Original Article
Loss of long noncoding RNA WT1-AS is associated with better survival in early stage of cervical cancer

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Abstract: Accumulating evidence suggests that long noncoding RNAs (lncRNAs) are crucial regulators for the development and progression of various cancers. However, the expression and clinical significance of lncRNA WT1-AS in cervical cancer remain unknown. The current study aimed to explore the correlation of WT1-AS with prognosis in cervical cancer patients. RT-qPCR assay was used to evaluate the expression of WT1-AS in 25 pairs of cervical cancer samples and adjacent normal tissues respectively. We found that WT1-AS was downregulated in cervical cancer and associated with tumor stage. Moreover, Kaplan-Meier analyses demonstrated that loss of WT1-AS was associated with better patient survival. The predictive value of WT1-AS expression was successfully validated in the TCGA dataset. In addition, WT1-AS and WT1 expressions were found positively correlated in cervical cancer. WT1 mRNA expression was also found significantly downregulated in cervical cancer. Collectively, our study indicated that WT1-AS played an important role in cervical cancer and loss of WT1-AS was indicative of better prognosis in patients with cervical cancer.

Keywords: Long noncoding RNA, WT1-AS, cervical cancer, prognosis

Introduction

Cervical cancer is the third most commonly diagnosed cancers among women. It is also the fourth lethal cancers with an estimated 500,000 new cases and 260,000 cases of death annually [1, 2]. Although notable progress has been made in the development of its treatment, the 5-year survival rate of advanced-stage patients remained poor [3]. Surgeries, chemotherapy, and radiotherapy have recently been used as standard treatment for patients with cervical cancer, while the efficacy and prognosis vary significantly in individual [4]. No sensitive biomarkers specific for the prognosis of cervical cancer are available to date. It is, therefore, imperative to discover novel molecular markers of cervical cancer, thus facilitating a more accurate prediction of efficacy and prognosis.

Long noncoding RNAs (lncRNAs) are a class of RNA molecules that are longer than 200 nucleotides and lack the capacity to code protein [5]. Increasing studies have demonstrated that lncRNAs play crucial regulatory roles in various biological processes such as transcriptional regulation, cellular proliferation and differentiation [6-8]. Dysregulated expressions of lncRNAs have been detected in various cancers and are generally related to the oncogenic or cancer-suppressive processes [9]. According to theses studies, it is possvile for lncRNA i to serve as a indicator for overall survival in multiple types of cancers. For instance, MALAT1 expression has been found in lung cancer and renal cell carcinoma, which is negatively correlated with survival outcomes [10, 11]. HOTAIR has been implicated in several cancers and show their potential as novel independent predictor for patient survival in breast cancer [12, 13].

WT1-AS is one of the antisense transcripts of the WT1 gene, which encodes a zinc finger transcription domain, which bears both tumor suppressor and oncogenic activities [14, 15]. WT1-AS has shown to be aberrantly expressed in various cancers. Although the oncogenic role of
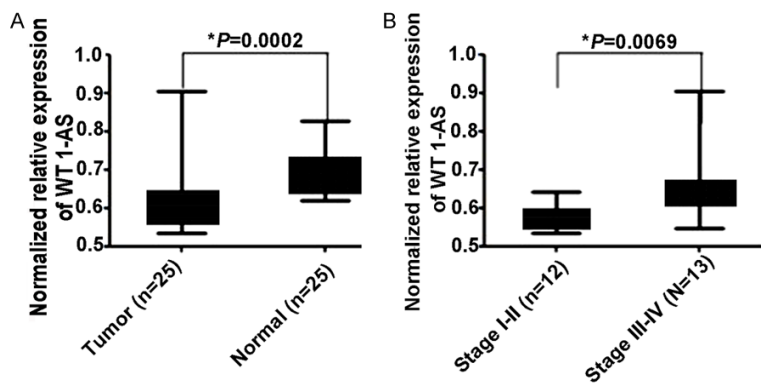
Figure 1. LncRNA WT1-AS is downregulated in cervival cancer and assoicated with tumor stage. A. The expression of WT1-AS was examined in the 25 pairs of cervival cancer tissue samples by RT-qPCR. B. The expression of WT1-AS was associated with tumor stage of cervival cancer.

