Overexpression of TPT1-AS1 and SAMMSON and down expression of LINC00961 associated with Advanced Grades of Gastric Cancer

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Abstract

Background

One of the deadliest cancers in the world is gastric cancer. Long non-coding RNAs play prominent roles in cancer. LINC00961, TPT1-AS1, and SAMMSON have recently been discovered, which significantly contribute in various cancers and can affect the tumor size, grade of tumors and the metastasis condition. The aim of this study was to determine LINC00961, SAMMSON and TPT1-AS1 expression in gastric cancer tissues in comparison with healthy adjacent tissues.

Methods

The number of cancerous tissues and control groups was calculated to be at 40 (n = 40) and were analyzed by Quantitative real-time polymerase chain reaction.

Results

We found that overexpression of TPT1-AS1 and SAMMSON, and downexpression of LINC00961 in cancerous tissues in comparison with healthy adjacent tissues. A positive association between TPT1-AS1 and SAMMSON expression and tumor grade was observed. The level of mRNA folding change increased in cancer group compared to control group and *P < 0.05 is considered for mRNA folding change.

Conclusion

Finally, we found that overexpression of TPT1-AS1 and SAMMSON, and downexpression of LINC00961 were observed significantly in gastric cancer tissues in comparison with adjacent non-cancerous tissues. These lncRNAs were suggested as potential tumor markers for the diagnosis and treatment of gastric cancer.

Background

Gastric cancer is one of the most dangerous malignancies in the world. Especially, in the eastern regions of Asia. Each year, the death toll is estimated to be around 950,000 in the world, accounting for almost half of the prevalence in the East Asia [1].Although the prevalence and mortality rate is reduced by this malignancy, but gastric cancer is the third cause of cancer-associated deaths worldwide [2]. Most patients with gastric cancer are identified in advanced stages of the disease. Although specific diagnostic and treatment methods for gastric cancer have been used, the survival rate of these patients is about 5 years, which is a very low survival rate [3–7]. Today, certain tumor markers are used such as CA19–9 and

carcinoma embryonic antigen (CEA) to diagnose gastric cancer, but they have no specificity to diagnose this malignancy [8].

Recently, non-coding RNAs such as long non-coding RNAs (lncRNAs) have been used as diagnostic tumor markers as well as therapeutic targets for malignancies [9, 10]. Today, it has been discovered that TPT1-AS1 can involve in various cellular processes, such as cellular differentiation, proliferation, migration, apoptosis, invasion and stem-cell biology [9]. For example, in people with non-small cell lung cancer (NSCLC), lncRNAs can play important roles in NSCLC tumorigenesis and progression [11].

LncRNA tumor protein translationally controlled 1 (TPT1) antisense RNA 1 (TPT1-AS1) is one of the latest LncRNAs that has recently been investigated in gliomas and cervical cancer. It has been observed that increased expression of this lncRNA can increase the tumor size, proliferation, metastasis, and more difficult treatment [9].TPT1-AS1 is associated with one of transcription factors which are associated with the transcription of some mitochondrial genes, and thus TPT1-AS1 can affect the regulation of the expression of these genes [9, 12]. However, there are few studies on this lncRNA.

The next lncRNA, which was measured in this study, is SAMMSON. This lncRNA is associated with the Wnt / B Catenin pathway, and it has been shown that by increasing the expression of this lncRNA, the pathway in cancer cells can be activated and leads to the growth, metastasis and self-renewal of these cells [13, 14]. According to the research, this lncRNA can make an important contribution in other cellular pathways.

Another lncRNA, which has been considered previously, is LINC00961. This lncRNA also plays a role in various cancer processes and this lncRNA affects a polypeptide called SPAR, which can inhibit mTORC1 [15]. It has been shown that the increase of LINC00961 expression can inhibit the metastasis and proliferation of the cancerous cells [11].

Methods

Patients And Tissue Specimens

In this study, tissue samples have been taken from the Cancer Institute of Imam Khomeini Hospital in Tehran. Patients' information and the written informed consent from participation were prepared by the center. Our exclusion criteria include any chemotherapy, radiotherapy, other cancers with gastric cancer and chronic or acute inflammatory diseases, and its progression confirmed by the pathologist by the relevant protocols. Cancer tissues and adjacent healthy ones were taken by the surgeon from 40 patients, and each of these specimens was divided into two parts. One part was immediately placed in liquid nitrogen and used for RNA extraction. cDNA generation and QRT-PCR technique were used to determine the expression of the related lncRNAs genes, and the other part was laid down in formalin for pathological examinations. Demographic information such as age, gender, site of primary neoplasm, date of diagnosis, race, marital status, family history of cancer, history of disease, specific drug use, were recorded for each patient.

