In Silico and Experimental Analyses of Long Non-Coding RNA TMPO-AS1 Expression in Gastric Cancer Tissues

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Abstract

Background

A large body of evidence has illustrated the prominent roles of non-coding RNAs, particularly long non-coding RNAs (lncRNAs), in cancer progression and diagnosis. In this study, we considered in silico and experimental analyses of long non-coding *TMPO-AS1* expression in adjacent nonmalignant tissues and cancerous tissues of the patients with gastric cancer.

Methods

In order to conduct the present study, 40 gastric tumor samples and 40 marginal noncancerous counterparts were collected. The *TMPO-AS1* expression levels in patients' samples were evaluated by qRT-PCR analysis. The Cancer Genome Atlas (TCGA) data were retrieved for *TMPO-AS1* and analyzed via Gepia and TANRIC online tools. Student t-test, one-way ANOVA, and chi-square test were accomplished via SPSS software.

Results

The data demonstrated that *TMPO-AS1* was overexpressed in cancerous tissues compared to nonmalignant adjacent ones ($p: 0.0076$). The TCGA data demonstrated that *TMPO-AS1* was upregulated in GC tissues in comparison to nonmalignant adjacent ones ($p = 0.001$).

Conclusion

Altogether, in our study, we demonstrated lncRNA *TMPO-AS1* could be considered as a biomarker in GC. However, further investigations are required to indicate the potential application of this lncRNA in diagnosis, prognosis and therapeutic target of GC.

Background

Gastric Cancer (GC) is considered as one of the most fatal gastrointestinal (GI) malignancies which is mostly diagnosed in male patients than females (2.2 times) (1). GC is the fifth most frequent neoplasm and the third leading cause of cancer-related deaths accounting for a high incidence in Eastern and Central Asia and Latin America (1)(2)(3). The 5-year overall survival (OS) rate of GC patients is very dismal (less than 25%) and differs among assorted ethnicities and geographic regions. It has been illustrated that the chance of curing GC patients can be greater if the disease is diagnosed at early stages. Despite advanced screening and clinical approaches, the diagnosis and therapy of GC are still challenging issues for therapists. Therefore, a new diagnostic marker and therapeutic target is required to pave the way for GC therapy (4). Recently, a large body of evidence has illustrated the prominent roles of

non-coding RNAs, particularly long non-coding RNAs (lncRNAs), in cancer progression and diagnosis (5). lncRNAs are an extensive family of non-coding RNAs with more than 200 nucleotides in length and possessing little or no protein-coding capability. lncRNAs have significant roles in several cell biological processes such as cell proliferation, differentiation, apoptosis, and the epigenetic regulation of gene expression (6)(7)(8). Accumulating investigations have discovered that lncRNAs play considerable roles in tumor progression and development of multiple human cancers such as GC. It has been indicated that aberrant expression of lncRNAs deregulate the important genes in cancer (9)(10). lncRNAs may act as tumor suppressor genes and/or oncogenes which are completely cancer type-and tissue context-dependent. For instance, *PANDAR* is one of the tumor suppressor lncRNAs which is down-regulated in non-small cell lung cancer tissues. Overexpression of *PANDAR* has been shown to facilitate apoptosis and promote tumor cell progression. On the other hand, *HOTAIR* is one of the oncogenic lncRNAs that has been demonstrated to be up-regulated in a variety of aggressive tumor tissues. Overall, deregulation of lncRNAs is one of the main contributors of carcinogenesis (11)(12)(13). However, the exact underlying mechanisms of lncRNAs in GC are unknown yet. Investigation on lncRNA can extend our basic information about the modulatory mechanisms for better management of GC diagnosis and treatment. Recent investigations have revealed that exploring the potential novel biomarkers has a putative impact on the diagnosis and management of GC from the earliest appearance to the ultimate stages (14). *Thymopoietin antisense transcript 1 (TMPO-AS1)* is one of the lncRNAs that shows oncogene activity in different kinds of human cancers. *TMPO-AS1* is transcribed in the antisense direction of the *TMPO* gene. Recent investigations have shown that *TMPO-AS1* is up-regulated in some cancer tissues such as prostate cancer (15), non-small cell lung cancer (16), esophageal squamous cell carcinoma (17), cervical cancer (18), hormone-refractory breast cancer (19), osteosarcoma (20), and colorectal cancer (21). For instance, overexpression of *TMPO-AS1* has been shown to significantly associate with tumorigenesis, whereas *TMPO-AS1* depletion attenuated carcinogenesis and promoted apoptosis in cervical cancer cells (18). Previous studies have cleared that *TMPO-AS1* was an important cell proliferation modulator and could be considered as a potential diagnostic and prognostic biomarker in multiple cancer types (15).

