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# Increased long non-coding RNA ARAP1-AS1 expression and its prognostic significance in human gastric cancer: a preliminary study

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**Abstract. – OBJECTIVE: Multiple studies have unveiled that long non-coding RNAs (lncRNAs) contribute to oncogenesis. LncRNA ARAP1 antisense RNA 1 (ARAP1-AS1) has been demonstrated to serve as an oncogene in bladder tumor and colorectal cancer. This study attempted to explore the correlation of ARAP1-AS1 expressions with clinical progress and prognosis in gastric cancer (GC) patients.**

**PATIENTS AND METHODS: RT-PCR was carried out to examine the levels of ARAP1-AS1 in 157 GC patients. The associations between ARAP1-AS1 expression and clinicopathologic features in GC patients were analyzed using the Chi-square test. The prognostic value of abnormally expressed ARAP1-AS1 in GC patients was further analyzed via Kaplan-Meier assays and multivariate survival assays.**

**RESULTS: The levels of ARAP1-AS1 were dramatically increased in GC samples compared with paired adjacent non-tumor specimens ($p$=0.01). The upregulation of ARAP1-AS1 was distinctly associated with TNM stage ($p$=0.010) and lymphatic metastasis ($p$=0.007). Further survival study revealed that patients with higher levels of ARAP1-AS1 had shorter overall survival ($p$=0.0020) and disease-free survival than those with lower levels of ARAP1-AS1. Finally, multivariate survival assay identified ARAP1-AS1 upregulation as an independent unfavorable prognostic factor in GC patients.**

**CONCLUSIONS: Our preliminary results identified a novel GC-related factor, ARAP1-AS1 which may be a potential prognostic biomarker for GC patients.**

*Key Words:*
LncRNA ARAP1-AS1, Gastric cancer, Prognosis.

## Introduction

Gastric cancer (GC) is the 4th most common cancer worldwide and it is the leading cause of cancer-associated death in China[1]. A range of risk factors for GC have been established, such as lymph nodes ratio, smoke, etc[2]. Although the great improvements of clinical treatment in surgery combined with radiotherapy and chemotherapy have been achieved, the long-term survivals of GC patients remain poor due to the diagnosis of many patients at an advanced stage accompanied by distant metastases which causes treatment failure[3,4]. In addition, the potential mechanisms involved in GC development remain largely unclear. Therefore, it is of great significance to identify sensitive biomarkers for early detection and targeted treatment of GC.

Long non-coding RNAs (lncRNAs) are a diverse class of non-proteins-coding transcripts longer than 200 nucleotides[5]. Previous, Human Genome Project suggests lncRNAs as "junk sequences" during evolution[6]. However, many studies in the last twenty years have confirmed that lncRNAs may be involved in various biological processes, such as embryonic developments, cellular growth, and embryogenesis, *via* modulating the expressions of various genes at various levels[7,8]. In recent years, the great advancement of novel sequencing technology involved in the gene expressions indicates a large number of abnormally expressed lncRNAs which are also demonstrated to be involved in the progression of several tumors, thus acting as tumor suppressors or oncogenes[9,10]. In addition, given the extensive roles of lncRNAs in tumor development and its frequent dysregulation in tumor tissues and blood, increasing attentions focus on the possible potential of some important lncRNAs used as novel and sensitive biomarkers[11-13]. Previously, several lncRNAs possessing the diagnostic and prognostic values have been identified in more and more clinical assays, such as lncRNA HOTAIR, lncRNA-ATB, and lnc01614[14-16].

LncRNA ARAP1 antisense RNA 1 (ARAP1-AS1), a recently identified lncRNA by Teng et al[17], was shown to be highly expressed in bladder can-

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cer. In their functional assays, ARAP1-AS1 was firstly demonstrated to serve as a tumor promoter in bladder cancer. Then, although the dysregulation of ARAP1-AS1 was also reported in several other tumors, the specific roles of ARAP1-AS1 and its clinical significance in those tumors remain largely unclear[18,19]. In this study, for the first time, we reported that ARAP1-AS1 was an upregulated lncRNA in GC. Then, we performed a series of clinical assays for the exploration of the clinical significance of ARAP1-AS1 in GC.