RNA extraction and real-time PCR

Total RNA was isolated from the samples using RNX- Plus solution (Cinnagen, Tehran, Iran) according to the instructions of the manufacturer. The extracted RNA is quantitatively and qualitatively evaluated by nano-drop spectrophotometer (Thermo Fisher Scientific Inc.,Wilmington, USA) and 0.8% agarose gel. cDNA was prepared by First Aid Reverse Transcription Kit (Fermentas). Real-time PCR was performed by SYBR Green master mix (Amplicon, Denmark) in the LightCycler96 instruments (Roche Life Science Deutschland GmbH, Sandhofer, Germany). Primer sequences were designed and synthesized by Takapou Zist Company. The primer sequences were as follows: LINC00961 (forward:5'-CTG TTC TGG ATG GGA GCG AA -3'; reverse: 5'-ACA GTC ACC ACG AAC AGC AC-3'), TPT1-AS1 (forward: 5'-CAC TCC CAG ATC TTC ACT TCA GG -3'; reverse: 5'-AAT TGG AGG CCA GTG CTC TG -3'), SAMMSON (forward: 5'-CCT CTA GAT GTG TAA GGG TAG T-3'; reverse: 5'- TTG AGT TGC ATA GTT GAG GAA-3') and beta-actin (forward: 5'-ACAGAGCCTCGCCTTTGC – 3'; reverse: 5'-ATCACGCCCTGGTGCCT – 3'). The difference in genes expression in comparison with the reference gene was determined by the 2^-ΔΔCt formula [16].

Data Analysis

Statistical analysis was carried out using the Statistical Package for Social Sciences version 16 (SPSS Inc., Chicago, IL). In order to compare the expression ratio of these genes in two groups, the distribution of data using the Kolmogorov-Smirnov test was examined and according to the proportions of the results of parametric and non-parametric tests (Student t tests and Mann Whitney). In this study, the significance level p was less than 0.05. The Pearson and Spearman tests were used to examine the relationship between variables in terms of parametric and non-parametric proportions.

Results

Characteristics Of The Participants

Properties of the patients are showed in Table 1. All study participants were Iranian, and the average age was 62 years (33 to 85). Twenty-nine (72.5%) were male patients, and 11 (27.5%) were female patients. In TNM staging, patients with low stage (I & II) were 5 (12.5%) and with high stage were 35 (87.5%). In this study, patients with adeno-carcinoma were 29 (72.5%) and non-adenocarcinoma was 11 (27.5%). In addition, primary site of neoplasm was determined in these patients and include 10 (25%) in Cardia, 10 (25%) in Antrum and 20 (50%) in the body of the stomach.

Table 1
Clinicopathological characteristics of the participants.

Characteristic	Categorization	(%) N	F.C of LINC00961 Mean ± SD	p Value LINC00961	F.C of TPT1-AS1 Mean ± SD	p Value TPT1-AS1	F.C of SAMMSON Mean ± SD	p Value SAMMSON
Age, years	60≥	23 (57.5)	0.43 ± 0.15	0.741	0.45 ± 4.22	0.632	1.57 ± 0.15	0.08
	> 60	17 (42.5)	0.62 ± 0.11		0.21± 4.33		1.85 ± 0.11	
TNM stage	low (I, II)	5 (12.5)	0.17 ± 0.54	0.562	0.39 ± 5.01	0.545	0.17 ± 1.44	0.527
	high	35 (87.5)	0.13 ± 0.63		0.31 ± 4.09		0.13 ± 1.76	
Gender	Female	11 (27.5)	0.16 ± 0.57	0.812	0.58 ±4.19	0.197	0.26 ± 1.23	0.562
	Male	29 (72.5)	0.19 ± 0.71		0.52 ± 4.51		0.29 ± 1.55	
Site of primary	Cardia	10 (25)	0.14 ± 0.60	0.264	0.32 ± 5.02	0.567	0.34 ± 1.32	0.554
	Antrum	10 (25)	0.19 ± 0.59		0.34 ± 4.12		0.29 ± 1.76	
	Body	20 (50)	0.09 ± 0.75		0.44 ± 4.29		0.29 ± 1.79	
Tumor size, cm	4 ≥	8 (20)	0.16 ± 0.48	0.233	0.58 ± 4.04	**0.046**	0.26 ± 2.63	0.04
	> 4	32 (80)	0.15 ± 0.54		0.45± 5.05		0.13 ± 3.28	
Histology	Adenocarcinoma	29 (72.5)	0.18 ± 0.58	0.323	0.27 ± 4.27	0.283	0.28 ± 1.78	0.638
	Non.Adenocarcinoma	11 (27.5)	0.16 ± 0.47		0.21± 4.56		0.24 ± 1.88	