using a reverse transcription kit (Takara). Quantitative real-time PCR (RT-qPCR) was performed using the SYBR Green PCR Kit (Takara) on ABI 7900HT (Applied Biosystems, California), according to the manufacture's protocol. The primers sequences were as follows: WT1-AS (forward): 5'-GCCTCTCTGTCCTCTTC-TTTGT-3', WT1-AS (reverse): 5'-GCTGTGAGTCCTGGTGCTT-AG-3'; WT1 (forward): 5'-AG-GGTACGAGAGCGATAACCAC-AC-3', WT1 (forward): 5'-TCAG ATGCCGACCGTACAAGA-3'; GA-PDH (forward): 5'-TTGGCATC-GTTGAGGGTCT-3', GAPDH (reverse): 5'-CAGT-GGGAACACGGAAAGC-3'.

The relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ equation, normalized with the average expression of GAPDH.

Statistical analysis

All experiments were repeated at least three times, and all data were presented as mean ± standard deviation, and analyzed with SPSS software (version 22.0; SPSS, Chicago, USA). Comparisons of the levels of WT1-AS between cervical cancer and adjacent normal tissues were performed using Student's paired t-test. Significant differences between two independent groups of samples were evaluated using Mann-Whitney U test. The Kaplan-Meier method and log-rank test were used to evaluate and compare the prognosis of cervical cancer patients. Association between WT1-AS expression and WT1 expression in cervical cancer was determined by using Pearson correlation test. $p < 0.05$ was considered statistically significant.

Results

LncRNA WT1-AS is downregulated in cervical cancer and associated with tumor stage

To evaluate the clinical significance of lncRNA WT1-AS, we first quantified WT1-AS expression in 25 pairs of cervival cancer samples and matched normal tissues by RT-qPCR assay. Our data showed that WT1-AS was significantly downregulated in cervical cancer samples in comparison with matched normal tissues

WT1-AS in cancers is gradually recognized, the clinical and prognostic significance of this lncRNA in cervical cancer has not been reported yet. In current study, we investigated the WT1-AS expression level in cervical cancer and further analyzed the relationship between WT1-AS expression and tumor stage and prognosis of cervical cancer. Furthermore, the correlation between WT1-AS and WT1 was also investigated. The results may suggest that WT1-AS is a potential biomarker for prognosis in cervical cancer.

Materials and methods

Clinical samples

A total of 25 pairs of cervical cancer samples and matched normal tissues were obtained from patients between January 2010 and January 2012 at Obstetrics and Gynecology Hospital Affiliated to Nanjing Medical University (Nanjing, China). The tissues were washed with sterile phosphate-buffered saline before being snap frozen in liquid nitrogen and then stored at -80°C until total RNA was extracted. All patients had not received any anti-cancer treatment prior to surgery. Written informed consent was obtained from all patients who participated in this study. The study started after obtaining approval from the hospital ethics committee. The disease-free survival was defined as the time between diagnosis and the date of death or the date last known alive.

Quantitative real-time PCR

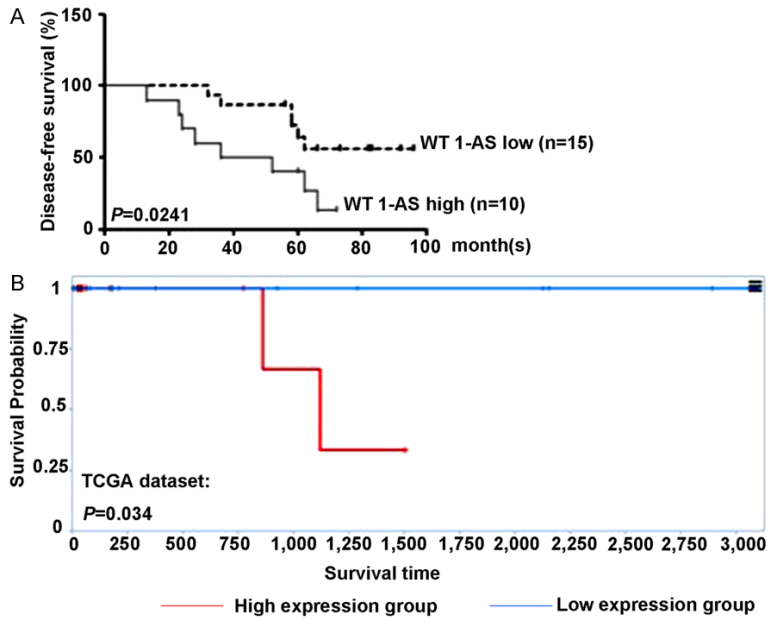Total RNA was extracted using TRIZOL (Takara, Dalian, China) and then cDNA was obtained

