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

CURRENT STATUS: UNDER REVIEW

Mohammad Amin Amini
Hamadan University of Medical Sciences Medical School

Jamshid Karimi
Hamadan University of Medical Sciences Medical School

Iraj Khodadadi
Hamadan University of Medical Sciences Medical School

Heidar Tavilani
Hamadan University of Medical Sciences Medical School

Seyed Saman Talebi
Hamadan University of Medical Sciences Medical School

Behrouz Afshar
Hamadan University of Medical Sciences Medical School

DOI: 10.21203/rs.3.rs-22694/v1

SUBJECT AREAS
Gastroenterology & Hepatology

KEYWORDS
Stomach neoplasm, RNA, Long non-coding (lncRNA), Biomarker
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 IncRNAs 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 IncRNAs 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’-ACAGAGCCTCGCCTTGC – 3’; reverse: 5’-ATCAGCCCTGGTGCCT – 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.

| Characteristic | Categorization | (%) | N      | F.C. of LINC00961 Mean ± SD | p Value | F.C. of TPT1-AS1 Mean ± SD | p Value | F.C. of SAMMSON Mean ± SD | p Value |
|----------------|---------------|-----|--------|----------------------------|---------|----------------------------|---------|----------------------------|---------|
| Age, years     | 60 ≥ > 60     | 23  | (57.5) | 0.43 ± 0.15 0.62 ± 0.11     | 0.741   | 0.45 ± 4.22 0.21 ± 4.33     | 0.632   | 1.57 ± 0.15 1.85 ± 0.11     | 0.08    |
| TNM stage      | low (I, II)   | 5   | (12.5) | 0.17 ± 0.54 0.13 ± 0.63     | 0.562   | 0.39 ± 5.01 0.31 ± 4.09     | 0.545   | 0.17 ± 1.44 0.13 ± 1.76     | 0.527   |
| Gender         | Female        | 11  | (27.5) | 0.16 ± 0.57 0.19 ± 0.71     | 0.812   | 0.58 ± 4.19 0.52 ± 4.51     | 0.197   | 0.26 ± 1.23 0.29 ± 1.55     | 0.562   |
| Site of primary| Cardia        | 10  | (25)   | 0.14 ± 0.60 0.19 ± 0.59 0.09 ± 0.75 | 0.264   | 0.32 ± 5.02 0.34 ± 4.12 0.44 ± 4.29 | 0.567   | 0.34 ± 1.32 0.29 ± 1.76 0.29 ± 1.79 | 0.554   |
| Tumor size, cm | 4 ≥ > 4       | 8   | (20)   | 0.16 ± 0.48 0.15 ± 0.54     | 0.233   | 0.58 ± 4.04 0.45 ± 5.05     | 0.046   | 0.26 ± 2.63 0.13 ± 3.28     | 0.04    |
| Histology      | Adenocarcinoma| 29  | (72.5) | 0.18 ± 0.58 0.16 ± 0.47     | 0.323   | 0.27 ± 4.27 0.21 ± 4.56     | 0.283   | 0.28 ± 1.78 0.24 ± 1.88     | 0.638   |
| Grade          | Grade I       | 3   | (7.5)  | 0.12 ± 0.55 0.11 ± 0.43 0.13 ± 0.44 0.13 ± 0.47 0.09 ± 0.62 | 0.233   | 0.26 ± 4.52 0.28 ± 4.79 0.43 ± 5.05 0.19 ± 5.29 0.32 ± 4.39 | 0.018   | 0.29 ± 1.64 0.21 ± 1.77 0.28 ± 1.83 0.16 ± 1.98 0.28 ± 1.74 | 0.012   |
| Necrosis       | Positive      | 11  | (27.5) | 0.15 ± 0.59 0.19 ± 0.49     | 0.413   | 0.28 ± 4.36 0.54 ± 4.60 0.381 | 0.17 ± 1.49 0.33 ± 1.52 | 0.628   |
| Lymphatic invasion | Positive | 31  | (77.5) | 0.19 ± 0.72 0.12 ± 0.81     | 0.534   | 0.41 ± 4.52 0.36 ± 4.97 0.179 | 0.28 ± 1.34 0.26 ± 1.46 | 0.329   |
| Vascular invasion | Positive  | 31  | (77.5) | 0.16 ± 0.64 0.12 ± 0.54     | 0.781   | 0.65 ± 4.61 0.64 ± 4.35 0.656 | 0.21 ± 1.44 0.15 ± 1.66 | 0.359   |
| Perineural invasion | Positive  | 22  | (55)   | 0.14 ± 0.56 0.19 ± 0.66     | 0.981   | 0.65 ± 5.09 0.46 ± 4.78 0.456 | 0.27 ± 1.74 0.25 ± 1.89 | 0.468   |