## Patients and Methods

### Patients and Clinical Samples

The tumor specimens and their adjacent non-tumor specimens were obtained from a panel of 157 GC patients pathologically diagnosed by two pathologists at the Zhuji People's Hospital of Zhejiang Province (China) from July 2011 to June 2013. The fresh samples including tumor and non-neoplasm samples were snap-frozen in liquid nitrogen and stored at -80°C for the subsequent RT-PCR experiments. 88 males and 69 females were collected in this tumor group, with a medium age of 66 years old. 107 cases diagnosed with stage I-II and 50 cases diagnosed with stage III-IV were clinically confirmed based on the TNM staging system stipulated by AJCC. All 157 GC patients provided written informed consents. This investigation was carried out under the approval of the Ethics Committee of the Zhuji People's Hospital of Zhejiang Province.

### RNA Isolation and Quantitative Reverse Transcriptase PCR

TRIzol reagent (Invitrogen, Hangzhou, Zhejiang, China) was used for the extraction of RNA in tissues based on the standardized operation steps. The 8000 Microvolume spectrophotometer purchased from Thermo Fisher Scientific (Haidian, Beijing, China) was used to determine the purity and concentration of RNA. Based on the manufacturer's recommendations, M-MLV reverse transcriptase (Promega, Haidian, Beijing, China) was used for the reverse transcription of 3 μg of total RNA. Real-time PCR was performed using the SYBR Green PCR Kit purchased from TaKaRa (Dalian, Niaoning, China). The conditions for ARAP1-AS1 amplification were established based on the guile of users for preliminary denaturation. The glyceraldehyde-3-phosphate dehydrogenase (GADPH) gene was used as a reference control for ARAP1-AS1. Comparative quantification was determined using the method of $2^{-\Delta\Delta CT}$. The primers for ARAP1-AS1, forward: 5'-AGCCACATAAATTCAGCAG-3', and reverse: 5'-CGATGTAGTAGGATTCCTTT-3', GAPDH, forward: 5'-GACTCATGACCACAGTCCATG-3', and reverse: 5'-AGAGGCAGGGATGATGTTCT-3'.

### Statistical Analysis

All statistical analyses were achieved by SPSS software (Abbott Laboratories, Chicago, IL, USA). The Student's *t*-test and Chi-square test were applied to determine the statistical significances of differences between the two groups of data. The Kaplan-Meier methods were utilized to analyze the cumulative survival time. Multivariate analyses were performed using Cox proportional hazards model. The differences were considered statistically significant for $p$-values $<0.05$.

## Results

### ARAP1-AS1 Expression is Increased in GC Tissue Samples

To determine whether ARAP1-AS1 had a functional effect in GC progress, we performed RT-PCR to examine its levels in 157 pairs of primary GC tissues and adjacent non-tumor tissues. Figure 1 displayed that the expression of ARAP1-AS1 in GC tissues was dramatically increased compared with matched normal gastric specimens ($p<0.001$), suggesting that ARAP1-AS1 may participate in the progression of GC.

### Association Between ARAP1-AS1 Expressions and Clinicopathological Characteristics

To explore the clinical correlation of ARAP1-AS1 levels with tumor progression, using the median ARAP1-AS1 expression in all 157 GC patients as a cutoff, all GC cases were divided into a high ARAP1-AS1 expression group (n=80) and a low ARAP1-AS1 expression group (n=77). Then, we performed the Chi-square test to analyze the collected data, finding that the upregulation of ARAP1-AS1 was distinctly associated with TNM stage ($p=0.010$) and lymphatic metastasis ($p=0.007$, Table I). However, no distinct difference was detected between ARAP1-AS1 expressions and other clinical features (all $p>0.05$, Table I).

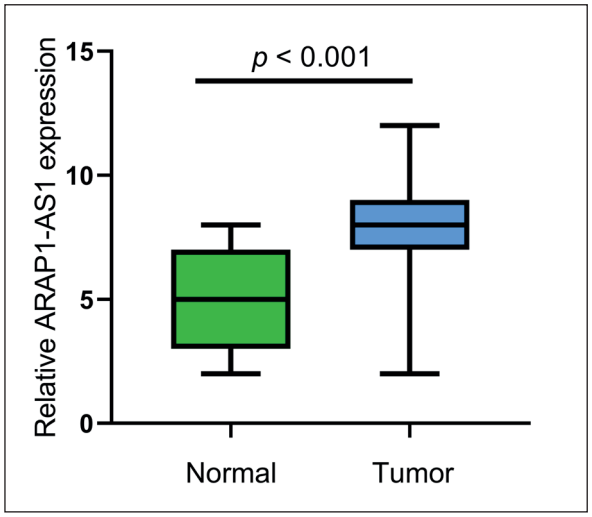


**Figure 1.** The relative expression level of ARAP1-AS1 in human GC tissues (n=157) and matched adjacent non-tumor gastric tissues (n=157) using RT-PCR.