TNM = Tumor/Node/Metastasis, F.C = Folding change of target genes *Grade I (Well differentiated), Grade II (Moderately differentiated), Grade III (Poorly differentiated), Grade IV (Undifferentiated).

Characteristic	Categorization	(%) N	F.C of LINC00961 Mean ± SD	p Value LINC00961	F.C of TPT1-AS1 Mean ± SD	p Value TPT1-AS1	F.C of SAMMSON Mean ± SD	p Value SAMMSON
Grade	Grade I	3 (7.5)	0.12 ± 0.55	0.233	0.26± 4.52	**0.018**	0.29 ± 1.64	0.012
	Grade II	10 (25)	0.11 ± 0.43		0.28 ± 4.79		0.21 ± 1.77	
	Grade III	18 (45)	0.13 ± 0.44		0.43 ± 5.05		0.28 ± 1.83	
	Grade IV	4 (10)	0.13 ± 0.47		0.19 ± 5.29		0.16 ± 1.98	
	Unknown	5 (12.5)	0.09 ± 0.62		0.32± 4.39		0.28 ± 1.74	
Necrosis	Positive	11 (27.5)	0.15 ± 0.59	0.413	0.28 ± 4.36	0.381	0.17 ± 1.49	0.628
	Negative	29 (72.5)	0.19 ± 0.49		0.54 ± 4.60		0.33 ± 1.52	
Lymphatic invasion	Positive	31 (77.5)	0.19 ± 0.72	0.534	0.41 ± 4.52	0.179	0.28 ± 1.34	0.329
	Negative	9 (22.5)	0.12 ± 0.81		0.36 ± 4.97		0.26 ± 1.46	
Vascular invasion	Positive	31 (77.5)	0.16 ± 0.64	0.781	0.65 ± 4.61	0.656	0.21 ± 1.44	0.359
	Negative	9 (22.5)	0.12 ± 0.54		0.64 ± 4.35		0.15 ± 1.66	
Perineural invasion	Positive	22 (55)	0.14 ± 0.56	0.981	0.65 ± 5.09	0.456	0.27 ± 1.74	0.468
	Negative	18 (45)	0.19 ± 0.66		0.46 ± 4.78		0.25 ± 1.89	

TNM = Tumor/Node/Metastasis, F.C = Folding change of target genes *Grade I (Well differentiated), Grade II (Moderately differentiated), Grade III (Poorly differentiated), Grade IV (Undifferentiated).

Quantitative Rt-pcr Analysis

TPT1-AS1 expression

In this study, to demonstrate the importance of Lnc RNA TPT1-AS1 in cancerous tissues of gastric samples, we evaluated TPT1-AS1 expression in 40 cancerous tissues (n = 40) and observed that the

TPT1-AS1 RNA levels significantly increased compared to the adjacent non-cancerous tissues (n = 40) ($p < 0.05$ Fig. 1-a).

Sammson Expression

We measured SAMMSON expression in cancer tissues (n = 40) and observed that the RNA levels enhanced compared to non-cancer tissues (n = 40) ($p < 0.05$ Fig. 1-b).

Linc00961 Expression

According to the LINC00961 expression and our findings, the RNA levels in gastric cancer tissues (n = 40) decreased significantly in comparison to the adjacent non-cancerous tissues (n = 40) ($p < 0.05$ Fig. 1-c).