Figure 2. Loss of WT1-AS is associated with better patents survival. A. The expression of WT1-AS was associated with patients survival of our dataset. B. The expression of WT1-AS was associated with survival in the TCGA dataset.

Kaplan-Meier using the log-rank test. Patients with cervical cancer were classified into low-WT1-AS group (n= 15) and high-WT1-AS group (n=10) according to the median level. We did a 5-year follow up study. The result indicated that patients with low WT1-AS expression had better disease-free survival (DFS) (**Figure 2A**, *p*=0.0241) than patients with high WT1-AS expression. In consistent with the findings in our dataset, significant differences in survival probability between the two groups were confirmed in TCGA dataset (**Figure 2B**, *p*=0.034). All these data demonstrated that loss of WT1-AS was correlated with better survival of cervical cancer patients.

The expression of WT1-AS is correlated with WT1 expression

WT1-AS, the antisense transcript of WT1, has been found to regulate WT1 protein levels *in vivo*. We then investigated the relationship between WT1-AS and WT1 in cervical cancer. We quantified the expression of WT1 in cervical cancer tissues by RT-qPCR assay and analyzed the correlation of WT1-AS and WT1 expression using Pearson correlation analysis. The results demonstrated a strong positive correlation between WT1-AS and WT1 expression (**Figure 3A**, r=0.83, p< 0.0001). Besides, positive correlation between WT1-AS and WT1 expression was also identified in Pearson correlation analysis of the TCGA dataset (**Figure 3B**, r=0.88, p<0.0001).
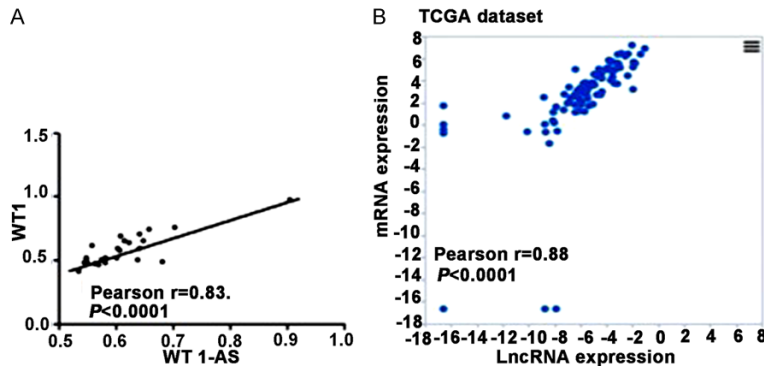

Figure 3. The expression of WT1-AS is correlated with WT1 expression. A. The expression of WT1-AS was significantly correlated with WT1 in our dataset. B. The expression of WT1-AS was significantly correlated with WT1 mRNA expression in the TCGA dataset.

(**Figure 1A**, *P*=0.0002). In addition, we found that low WT1-AS expression was negatively related to early tumor stages (**Figure 1B**, *p*= 0.0069) when the stages of disease were classified into early stages I-II (n=12) and advanced stages III-IV (n=13).

