Original Article
Running Title: LncRNA TMPO AS-1 Expression Level in Melanoma Tissues
Received: February 28, 2022; Accepted: July 31, 2022

Evaluation of the LncRNA TMPO AS-1 Expression Level in Melanoma Tissues in Comparison to Paired Normal Marginal Tissues

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Abstract
Background: Long noncoding RNA (lncRNA) is recognized as an essential controller of gene expression and other activities of the cells. Additionally, lncRNAs have a critical role in the progression and growth of human malignancies, like melanoma. Among lncRNAs, thymopoietin (TMPO)-antisense RNA 1 (TMPO AS-1) has a significant role in melanoma. The current study aimed to determine the expression level of TMPO AS-1 in melanoma patients.
Method: In this case-control research, 50 pairs of tumor and non-tumor tissues of melanoma patients were separated by the surgeon. Subsequently, TMPO AS-1 expression level in the tissues was evaluated. We used TRIzol to extract total RNA from the tumor and non-tumor tissues, following which complementary DNA was synthesized. The TMPO AS-1 expression level of TMPO AS-1 was evaluated via quantitative reverse transcription- polymerase chain reaction (qRT-PCR) technique. Moreover, clinicopathological features of the melanoma patients were evaluated.
Results: Our findings revealed that TMPO AS-1 expression level was upregulated in the melanoma tissues in comparison to the non-tumor ones. Remarkably, the TMPO AS-1 expression level was considerably correlated to the clinicopathological characteristics of the patients, including lymph nodes and distant metastasis.
Conclusion: Upregulation of TMPO AS-1 in melanoma indicated that TMPO AS-1 and its downstream signal pathways could be used as a new target treatment option and prognostic tumor marker for melanoma.
Keywords: Biomarkers, TMPO AS-1, Melanoma, RNA, Long noncoding

Introduction

Despite decades of clinical research and the improvement in different methods of treatment, malignancy still remains the leading cause of mortality. According to the World Health Organization (WHO), in 2019, there were 10 million deaths and 19.3 million new cases of cancer worldwide.[1,2,3] Melanoma is a kind of skin malignancy that progresses when melanocytes begin to

develop without a controller. The incidence rate of this type of skin malignancy is low, but the treatment of the melanoma is difficult with a low survival rate. Family history, having fair skin, and overexposure to the sun are important factors leading to melanoma.[4] In 2020, the incidence and mortality rates of melanoma was 1.7% (324,635 new cases) and 0.6% (57,043), respectively, worldwide.[1,5] The treatment options for melanoma may include surgical removal, chemotherapy, radiotherapy, immunotherapy, or targeted therapy. Thus, we need new insight into how to improve the survival rate in advanced stages of this malignancy.[4,6] Surgery of the lesion could be conducive to the treatment of melanoma patients if diagnosed in an early stage. However, melanoma is an invasive illness that tends to spread beyond its original location. Once melanoma progresses, surgery is not effective and treatment becomes more challenging.[7] Long noncoding RNAs (lncRNA) are a kind of RNA with more than 200 nucleotides that are not translated into protein.[8] lncRNAs are broadly studied in malignancy pathogenesis. Previous research has confirmed that some lncRNAs are involved to malignancy, including cell proliferation, migration, invasion, and apoptosis.[8,9] TMPO Antisense RNA 1 or TMPO AS-1 is an lncRNA situated on the opposite strand of TMPO gene. Uncontrolled TMPO AS-1 expression has confirmed to be associated with tumor development and could serve as a favorable target for treatment and a prognostic or diagnostic marker in certain human malignancies.[8] The functional and potential role of TMPO AS-1 in melanoma is not yet fully understood. In this research, we detected expression of the levels of TMPO AS-1 via quantitative reverse transcription- polymerase chain reaction (qRT-PCR) in tumor and peripheral tissues.