Correlation Study

To verify the correlation of TPT1AS1 and SAMSON expression with the grade of tumors, we analyzed the correlation of these Lnc RNAs expression with the grades of tumors. A significant and positive association between TPT1AS1 expression and tumor grade was found ($r = 0.170$, $p = 0.018$, Fig. 2). In addition, SAMSON expression showed a significant association with the tumor grade ($r = 0.655$, $p = 0.012$, Fig. 3).

Roc Curve Analysis

ROC curves analysis were drawn to find out whether the genes expression levels of TPT1AS1, SAMSON and LINC00961 might be considered as potential tumor biomarkers for Gastric cancer. The area under curve (AUC) of ROC analysis for TPT1AS1as plotted for Gastric cancer tissues compare to control tissues was obtained as [0.543 (95% CI, 0.428–0.655)], AUC of ROC analysis for SAMMSON [0.879 (95% CI, 0.787–0.941)], and AUC of ROC analysis for LINC00961 [0.772 (95% CI, 0.664–0.858)] in 40 pairs of Gastric cancer patient samples as shown in respectively Fig. 4d–f.

Discussion

Gastric cancer is one of the most dangerous and fatal cancers in the world, which is ranked third in terms of the deadliest among all types of neoplasms. This type of neoplasm is increasing in the world today, and different cognitive ways have been expressed. However, extensive studies are required to identify and cure it [17, 18].

Long non-coding RNAs (lncRNAs) contribute greatly in many diseases, for example cancer. Genomic studies of transcriptomes (a study of all coding and non-coding RNAs) have shown that transcription is

abundant in the mammalian genome, which at least 80% of this transcription is exclusively related to non-coding RNAs (ncRNAs) [19]. ncRNAs are classified into two groups based on their sequencing length: short ncRNAs with less than 200 nucleotides (sncRNAs) and long non-coding RNAs with more than 200 nucleotides (lncRNAs). Until now, according to numerous studies, more than 58,000 lncRNAs have been identified in the human genome. A large number of lncRNAs have not been found to function and role and they seem to act only as transcriptional noises. However, some of them have different functions in gene transcription and protein translation. Unlike microRNAs, lncRNAs are able to activate the gene expression and others have the ability to suppress it. lncRNAs are able to interact with a variety of macromolecules, such as DNA, RNA, and proteins, and play vital roles in regulating gene expression at transcriptional, post transcriptional and epigenetic levels. lncRNAs are mainly located within the nucleus and affect the expression of gene at the epigenetic level, and a small number (approximately 15%) are present in the cytoplasm and regulate protein translation. An important role of lncRNAs in many diseases, especially cancer, has been reported in numerous studies [20]. Recently, a number of lncRNAs have proven to play a significant part in the proliferation, cell cycle, apoptosis, invasion, migration, metastasis and tumorigenicity of gastric cancer cells [21]. As previously mentioned, less than thousands of lncRNA mechanisms have been discovered. Recently, three lncRNAs have been identified including TPT1-AS1, SAMMSON and LINC00961, which have been subjected to limited studies on their action mechanism in cancer.

TPT1-AS1 is one of the most recent lncRNAs that is located on the 13q14.13 gene. This lncRNA has recently been investigated in glioma and cervical cancer. It is observed that increased expression of this lncRNA can enhance the tumor size, proliferation, metastasis, and requires treatments that are more difficult. [9]. On the other hand, this lncRNA is associated with a protein called SP1 [9]. SP1 along with zinc finger protein 179 (ZnF 179) create a route which can affect the protection against oxidative stress and mitochondria-induced ROS. This protein is one of the factors involved in transcription and also regulates the expression of some subunits in the complex of electron transport chain [12]. It has been observed that overexpression of TPT1-AS1 can increase the expression of gene and SP1 protein, which eventually results in the disruption of the electron transfer chain, ultimately damaging the mitochondria and cells and exacerbating oxidative stress condition [9].

SAMMSON locus is located on the chromosome 3p13. This lncRNA is associated with the Wnt/B Catenin pathway. It has been shown that increased expression of this lncRNA can activate the pathway in cancerous cells and accelerate the growth of the cells, metastasis, and self-renewal of these cells [14]. This lncRNA is also associated with a mitochondrial protein called P32, which is effective both in the integrity of the mitochondrial structure and in the production of energy through oxidative phosphorylation. It can also contribute to the progression of certain cancers and the transformation of the cell metabolism from oxidative phosphorylation to glycolysis and the increased oxidative stress, due to its ineffectiveness and lack of proper production [22]. SAMMSON, as mentioned above, is associated with this protein, and by deactivating P32, it can disrupt the mitochondrial function and structure and also leads to an oxidative stress condition[23] [14].

