MIR4697HG was significantly increased in human ovarian cancer tissues. Knockdown of lncRNA MIR4698HG significantly suppressed cell proliferation, clonogenic potential, and motility partly by regulating ERK/AKT/MMP9 pathway[29]. Li et al showed that the expression of lncRNA SPRY4-IT1 was significantly upregulated in ovarian tumor tissues and high level lncRNA SPRY4-IT1 expression acted as an independent prognostic factor for progression-free survival and overall survival in ovarian cancer patients. Also, knockdown of lncRNA SPRY4-IT1 suppressed ovarian cancer cell proliferation and arrested cell cycle at a G0/G1 stage[30]. However, the role of lncRNA GAS5 in ovarian cancer is still poorly studied in ovarian cancer.

In this study, we aim to investigate the relationship of lncRNA GAS5 expression with ovarian cancer progression and prognosis. We found that the expression levels of lncRNA GAS5 were significantly decreased in ovarian cancer patients compared with those in adjacent normal subjects. Furthermore, low-GAS5 expression group was correlated with larger tumor size, deeper depth of invasion, and higher FIGO stage. Importantly, patients with low GAS5 expression level had poorer disease-free survival and overall survival. However, GAS5 expression level was not associated with other parameters such as age, histologic differentiation, lymphatic metastasis, and distant metastasis. Taken together, our findings indicate that lncRNA GAS5 acts as a functional anticancer gene in ovarian cancer development, which is an extensional research in ovarian cancer of our previously study [17]. Moreover, the precise role of lncRNA GAS5 in development and progression of ovarian cancer needs to be further elucidated.

In conclusion, we found lncRNA GAS5 was decreased in human ovarian cancer and its down-regulation may be indicative of poor survival rates and a

higher risk for ovarian cancer progression, and it could be play as a potential prognostic marker in ovarian cancer patients.

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（编辑　王晓鹰）