SUMO1 pseudogene 3 (SUMO1P3) expression in human gastric cancer and its clinical significance

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Abstract

Introduction/Aim: The aim of this study was to investigate SUMO (small ubiquitin-like modifier) 1 pseudogene 3, SUMO1P3 expression, as one of the pseudogene-expressed long non-coding RNA (lncRNAs) in gastric cancer (GC) patients.

Materials and Methods: Fresh gastric cancer and adjacent non-tumor tissues were collected from 182 GC patients, who were admitted to the Alzahra Hospital, Isfahan, Iran on December 2014 to January 2016. Quantitative reverse transcription-polymerase chain reaction was used to investigate the SUMO1P3 levels. Then, the association between the level of SUMO1P3 in gastric cancer tissues and the clinicopathological features of patients with gastric cancer was analyzed. To find the differences of SUMO1P3 levels between gastric cancer tissues and adjacent non-tumor tissues, one-way analysis of variance (ANOVA) was applied. A significance level of 0.05 was considered for the tests.

Results: The results showed that SUMO1P3 levels in males were not significantly higher than those in females ($p = 0.485$). No significant difference of SUMO1P3 expression was observed between patients under 64 years old and above ($p = 0.155$). The SUMO1P3 levels were not associated with perineural invasion ($p = 0.319$), lymphatic invasion ($p = 0.797$), invasion depth ($p = 0.790$), location of the tumor ($p = 0.811$), tumor size ($p = 0.635$), and grading ($p = 0.289$).
Conclusions: These results indicated that in our patient population and according to the used method in this study, pseudogene-expressed lncRNA SUMO1P3 may not be a potential biomarker in the diagnosis of gastric cancer.

Key words: SUMO1P3, long non-coding RNA, tumor marker, gastric cancer

Introduction

Gastric cancer (GC) is one of the most leading causes of cancer death nowadays and is considered as the most common gastrointestinal malignancy in some parts of the world, especially; East Asia, Eastern Europe, and parts of Central and South America (1-3). Nevertheless, since there are no specific symptoms for patients with early stage of GC, it is usually diagnosed at advanced stage and, accordingly, the prognosis for advanced stage GC is considerably poor for most of the patients (4, 5).

For GC prognostic prediction, there is still no commonly-accepted biomarker to facilitate the management of GC patients (2, 6). Therefore, detection of the new biomarkers for GC may play a significant role in improving diagnosis and also treatment of human GC. In addition, a detailed evaluation of the molecular mechanisms underlying gastric carcinogenesis can open new horizons for GC treatment.

Recent studies have shown that, large size long noncoding RNA (lncRNA) [size > 200 nt], is a new class of the noncoding RNA that contributes in cellular development, differentiation, and many other biological processes (7). Moreover, it has been stated that expression of lncRNA is associated with cancer development and progression (8, 9).

According to the recent reports, several types of lncRNAs have been detected and most of them have specific names (10, 11). Among the lncRNA family, the pseudogene-expressed lncRNAs are one of the major types. For this family, the 'P' suffix is used for pseudogenes of the both lncRNA classes and protein-coding genes. It should be noted that pseudogenes, considered as defunct relatives of functional genes, are nonfunctional genomic DNA sequences which are similar to normal genes. However, there is still very limited evidence of the clinical association between pseudogene expressed lncRNAs and GC.

The aim of this study was to investigate SUMO (small ubiquitin-like modifier) 1 pseudogene 3, SUMO1P3 expression, as one of the pseudogene-expressed lncRNAs in GC patients.

Materials and Methods

The study protocol was approved by the Ethical Committee of Shahid Beheshty University of Medical Sciences in accordance with standards set by the committee and in compliance with the 1975 Helsinki Declaration and its revision in 2000. Fresh gastric cancer and adjacent non-tumor tissues were collected from 182 GC patients, who were admitted to the Alzahra Hospital, Isfahan, Iran between December 2014 to January 2016. Before the study, patients gave their informed consent.

The study protocol was in accordance with Mei et al (6). After performing the biopsies, the specimens were immediately soaked in RNA-fixer Reagent (Exiqon, Helsinki, Denmark) and stored at -80 °C until performing the laboratory tests.

In this study, noncancerous tissues biopsies were taken from the adjacent tissues located 5 cm away from the edge of gastric cancer. An expert pathologist reviewed the samples and found the border where there were no obvious tumor cells.

For each sample, the total RNA was extracted using TRIzol reagent (Exiqon, Helsinki, Denmark) according to the instructions published by the manufacturer. Next, reverse transcription (RT) was performed using random primers and oligo(dT)15 primer in the GoScript RT System (Exiqon, Helsinki, Denmark).