Material and Method

Ethical approval

This study was conducted in accordance with the amended declaration of Helsinki. The institutional review board approved the study, and all the participants provided written informed consent. All the patients signed forms, and their personal information was confidential. The Academic Committee of Research Ethics (Studies in Human Subjects) of Tabriz University of Medical Sciences evaluated and confirmed this study as a research project (trial registration number: IR.TBZMED.REC.1399.944).

Study population and sampling method

The Ethics Committee of Tabriz University of Medical Sciences approved the current work (code: IR.TBZMED.REC.1399.944). All the participants read and signed written consent. This case-control study was conducted between 2019 and 2020 in Imam Reza Hospital (Tabriz-Iran). We recruited 40 patients (21 men and 19 women) with melanoma, whose malignancy was diagnosed based on paraclinical examinations and clinical signs by a pathologist. During the surgery, a small part of the tumor and peripheral tumor tissue were detached. Table 1 depicts the clinicopathological characteristics of the patients. Following the surgery, the samples were directly moved into an RNase inhibitor solution (Biocompare) and kept at -80 ° C until RNA extraction. Prior to the sampling, written informed consent was signed by all the patients.

RNA extraction

Total RNA was extracted with TRIZOl reagent (Thermo Fisher Scientific) as stated by the manufacturer. The total RNA quantity and quality were affirmed via NanoDrop spectrophotometer (Thermo Fisher Scientific) at 260/280. Moreover, we evaluated RNAs quality with gel electrophoresis on 1% agarose.

Subsequently, RNAs were stored in a temperature of -80 until cDNA synthesis.

Syntheses of cDNA and qRT-PCR

We synthesized CDNA using cDNA synthesis kits (Biofact, Seoul) with a volume of 20 µl. The internal control gene was GAPDH. qRT-PCR was performed with master mix SYBER Green using Roche Real-time PCR Light Cycler 96 (Germany) and was reported by 2-ΔΔCT method. Table 2 represents the details of a program of the PCR and sequences of the primers.

Explanation of TMPO AS-1 analysis as a tumor marker

TMPO AS-1 relative expression analysis was performed with REST (randomization test applying the Relative Expression Software Tool). We evaluated the sensitivity and specificity of TMPO AS-1 expression levels to distinguish melanoma tissues from normal tissues via ROC curve plot (Sigma Plot 12.5 software). This plot is a graph presenting the false positive rate and true positive rate for unalike descriptors threshold.[10] Afterwards, the horizontal axis in the ROC curve displays a false positive rate and the vertical axis shows a true positive rate.

Statistical analysis

Statistical study was done with GraphPad Prism 6 software (San Diego, CA, USA). We used the unpaired Student's T- test to estimate the statistical value of the differences among the variables (P-value < 0.05, mean ± SD).

Results

TMPO AS-1 expression level in melanoma tissues

We analyzed the TMPO AS-1 expression level in 40 melanoma tissues and compared them to 40 peripheral non-tumor tissues. Additionally, the clinicopathological features of the melanoma patients, like age and sex, as well as tumor-related features, such as distant metastasis, were evaluated. The results revealed a considerable overexpression in TMPO AS-1 level in cancerous tissues compared to that of the peripheral non-tumor tissues (fold change = 1.36, P = 0.0010) (Figure 1). TMPO AS-1 expression level was significantly correlated with some clinicopathological characteristics, like distant metastasis (P = 0.0055) (Figure 2), yet there was no considerable relationship between TMPO AS-1 expression level and sex or age

TMPO AS-1 as a diagnosis marker in melanoma

We utilized ROC curve to analyze the specificity and sensitivity of TMPO AS-1 as a new biomarker in melanoma. The results showed a ROC region biomarker index (0.7) for the TMPO AS-1 in the melanoma patients (Table 3) (Figures 3 and 4).

Discussion

In the current research, TMPO AS-1 expression considerably increased in the melanoma tissues compared to that in the peripheral healthy tissues. These results suggested that TMPO AS-1 may be associated with tumorigenesis in melanoma. We also found that TMPO AS-1 expression level was significantly correlated with some clinicopathological characteristics, like distant metastasis. Nonetheless, there was no considerable correlation between TMPO AS-1 expression level and sex or age. Furthermore, the ROC analysis showed that TMPO AS-1 expression level might be used as a prognosis and diagnosis biomarker in melanoma.