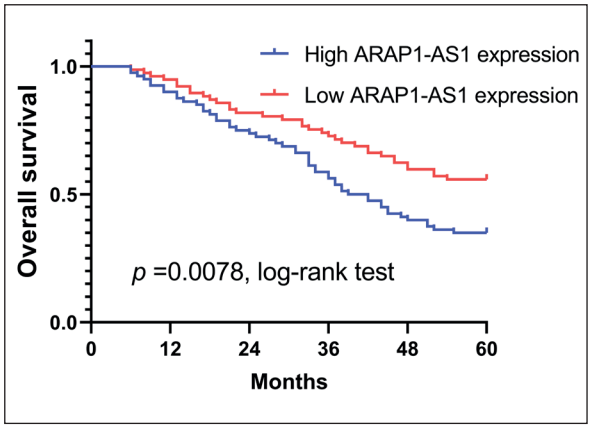


**Figure 2.** The overall survival of GC patients who had lower or higher ARAP1-AS1 expressions analyzed by Kaplan-Meier assays and log-rank test.

### *The Clinical Prognostic Influence of ARAP1-AS1 Expressions in GC Patients*

During the entire follow-up periods, 87 of the 157 patients (55.4 %) with GC died, and the median overall survival time of all the recruited patients was 44 months. Then, we performed Kaplan-Meier assays to analyze the prognostic values of ARAP1-AS1 levels in GC patients. As shown in Figure 2, patients with high ARAP1-AS1 expression in GC had distinctly longer overall survival (OS) than those with low ARAP1-AS1 expression ($p$=0.078). In addition, disease-free survival (DFS) of patients with high ARAP1-AS1 expression displayed a similar trend ($p$=0.0020, Figure 3). Additionally, based on the data of multivariate assays, ARAP1-AS1 expression, as well as lymphatic metastasis and TNM stage, was supposed to be an independent poor prognostic factor for both OS (HR=2.981, 95% CI: 1.374-4.368, $p$=0.017) and DFS (HR=3.159, 95% CI: 1.547-4.887, $p$=0.007) in GC patients, indicating that ARAP1-AS1 may be an independent prognostic factor for GC (Table II).

**Table I.** ARAP1-AS1 expression and clinicopathologic features in GC patients.

| Characteristics | All cases | ARAP1-AS1 expression | | *p*-value |
|---|---|---|---|---|
| | | High | Low | |
| ***Age*** | | | | 0.577 |
| ≥60 | 79 | 42 | 37 | |
| <60 | 78 | 38 | 40 | |
| ***Gender*** | | | | 0.959 |
| Male | 88 | 45 | 43 | |
| Female | 69 | 35 | 34 | |
| ***Differentiation*** | | | | 0.356 |
| Well-moderate | 90 | 43 | 47 | |
| Poor | 67 | 37 | 30 | |
| ***Tumor size*** | | | | 0.161 |
| ≥5 cm | 68 | 39 | 29 | |
| <5 cm | 89 | 41 | 48 | |
| ***TNM stage*** | | | | 0.010 |
| I/II | 107 | 47 | 60 | |
| III/IV | 50 | 33 | 17 | |
| ***Lymphatic metastasis*** | | | | 0.007 |
| Negative | 113 | 50 | 63 | |
| Positive | 44 | 30 | 14 | |

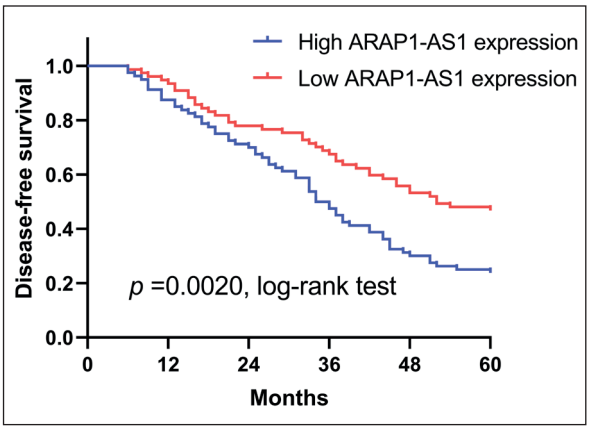


**Figure 3.** The disease-free survival of GC patients who had lower or higher ARAP1-AS1 expressions analyzed by Kaplan-Meier assays and log-rank test.