TQM = 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 (IncRNAs) 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 (IncRNAs). Until now, according to numerous studies, more than 58,000 IncRNAs have been identified in the human genome. A large number of IncRNAs 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, IncRNAs are able to activate the gene expression and others have the ability to suppress it. IncRNAs 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. IncRNAs 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 IncRNAs in many diseases, especially cancer, has been reported in numerous studies [20]. Recently, a number of IncRNAs 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 IncRNA mechanisms have been discovered. Recently, three IncRNAs 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 IncRNAs that is located on the 13q14.13 gene. This IncRNA has recently been investigated in glioma and cervical cancer. It is observed that increased expression of this IncRNA can enhance the tumor size, proliferation, metastasis, and requires treatments that are more difficult. [9]. On the other hand, this IncRNA 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 IncRNA is associated with the Wnt/B Catenin pathway. It has been shown that increased expression of this IncRNA can activate the pathway in cancerous cells and accelerate the growth of the cells, metastasis, and self-renewal of these cells [14]. This IncRNA 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 IncRNAs 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 IncRNAs. On the other hand, increased expression of these genes can damage mitochondria and increase oxidative stress circumstance.

Another IncRNA, which has been investigated in this study, is LINC00961. This IncRNA, which is located on the 9p13.3 gene, significantly contribute in various cancer processes and has 1546 nucleotides. This IncRNA 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 IncRNA 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 IncRNA 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 IncRNA 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 IncRNA 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 IncRNA 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 IncRNA, 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 IncRNAs mentioned above, if a mutation takes place in the
expression of these IncRNAs, 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 IncRNAs 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 IncRNAs 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**

**Acknowledgements**

The results presented in this article were part of M.A. Amini MSc thesis. We would like to thank H. Moridi and H. Rezaie for their assistance.

**Funding**

This study was financially and ethical supported by the Hamadan University of Medical Sciences (No: 9709135418).
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 surgery.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no financial competing interests.

Author details

1Department of Clinical Biochemistry. Hamadan University of Medical Sciences, Hamadan, Iran. 2Department of Internal Medicine. Hamadan University of Medical Sciences, Hamadan, Iran

References

1. Kamangar F, Dores GM, Anderson WF. Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world. Journal of clinical oncology. 2006;24(14):2137–50.

2. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. Cancer J Clin. 2015;65(2):87–108.

3. Rahman R, Asombang AW, Ibdah JA. Characteristics of gastric cancer in Asia. World journal of gastroenterology: WJG. 2014;20(16):4483.

4. Van Cutsem E, Sagaert X, Topal B, Haustermans K, Prenen H. Gastric cancer. The Lancet. 2016;388(10060):2654–64.

5. Tan YK, Fielding JW. Early diagnosis of early gastric cancer. Eur J Gastroenterol Hepatol. 2006;18(8):821–9.
6. Cho JY. Molecular diagnosis for personalized target therapy in gastric cancer. J Gastric Cancer. 2013;13(3):129–35.

7. Amini MA, Karimi J, Khodadadi I, Tavilani H, Talebi SS, Afshar B. Overexpression of ROMO1 and OMA1 are Potentially Biomarkers and Predict Unfavorable Prognosis in Gastric Cancer. Journal of gastrointestinal cancer 2019:1–8.

8. Shimada H, Noie T, Ohashi M, Oba K, Takahashi Y. Clinical significance of serum tumor markers for gastric cancer: a systematic review of literature by the Task Force of the Japanese Gastric Cancer Association. Gastric cancer. 2014;17(1):26–33.

9. Jiang H, Huang G, Zhao N, Zhang T, Jiang M, He Y, Zhou X, Jiang X. Long non-coding RNA TPT1-AS1 promotes cell growth and metastasis in cervical cancer via acting AS a sponge for miR-324-5p. Journal of Experimental Clinical Cancer Research. 2018;37(1):169.