The ROC curve results showed a relatively appropriate specificity and sensitivity for SAMMSON and LINC00961 RNA levels in cancerous and non cancerous tissues, indicating that these two genes expression levels may be used for gastric cancer diagnosis.

As it is shown in our finding, overexpression of TPT1-AS1 and SAMMSON have a significant association with the advanced grades of cancerous tissues. According to the previous studies, increased expression of these lncRNAs may enhance the growth of the cancer cell and tumor tissue proliferation. It can be concluded that TPT1-AS1 and SAMMSON take a leading role in the development of gastric cancer, especially in the higher grades, and increased expression of these genes can be considered as a diagnosis pathway in gastric cancer. In the future, it can be used to treat this lethal cancer by inhibiting lncRNAs. On the other hand, increased expression of these genes can damage mitochondria and increase oxidative stress circumstance.

Another lncRNA, which has been investigated in this study, is LINC00961. This lncRNA, which is located on the 9p13.3 gene, significantly contribute in various cancer processes and has 1546 nucleotides. This lncRNA acts on a polypeptide called Small Regulatory Polypeptide of Amino Acid Response (SPAR), which can inhibit (mammalian Target Of Rapamycin Complex 1) mTORC1, but nevertheless this lncRNA can affect the activity of mTORC1, and this complex can also control mitochondria in electron transport chain complex proteins and ATP production and consumption [15]. It is expected to modify the toxicity of mitochondrial oxidative stress and contribute to the onset of cancer. It has been shown that increased expression of LINC00961 can have an inhibitory role in the metastasis and proliferation of cancerous cells [11].

Based on the findings of the present study, a significant overexpression of TPT1-AS1 and SAMMSON in cancerous tissues has been shown in comparison with healthy adjacent tissues. On the other way, decreased expression of LINC00961 in patients with gastric cancer, as compared to the healthy adjacent group, revealed that in the pathologic condition, overexpression of TPT1-AS1 and SAMMSON and down expression of LINC00961 might take a leading role in the development of gastric cancer as well as in higher grades.

Recently, studies have been done to increase the expression of TPT1-AS1 gene, for example, in 2017, Jiang et al. studied cervical cancer both in vivo and in vitro, and observed that lncRNA TPT1-AS1 could be involved as an oncogenic agent in the development of cervical cancer, and also, they introduced it as an effective therapeutic goal [9]. In addition, in 2019, Wu et al. studied epithelial ovarian cancer, and observed that lncRNA TPT1-AS1 could lead to tumorigenesis and metastasis in epithelial ovarian cancer [24].

In 2017, Li et al. Conducted a study on liver cancer both in vivo and in vitro, and found that SAMMSON lncRNA activates Wnt / B Catenin pathway in cancer cells and can activate these cells in their growth and self-renewal of cancer cells [14] .

In 2018, Huang et al. investigated the cancer tissues of patients with lung cancer and found that the expression of LINC00961 gene has declined significantly in these patients, and in healthy subjects, this lncRNA had an inhibitory effect on the cell growth, proliferation and metastasis [25]. In 2019, Lixia Zhang et al. studied on the cancer tissues of patients with Tongue Squamous Cell Carcinoma (TSCC) and observed that the expression of LINC00961 has significantly decreased and this lncRNA, with its own function, inhibits the growth, proliferation and metastasis of cancerous cells by regulating the Wnt/β-Catenin signaling pathway [26].

Considering these functions of the lncRNAs mentioned above, if a mutation takes place in the expression of these lncRNAs, it could affect different signaling pathways. With the effect on various genes and proteins presented in these pathways, they can contribute to the progression, invasion and metastasis in cancerous cells, which is due to the increased expression of TPT1-AS1 and SAMMSON and reduced expression of LINC00961 [9, 11, 12]. In this study, using genetic techniques and biochemical tests, it has been found that overexpression of TPT1-AS1 and SAMMSON and down expression of LINC00961 could increase proliferation, invasion and metastasis in cancerous cells compared to healthy subjects. According to the previous studies and the present study, it can be concluded that these important lncRNAs may cause gastric cancer and can be used as tumor markers of this cancer. However, more research and large numbers of samples are required to reveal the exact mechanisms of this LncRNAs and better understanding of their potential use in gastric cancer.