Loss of WT1-AS is associated with better patients' survival

Additionally, we analyzed the relationship between the WT1-AS expression and the clinical outcomes of cervical cancer patients by

WT1 is significantly downregulated in cervical cancer

We further evaluated WT1 expression in 25 pairs of cervical cancers and adjacent nontumor tissues by RT-qPCR. As shown in **Figure**
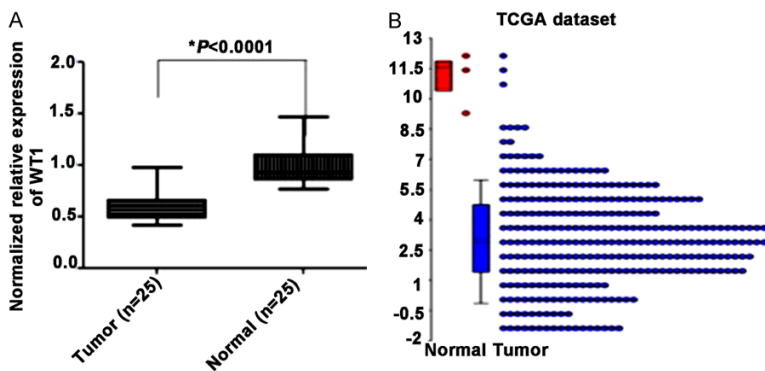
Figure 4. WT1 is significantly downregulated in cervival cancer. A. The expression of WT1 was examined in the 25 pairs of cervival cancer tissue samples by RT-qPCR. B. The expression of WT1 in the TCGA-CESC dataset.

4A, WT1 expression was significantly downregulated in cervical cancer samples when compared to matched normal tissues ($p<0.0001$). Another validation of the abnormal WT1 expression was conducted using TCGA-CESC dataset. Similarly, WT1 expression was again found downregulated in cervical cancer samples in comparison with the normal tissues (**Figure 4B**).

Discussion

Despite the development of advanced therapeutic strategies, the prognosis of patients with cervical cancer varies significantly and is hard to be predicted [4]. In order to individualize therapy for improving overall survival of patients with cervical cancer, it is critical to identify novel biomarkers for the early identification of patients with a high risk of treatment failures, With the advances in high-throughput transcriptome analysis, lncRNAs have been gradually proved to play an important role in biological function in various cancers and have a great potential to serve as a prognostic biomarker. Furthermore, some lncRNAs have been found to be associated with cervical cancer outcomes [16-18]. Jiang *et al.* showed that low expression of lncRNA LET is significantly associated with poor prognosis of patients with cervical cancer [19]. Ouyang *et al.* demonstrated that GINS2 downregulation markedly suppresses cervical cancer progression and it may be a valuable prognostic biomarker in early-stage cervical cancer [20].

Several previous studies have reported that WT1-AS expression is correlated with cancer progression. For example, WT1-AS expression was found significantly down-regulated in gastric cancers and associated with tumor size and the clinico-pathological stage [21]. In addition to gastric cancer, abnormal WT1-AS expression has also been found in acute myeloid leukemia [22]. In our study, we showed that expression of WT1-AS was downregulated in cervical cancer samples compared with matched normal tissues in 25 pairs of patients. Furthermore, WT1-AS expression was associated with tumor stage. In addition, we investigated the correlation of WT1-AS expression and the clinical outcomes of cervical cancer patients by Kaplan-Meier using the log-rank test. Our results showed that patients with low expression of WT1-AS displayed significantly better DFS than those with high WT1-AS expression. These findings were confirmed by TCGA dataset.

WT1-AS is the antisense transcript of WT1 and it has previously been implicated in tumorigenesis by interacting with WT1. Studies reported that expression of WT1-AS mRNA was parallel to the expression of WT1 mRNA and WT1-AS, which could positively regulate WT1 protein levels in vivo [23]. WT1 gene has been reported to regulate both oncogenic and tumor suppressor functions. Researchers found that aberrant expression of WT1 is associated with poor prognosis, enhanced tumor progression and resistance to chemotherapy [24-26]. In our study, we found that WT1-AS and WT1 expression were positively correlated in cervical cancer. Furthermore, WT1 expression was also found significantly downregulated in cervival cancer compard to matched normal tissues. However, further studies to explore the molecular and biological mechanism underlying the association of WT1-AS and WT1 in cervical cancer is still needed.

In summary, our study showed that LncRNA WT1-AS is downregulated in cervical cancer and associated with tumor stage, and patients with low WT1-AS level have better prognosis. However, the regulatory mechanisms of WT1-AS remain to be elucidated.

Disclosure of conflict of interest

None.

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