10. Moridi H, Karimi J, Tavilani H, Khodadadi I, Emami Razavi AN. Overexpression of PURPL and downregulation of NONHSAT062994 as potential biomarkers in gastric cancer. Life Sci. 2019;237:116904.

11. Jiang B, Liu J, Zhang Y-h, Shen D, Liu S, Lin F, Su J, Lin Q-f, Yan S, Li Y. Long noncoding RNA LINC00961 inhibits cell invasion and metastasis in human non-small cell lung cancer. Biomed Pharmacother. 2018;97:1311–8.

12. Ben-Shachar D, Karry R. Sp1 expression is disrupted in schizophrenia; a possible mechanism for the abnormal expression of mitochondrial complex I genes, NDUFV1 and NDUFV2. PLoS One. 2007;2(9):e817.

13. Li X, Li M, Chen J, Dai H, Wang L, Xiong Y, Zhong Y, Zhang L. SAMMSON drives the self-renewal of liver tumor initiating cells through EZH2-dependent Wnt/β-catenin activation. Oncotarget. 2017;8(61):103785.

14. Liu D, Liu Y, Xia Z, Dong H, Yi Z. Reactive oxygen species modulator 1 regulates
oxidative stress and induces renal and pulmonary fibrosis in a unilateral ureteral obstruction rat model and in HK-2 cells. Mol Med Rep. 2017;16(4):4855–62.

15. Morita M, Gravel S-P, Chénard V, Sikström K, Zheng L, Alain T, Gandin V, Avizonis D, Arguello M, Zakaria C. mTORC1 controls mitochondrial activity and biogenesis through 4E-BP-dependent translational regulation. Cell Metabol. 2013;18(5):698–711.

16. Livak KJ, Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2–ΔΔCT method. methods 2001, 25(4):402–408.

17. Wu H, Gu Y-h, Wei L, Guo T-k, Zhao Y, Su G, Li J, Xie X-d. Association of Romo1 gene genetic polymorphisms with risk of gastric cancer in northwestern Chinese population. Pathology Oncology Research. 2015;21(3):581–7.

18. Sudo G, Nasuno H, Nakachi K, Nakase H. Gastrointestinal: Secondary gastric linitis plastica: A peritoneal recurrence of breast cancer. Journal of gastroenterology and hepatology 2019.

19. Ma L, Bajic VB, Zhang Z. On the classification of long non-coding RNAs. RNA Biol. 2013;10(6):924–33.

20. Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. Nature reviews genetics. 2009;10(3):155.

21. Hao N-B, He Y-F, Li X-Q, Wang K, Wang R-L. The role of miRNA and IncRNA in gastric cancer. Oncotarget. 2017;8(46):81572.

22. Hu M, Crawford SA, Henstridge DC, Ng IH, Boey EJ, Xu Y, Febbraio MA, Jans DA, Bogoyevitch MA. p32 protein levels are integral to mitochondrial and endoplasmic reticulum morphology, cell metabolism and survival. Biochem J. 2013;453(3):381–91.

23. Amini MA, Talebi SS, Karimi J. Reactive Oxygen Species Modulator 1 (ROMO1), a New Potential Target for Cancer Diagnosis and Treatment. Chonnam medical journal. 2019;55(3):136–43.
24. Wu W, Gao H, Li X, Zhu Y, Peng S, Yu J, Zhan G, Wang J, Liu N, Guo X. LncRNA TPT1-AS1 promotes tumorigenesis and metastasis in epithelial ovarian cancer by inducing TPT1 expression. Cancer Sci. 2019;110(5):1587.

25. Huang Z, Lei W, Tan J, Hu HB. Long noncoding RNA LINC00961 inhibits cell proliferation and induces cell apoptosis in human non-small cell lung cancer. Journal of cellular biochemistry. 2018;119(11):9072–80.

26. Zhang L, Shao L, Hu Y. Long noncoding RNA LINC00961 inhibited cell proliferation and invasion through regulating the Wnt/β-catenin signaling pathway in tongue squamous cell carcinoma. Journal of cellular biochemistry. 2019;120(8):12429–35.

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 change increased in cancer group compared to control group. b) The level of mRNA folding change of SAMMSON gene in patients and control group. 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 control group. 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.
Correlation analysis showed significant relationship between overexpression of TPT1-AS1 and grade of tumor.
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.