Conclusion

Finally, according to our findings, we observed that in patients with Gastric Cancer compared to group control, the increased expression of TPT1-AS1 and SAMMSON genes and down expression of LINC00961 gene and changes in the different signaling pathways, which can ultimately cause gastric cancer to start and exacerbate. However, we can recognize one of the causes of the promotion and exacerbation of this fatal cancer as a disorder in expression and function of TPT1-AS1, SAMMSON and LINC00961 genes, and we introduce these lncRNAs as tumor markers for gastric cancer but in this study is required more researches to confirm it.

Abbreviations

GC: Gastric Cancer; qRT-PCR: Quantitative real-time polymerase chain reaction; LncRNAs: Long noncoding RNAs

Declarations

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Availability of data and materials

Not applicable.

Ethics approval and consent to participate

The study protocol was approved by Hamadan University of Medical Sciences Ethics Committee (code: IR.UMSHA.REC.1397.506 approved on 4 Dec 2018), and all patients provided written informed consent for the procedures before endoscopy and surgury.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no financial competing interests.

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Figures

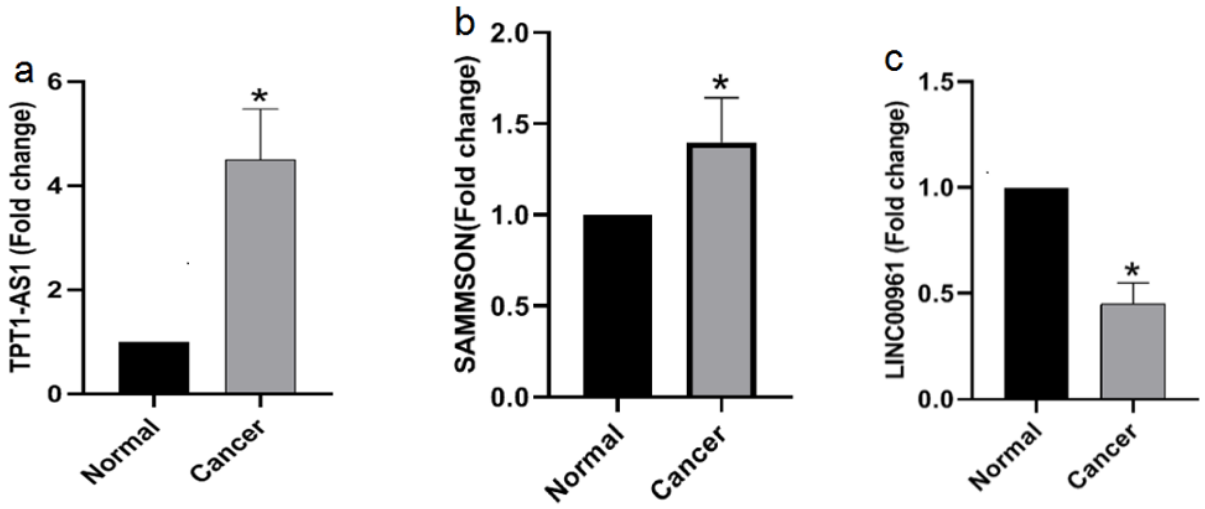

Figure 1

The level of mRNA folding change of the genes in patients and control group. a) The level of mRNA folding change of TPT1-AS1 gene in patients and control group. As shown in the figure, the level of mRNA folding hange increased in cancer group compared to control group. b) The level of mRNA folding change of SAMMSON gene in patients and group control. As shown in the figure, the level of mRNA folding change increased in cancer group compared to control group. c) The level of mRNA folding change of LINC00961 gene in patients and group control. As shown in the figure, the level of mRNA folding change decreased in cancer group compared to control group. *P<0.05 is considered for mRNA folding change.