For the polymerase chain reaction (PCR), the GoTaq qPCR master mix (Exiqon, Helsinki, Denmark) was used on the Mx3005P QPCR System (Corbet, Sydney, Australia). Similar to the other publications, the "b-Actin was amplified to normalize the relative levels of lncRNA". Sangon Biotech (Exiqon, Helsinki, Denmark) was used to synthesize the primers for SUMO1P3 and b-actin. Their sequences were as follows:

"50-ACTGGGAATGGAGGAAGA-30 (sense) and 50-TGAGAAAGGATTGAGGGAAAAG-30 (antisense) for SUMO 1P3; 50-AAGCCACCCCACTTCTCTCTAA-30 (sense) and 50-AATGCTATCACCTCCCCTGTGT-30 (antisense) for b-actin". The data were analyzed by the DCt method [8]. All results are expressed as the mean ± SD of three independent experiments.
Pathological characteristics of the patients including; perineural invasion, lymph metastasis, invasion depth, location of the tumor, tumor size, staging and grading were also recorded.

Histological grading was performed according to the National Comprehensive Cancer Network clinical practice guideline of oncology (V.1.2011).

Statistical analysis
To find the differences of SUMO1P3 levels between gastric cancer tissues and adjacent non-tumor tissues, one-way analysis of variance (ANOVA) was applied. The correlation between SUMO1P3 level and clinicopathological factors was further analyzed by ANOVA and t-test. Statistical analysis was performed using SPSS version 16.0 (Chicago, IL). A significance level of 0.05 was considered for the tests.

Results

Table 1, illustrates the SUMO1P3 expression levels and demographic characteristics of the patients including age and gender.

Table 2, shows the relationship between SUMO1P3 expression levels (Ct) in GC diagnosed patients.

Figure 1, gives the ROC curve of the SUMO1P3 levels between gastric cancer tissues and adjacent non-tumor tissues.

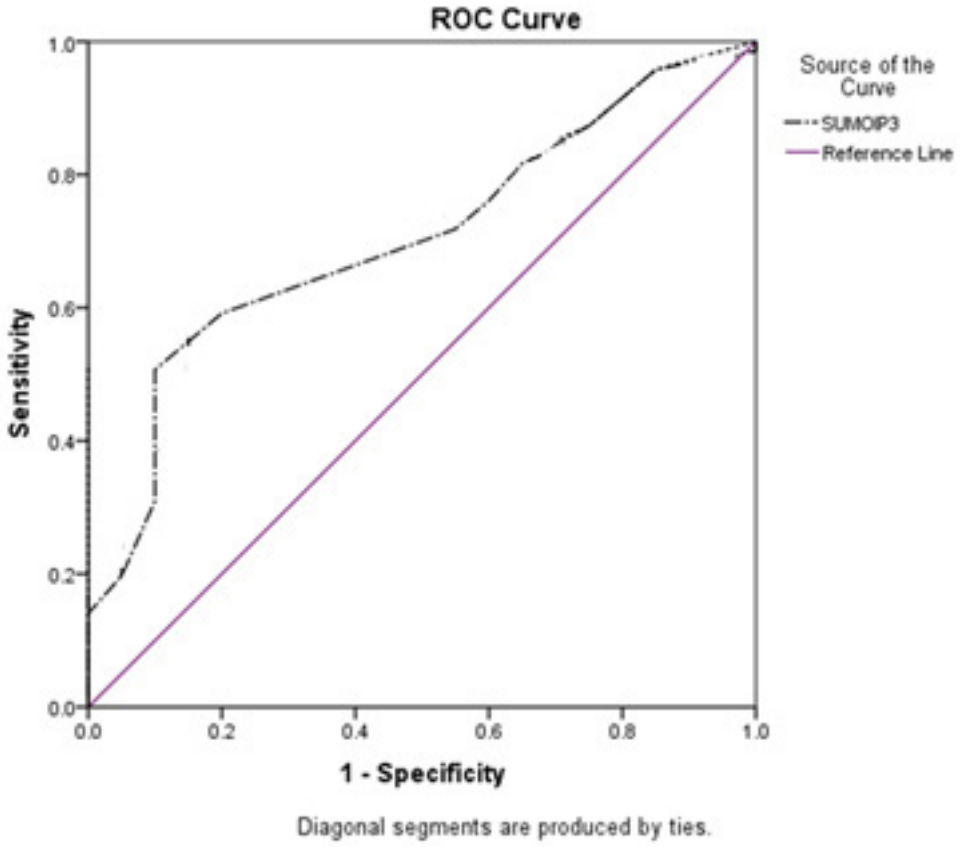
Table 1: The SUMO1P3 expression levels and demographic characteristics of the patients including age and gender

P-value	Non-cancerous		Cancerous			Characteristics
	Percentage	Quantity	Percentage	Quantity		
0.001	70	28	39.4	56	< 64	Age (year)
	30	12	60.6	86	> 64	
< 0.001	65	26	31	44	Female	Gender
	35	14	69	98	Male	

Table 2: The relationship between SUMO1P3 expression levels (ΔCt) in GC diagnosed patients

P-value	Non-cancerous		Cancerous		ΔC_t
	SD	Mean	SD	Mean	
0.005	2.8	-29.1	11.7	-23.6	SUMOIP3

Figure 1: The ROC curve of the SUMO1P3 levels between gastric cancer tissues and adjacent non-tumor tissues



The results showed that SUMO1P3 levels in males were not significantly higher than those in females (p = 0.485, Table 3). No significant difference of SUMO1P3 expression was observed between patients under 64 years old and above (p = 0.155, Table 3). In other words, patients below 64 years-old showed higher SUMO1P3 levels compared to those older than 64.