Materials And Methods

Patients and sampling

In the present study, 40 gastric tumor samples and 40 margin noncancerous counterparts were collected from 40 GC patients (21 males and 19 females) whose disease (GC) was confirmed by the physicians and pathologists at Imam Reza Hospital, Tabriz University of Medical Sciences, Tabriz, Iran. Due to tumor infiltration possibility in marginal tissues, the samples were examined and confirmed by pathologists. The ethical committee of Tabriz University confirmed the protocol of this study (N: IR.TABRIZU.REC.1399.009). The samples were frozen in liquid nitrogen immediately after tissue sampling during surgery to prevent RNA degradation.

RNA isolation and cDNA synthesis

Total RNA was extracted by TRIzol isolation solution (GeneAll Biotechnology, Seoul, Korea) using the guanidinium-isothiocyanate method. The quality evaluation of extracted RNAs was verified via Nano-drop (Thermo Fisher Scientific, USA) spectrophotometer by measuring absorption at 260/280 nm. Then, the isolated RNAs were reverse-transcribed to Complementary DNA (cDNA) using cDNA synthesis kits (Biofact, Seoul, South Korea) according to the manufacture's instrument.

Quantitative real-time polymerase chain reaction (qRT-PCR)

The *TMPO-AS1* expression levels in patients' samples were determined by qRT-PCR analysis (Roche real-time PCR Light Cycler 96 device). *Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* was considered as the internal control gene. The sequences of gene-specific primers for *TMPO-AS1* and *GAPDH* are summarized in Table 1. The total volume was 20µL including 10µL SYBR Green, 1µL primer, and 2µL cDNA and DEPC water. Thermal cycling conditions comprised an activation step for 15 min at 95°C followed by 40 cycles including a denaturation step for 10 seconds at 95°C and 1 min at 60°C for annealing and extension. The evaluation of the lncRNA expression was conducted by $\Delta\Delta CT$ formula and relative quantification (RQ) = $2^{-(\Delta\Delta CT)}$.

Table 1
List of primers.

Gene	Primer sequence	Product size
TMPO-AS1-Forward	5'-AGACGCCGATAAGGGACAG-3'	139 bp
TMPO-AS1-Reverse	5'-AGCCAAGGGTCCTCACA-3'	166 bp
GAPDH-Forward	5'-CAAGATCATCAGCAATGCCTCC-3'	
GAPDH-Reverse	5'-GCCATCACGCCACAGTTTCC-3'	

Bioinformatics analysis of TCGA dataset

The Cancer Genome Atlas (TCGA) data were retrieved and analysed via Gepia (<http://gepia.cancer-pku.cn/>) and TANRIC (https://ibl.mdanderson.org/tanric/_design/basic/main.html) online tools. *TMPO-AS1* expression and corresponding disease free survival (DFS) and overall survival (OS) were analysed by Gepia online tool and subgroup analyses of the gene expression were conducted via TANRIC online software. The results were shown as |Fold change (FC) > 2| and p-value < 0.05.

Statistical Analysis

All the data are presented as mean and SD. The study employed student t test and one-way ANOVA to analyze the obtained data. Chi-square test (Spearman) was also conducted to evaluate the association between lncRNA expression level and clinicopathological characteristics. All the statistical analyses were done via SPSS v23.0 software (IBM Corp., Armonk, NY, USA). A p-value < 0.05 was considered as significant.

Results

TMPO-AS1 expression in GC patients

The lncRNA *TMPO-AS1* expression was evaluated in 40 tissues of GC patients in comparison to noncancerous counterparts. The data demonstrated that *TMPO-AS1* was overexpressed in the tissues (median: 4.8, p: 0.0076). The results are presented in Fig. 1.

Furthermore, the patients were divided into two groups according to the *TMPO-AS1* expression (Median expression). The results revealed that there was not any significant correlation between *TMPO-AS1* expression and demographic and clinicopathological data including age, gender, smoking, lymph node metastasis, distant metastasis, H. pylori infection, alcohol abuse, stages, and differentiation. The results are summarized in Table 2.

Table 2
The results of analyzing the correlation
between *TMPO-AS1* expression and
demographic and clinicopathological data.

Parameters	<i>TMPO-AS1</i>		
	Low	High	p-value
Age (Year)			
< 60	15	19	0.182
60≤	5	1	
Gender			
Female	12	7	0.113
male	8	13	
Smoking			
No	13	15	0.49
Yes	7	5	
Lymph node metastasis			
Negative	13	12	0.744
Positive	7	8	
Distant metastasis			
Negative	17	18	0.633
Positive	3	2	
H. Pylori infection			
Negative	12	7	0.113
Positive	8	13	
Alcohol abuse			
Negative	16	16	1
Positive	2	1	
Stage			
I + II	12	7	0.113
III + IV	8	13	

Parameters	<i>TMPO-AS1</i>		
Differentiation			
Poor	7	4	
Moderate	7	9	0.31
Good	6	7	0.392

TCGA data of *TMPO-AS1* in GC patients

The TCGA data of 408 GC patients' tissues and 211 normal counterparts were retrieved and analysed via Gepia and TANRIC online tools. The data demonstrated that *TMPO-AS1* was upregulated in GC tissues in comparison to normal counterparts ($p = 0.001$) (Fig. 2).