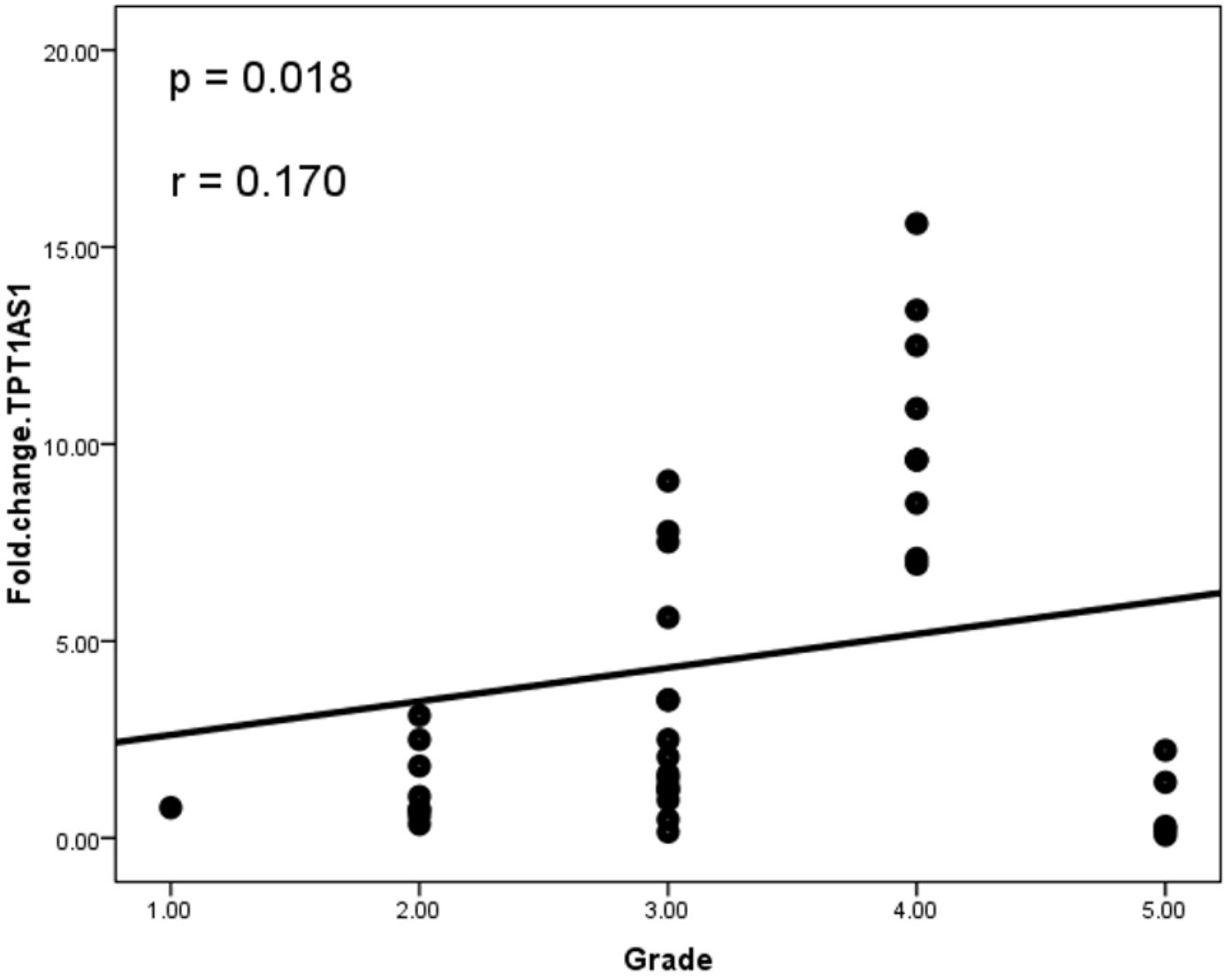

Figure 2

Association between grade of tumor and TPT1-AS1 gene expression in cancerous tissues. Correlation analysis showed significant relationship between overexpression of TPT1-AS1 and grade of tumor.