As shown in Table 3, the SUMO1P3 levels were not associated with perineural invasion (p = 0.319), lymphatic invasion (p = 0.797), invasion depth (p = 0.790), location of the tumor (p = 0.811), tumor size (p = 0.635), and grading (p = 0.289).

Table 3: The relationship between SUMO1P3 expression levels (ΔCt) and pathological factors among the studied patients

p value	Mean ± SD	No. of patients (%)	Factors		
0.485	5.768±1.389	4 (40%)	< 64	Malignant	Age (year)
	5.2636±2.090	6 (60%)	≥ 64		
0.155	5.821±1.563	2 (20%)	Male	Malignant	Gender
	4.924±2.211	8 (80%)	Female		
0.319	5.150±2.029	6 (60%)	Positive		Perineural invasion
	6.086±1.473	4 (40%)	Negative		
0.797	5.408±2.083	6 (60%)	Positive		Lymph invasion
	5.375±1.696	4 (40%)	Negative		
-	5.434±2.048	10 (100%)	Positive		Lymph node metastasis
0.750	-	0 (0 %)	T1-T2		Invasion depth
	5.323±1.971	10 (10%)	T3-T4		
0.811	5.429±2.066	10 (10%)	NON CARDIA		Location of the tumor
	-	0 (0 %)	CARDIA		
0.625	5.095±0	2 (20 %)	Small		Tumor size
	5.476±2.019	8 (80%)	Large		
-	-	0 (0%)	1-2		Staging
	5.566±2.068	10 (10%)	3-4		
0.289	5.196±0	2 (20%)	1		Grading
	-	0 (0%)	2		
	4.910±2.076	8 (80%)	3		

Discussion

In this study, we were interested in evaluating the expression of lncRNA SUMO1P3 at a molecular level as one of the pseudogene-expressed lncRNAs in GC patients.

Recent studies have shown that, lncRNA plays an important role in gastric cancer (9, 12). However, considering the pseudogene expressed lncRNAs, the potential of lncRNAs as a clinical diagnostic marker for clinical applications is still basically unknown.

Our results revealed that the expression levels of SUMO1P3, one of the transcripts of pseudogene, were not up-regulated in gastric cancer. As opposed to our findings, a recent publication by Mei al (6). indicated that "pseudogenes might play their cancer-associated roles in RNA level".

We also followed different parameters affecting the SUMO1P3 expression in our patients including; age, gender, tumor size, differentiation, lymphatic metastasis, invasion (13, 14). No significant up-regulation of SUMO1P3 expression in our patients with GC was found for the mentioned factors (Table 3).

We found that SUMO1P3 expression is independent of age. This result was in agreement with previous reports, stating that some lncRNAs such as gastric-cancer-associated transcript 1, GACAT1, have been proved to be independent of age (9, 15, 16). It should be noted that, for some types of cancer, gender is concerned to be a factor to influence its incidence (9, 15, 16). In our study, we investigated that gender was not a factor that is significantly related to SUMO1P3 expression in patients with GC ($p = 0.485$, Table 3).

In the previously published papers, the relationship between invasion and lymphatic metastasis in GC and miRNA expression has been reported (17). Our results indicated a non-significant relationship between invasion and lymphatic metastasis in GC and lncRNA expression (Table 3).

In recent years, the understanding of GC biomarkers has undergone a marked change (1, 18-24). Descriptions of gastric wall function have evolved from an impermeable and passive barrier to a multifunctional tissue layer with an active role in dynamic cellular communication and adaptive permeability (1, 7, 25).

On the basis of the present results and according to the used method for our patient population, we can believe that lncRNA SUMO1P3 may not be a potential biomarker in the diagnosis of gastric cancer. However, more accurate follow-up studies are needed for the evaluation of the variations of lncRNA SUMO1P3 expression for gastric cancer patients. The results here should be confirmed in larger series, considering confounding factors (26, 27), and providing a more detailed assessment of lncRNA SUMO1P3 levels using other modalities.

Conclusions

In this work, expression of lncRNA SUMO1P3 in gastric cancer patients was evaluated. No statistical significant change of pseudogene-expressed lncRNA SUMO1P3 was seen according to the used method in this study. Therefore, pseudogene-expressed lncRNA SUMO1P3 may not be a potential biomarker in the diagnosis of gastric cancer.

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Conclusions

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