## Discussion

Although several great developments for the treatment of tumors have been achieved in recent years, a great part of GC patients still exhibited an unfavorable clinical outcome mainly due to their diagnosis at the late stages[20]. In recent years, the heterogeneous characteristics of GC have contributed to the developments of personalized medicine and molecular diagnosis, potentially allowing new concepts for the clinical challenge of current clinical treatments[21,22]. These advancements encouraged the identification of the novel and sensitive biomarkers. The rapid development of RNA sequencing technology highlighted the abnormal expressions of a large number of lncRNAs in tumors tissues and provided convincing proof that several dysregulated lncRNAs were closely correlated with the tumor progress, including GC[23,24]. In addition, growing studies suggested lncRNAs as novel potential biomarkers for GC patients.

Recently, increasing investigations suggested important multiple roles of lncRNAs in GC. For instance, the lower levels of lncRNA SLC25A5-AS1 were observed in GC tissues, especially in those with advanced clinical stages. The overexpression of lncRNA SLC25A5-AS1 distinctly inhibited the proliferation of GC cells *via* regulating miRNA-19a-3p/PTEN/PI3K/AKT pathway[25]. LncRNA FLVCR1-AS1, a potential prognostic biomarker in GC reported by Liu et al[26], was shown to be distinctly highly expressed in GC, and its knockdown resulted in the suppression of the proliferation and migration in GC cells *via* sponging miRNA-155 to decrease c-Myc expression. ARAP1-AS1 was a novel functionally characterized lncRNA. First, in bladder cancer, ARAP1-AS1 expressions were found to be upregulated and to predict a poor clinical outcome. Functional assays indicated that ARAP1-AS1 promoted the proliferation and metastasis of bladder cancer cells *via* the modulation of miRNA-4735-3p/NOTCH2 axis[17]. In addition, Ye et al[27] provided evidence that ARAP1-AS1 expression was increased in colorectal cancer, which was induced by YY1 transcription factor. Moreover, forced ARAP1-AS1 expression was found to strengthen the metastasis ability of colorectal cancer cells through Wnt/β-Catenin pathway. These findings suggested the positive roles of ARAP1-AS1 in some solid tumors. However, whether ARAP1-AS1 was also abnormally expressed in GC, and the possible clinical significance of ARAP1-AS1 in GC patients has not been investigated.

In this study, we first examined ARAP1-AS1 expressions in 157 GC patients and found that ARAP1-AS1 levels were distinctly upregulated in GC tissues compared to matched normal gastric tissues, suggesting ARAP1-AS1 as a novel potential oncogene for GC. Then, we analyzed the clinical significance of ARAP1-AS1 in GC

**Table II.** Multivariate Cox proportional hazard model analysis of overall survival and disease-free survival in GC patients.

| Variable | Overall survival | | | Disease-free survival | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | *p* | HR | 95% CI | *p*-value |
| Age | 0.987 | 0.458-1.985 | 0.218 | 1.261 | 0.784-1.758 | 0.298 |
| Gender | 1.275 | 0.764-1.875 | 0.387 | 1.018 | 0.572-1.933 | 0.302 |
| Differentiation | 1.562 | 0.784-2.653 | 0.124 | 1.387 | 0.898-2.342 | 0.249 |
| Tumor size | 1.745 | 0.842-2.237 | 0.212 | 1.475 | 0.947-2.441 | 0.185 |
| TNM stage | 3.362 | 1.327-4.765 | 0.007 | 3.147 | 1.147-4.417 | 0.011 |
| Lymphatic metastasis | 3.114 | 1.417-5.127 | 0.002 | 3.314 | 1.578-5.745 | 0.001 |
| ARAP1-AS1 expression | 2.981 | 1.374-4.368 | 0.017 | 3.159 | 1.547-4.887 | 0.007 |

patients, finding that the upregulation of tissue ARAP1-AS1 expression was positively associated with TNM stage and lymphatic metastasis. Moreover, GC patients with high ARAP1-AS1 expressions exhibited shorter OS and DFS compared with patients showing low ARAP1-AS1 expressions. To further explore the potential application of ARAP1-AS1 used as a novel biomarker, we performed multivariate assays, revealing that high ARAP1-AS1 expression was an independent prognostic marker for both OS and DFS for GC patients. However, the small sample size of our assays was also a limitation which may influence the accuracy of our conclusion.

## Conclusions

These preliminary results identified a novel GC-associated lncRNA ARAP1-AS1 which was proved to have a positive association with positively clinical progress and clinical prognosis of GC patients. In the future, ARAP1-AS1 may serve as a prognostic biomarker for clinical management.

### Conflict of Interests

The Authors declare that they have no conflict of interests.

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