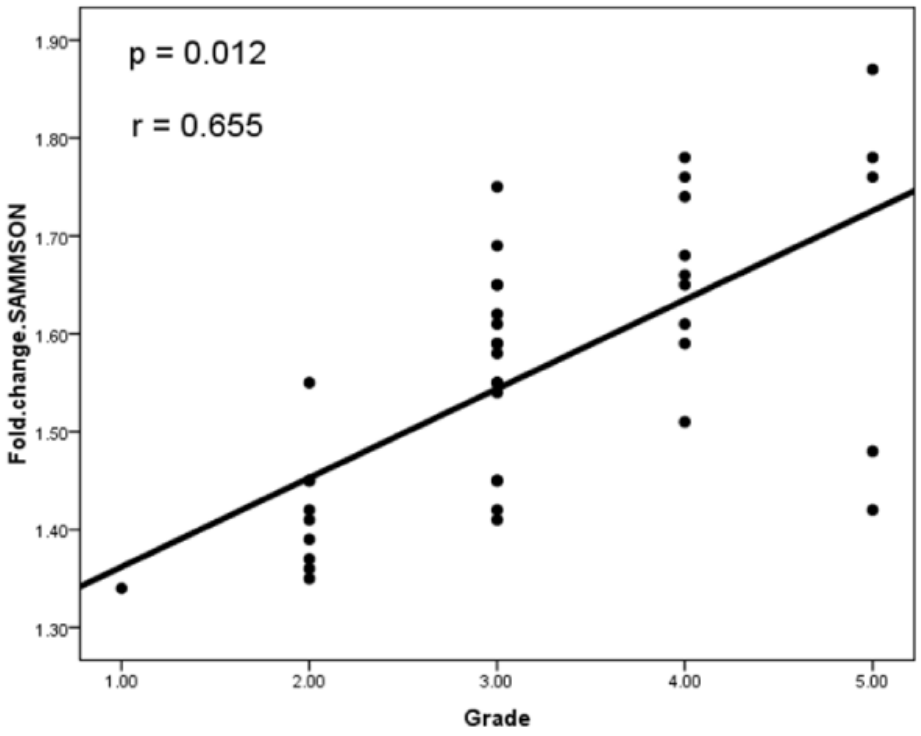

Figure 3

The relationship between grade of tumor and SAMMSON gene expression in cancerous tissues. Correlation analysis showed a significant relationship between overexpression of SAMMSON and the grade of tumor.

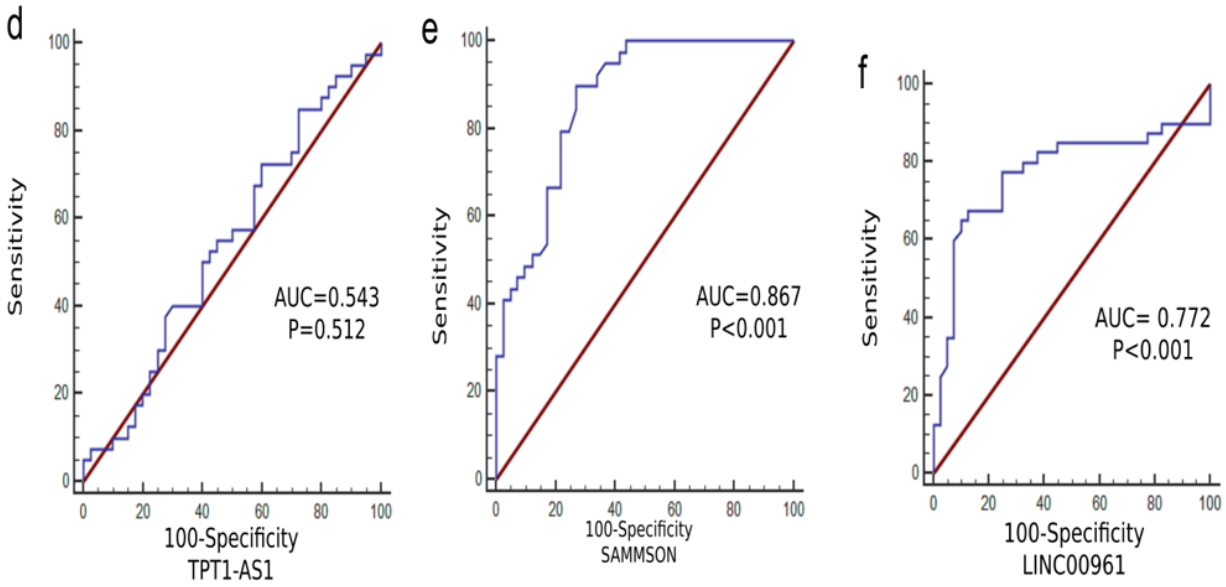

Figure 4

d–f. ROC of TPT1-AS1 (a), SAMMSON (b) and LINC00961 (c) RNA levels for Gastric Cancer detection in different GEO data and in the clinical data.