Furthermore, it was demonstrated that *TMPO-AS1* expression did not correlate with tumor stage and grade ($p = 0.147$, $p = 0.86$, respectively), whereas it correlated with molecular and Lauren classification ($p = 0.0001$). The data are presented in Fig. 3. Moreover, Kaplan-Meier curve analyses did not show any significant correlation between *TMPO-AS1* expression and overall survival (OS) ($p = 0.62$) as well as disease-free survival (DFS) ($p = 0.73$) in the patients (Fig. 4).

Discussion

Even though investigations on the molecular mechanisms of GC in occurrence and progression stages have recently been developed (22), GC is still the third leading cause of cancer-related deaths worldwide (3). It has been suggested that it is due to the tumor heterogeneity or genomic complexities of GC. Therefore, it is necessary to improve diagnosis and prognosis and ultimately treatment of GC patients by finding new molecular biomarkers effective in tumorigenesis and tumor progression in GC (23). LncRNAs have been identified as a class of noncoding RNAs that have various modulatory roles in several biological processes. The ectopic expression of lncRNAs has been explored as a potential diagnostic and prognostic biomarker in GC. For instance, lncRNA *H19* is overexpressed in GC tissues compared with paired tumor-free adjacent tissues. Aberrant expression of lncRNA *H19* in GC has been shown to elevate tumorigenesis; investigations have revealed that *H19* has a potential utilization in GC therapy (24). Also, up-regulation of *H19* in GC patients' plasma mainly supports its significant potential as a diagnostic biomarker in GC (25). *TMPO-AS1* is one of the oncogenic lncRNAs in several cancers that is involved in cancer carcinogenesis. Investigations have previously shown that *TMPO-AS1* overexpression results in increased cancer cell proliferation. Conversely, knockdown of *TMPO-AS1* results in enhanced cancer cell apoptosis and attenuates cell proliferation in some cancer types such as osteosarcoma and breast cancer (20)(19).

In this study, we demonstrated overexpression of *TMPO-AS1* in tumor tissues of GC patients relative to noncancerous adjacent tissues, which, in turn, is related to the prognosis of GC patients. Also, up-regulated *TMPO-AS1* expression in tumor tissues compared with adjacent tissues has been revealed by

TCGA analysis. These two different results reinforce each other and confirm the importance of studying the potential role of *TMPO-AS1* as a diagnostic or therapeutic target in GC patients.

Previous studies have shown that lncRNAs can exert their impact on the expression of protein-coding genes through their interaction with other molecules such as DNAs, miRNAs, transcriptional factors, etc. Androgen receptor (AR) and estrogen receptor (ER) are molecules that can be affected by lncRNAs (26). *TMPO-AS1* is known as an antisense lncRNA that is markedly overexpressed in several cancers such as prostate and breast cancers. *Huang et al.* have shown that *TMPO-AS1* is effectively modulated by AR in prostate cancer. In addition, a study in Japan on hormone-refractory breast cancer showed that *TMPO-AS1* stabilizes ESR1 mRNA. By contrast, the estrogen signaling is inhibited by *TMPO-AS1* knockdown. Therefore, *TMPO-AS1* is affected by some sex hormone receptors such as AR and ER in prostate and breast cancers, respectively (15)(19).

GC is one of the male-predominant cancers. Decoding the factors that contribute to this sex-related disparity can unveil novel GC tumorigenesis pathways (27). For the first time in 1983, Tokunaga *et al.* demonstrated the association between sex hormone receptors and GC (28). It has been postulated that high GC incidence rate in men compared to women is due to estrogen effects. There are some findings supporting or refuting this postulate (29).

Conclusion

Taken together, we demonstrated that *TMPO-AS1* is significantly more expressed in cancerous tissues than in the marginal noncancerous tissues of GC patients. Based on the preservative role of ER and AR in the etiology of GC postulate, and the relationship between *TMPO-AS1* and sex hormone signaling pathways, further investigations on the possible roles of *TMPO-AS1* in the sex hormone signaling pathways in GC are required.

Declarations

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Author's contributions

E.M. R.S. and A.P. conducted the research; E.M. R.S. T.A. and A.P. analyzed the data; T.A. and B.B. wrote the paper; B.B. had primary responsibility for the final content. All authors have reviewed and approved the final manuscript.

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Availability of data and materials

The authors declare that the datasets on which the conclusions of this manuscript rely are deposited in publicly available repositories.

Ethics approval and consent to participate

The authors declare that there is no conflict of interest.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

The lncRNA *TMPO-AS1* expression in GC patients

Figure 2

TMPO-AS1 differential expression in TCGA GC patients

Figure 3

TMPO-AS1 correlation with clinicopathological characteristics. A. stage, B. pathological grade, C. molecular classification, D. Lauren classification

Figure 4

Kaplan-Meier curve analysis of *TMPO-AS1* expression with OS and DFS