In spite of various methods of therapy, the incidence and mortality rate of melanoma has increased in all cancer types worldwide.[11] Currently, the diagnosis, prognosis, and treatment of patients with melanoma are mainly related to pathological and clinical factors, which necessitates finding new molecular markers involved with melanoma.[12,13] Our findings are closely consistent with several reports around the

world. Fang et al. revealed that the TMPO AS-1 expression level increases in NSCLC cell lines and tissues. Overexpression of TMPO AS-1 at protein and mRNA levels was involved with NSCLC development. Knockdown of TMPO AS-1 led to significant suppression of cell growth, invasion, and metastases in vivo and in vitro.[14] Liu et al. exhibited that up-regulation of lncRNA TMPO AS-1 induces osteosarcoma cells apoptosis via controlling E2F1and miR-329 expression.[15] Cheng et al. reported that abnormal expression of TMPO AS-1 in retinoblastoma tissues induces retinoblastoma cell growth and metastases, and promotes malignant retinoblastoma cell phenotypes via sponging miR-199a-5p.[16] Zhao et al. also showed that lncRNA TMPO AS-1 could potentially enhance LCN2 expression via binding to transcription factor E2F6, thereby stimulating the development of ovarian malignancy.[17] Wang et al. revealed that TMPO AS-1 was considerably overexpressed in breast cancer and suppressing this lncRNA significantly inhibits tumor cell progression and metastasis via sponging miR-4731-5p.[18] In another study, Li et al. found that high expression level of TMPO AS-1 in thyroid tumor induces cancer cell proliferation, migration, and growth via sponging miR-498 to regulate the TMPO gene in tissues and cell lines.[19] Moreover, Mitobe et al. suggested that TMPO AS-1 could positively regulate estrogen receptor 1 (ESR1) mRNA expression by stabilizing ESR1 mRNA through interaction with ESR1 mRNA. Enhanced expression of ESR1 mRNA by TMPO AS-1 could play a critical role in the proliferation of ER-positive breast cancer.[20] Zhang et al. indicated that TMPO AS-1 expression level considerably increases in human LSCC tissues compared to normal samples. TMPO AS-1 up-regulation was positively related to lymph node metastasis and clinical stage, which might be used as an independent prognosis factor for the overall survival of LSCC patients.[21]

Ultimately, the present research showed that TMPO AS-1 is highly expressed in melanoma and its up-regulation is associated with the pathogenesis and tumorigenesis of melanoma. Treatments for these patients are very limited and the therapeutic outcome is not effective in advanced stages. Therefore, early prognosis, diagnosis, and treatment are of particular importance.

As we know, this was the first study to investigate the TMPO AS-1 expression level. In order to obtain better understanding in this field, we need further research with a higher number of patients and related genes associated with TMPO AS-1 to be used as a prognostic and diagnostic factor in melanoma malignancy.

Conclusion

Overall, in the current study, we found that TMPO AS-1 is highly expressed in melanoma, which is associated with the pathogenesis and tumorigenesis of melanoma. Treatments for these patients are very limited and the therapeutic outcome is not effective in advanced stages. Therefore, early prognosis, diagnosis, and treatment are of particular importance.

Funding

This study was financially supported by Tuberculosis and Lung Disease Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

Conflict of Interest

None declared.

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Table 1. Clinicopathological features of the patients with melanoma		
Number of sample	40	
Age (mean: 59.7)	60>22	60<18
Sex	Male: 21	Female: 19
Metastases	Positive: 29	Negative: 11
Chemotherapy	Positive: 30	Negative: 10
Family history	Positive: 5	Negative: 35
Age ± SD	60 ± 14.7	

Table 2. Primer sequence and characteristics used in the quantification of the target genes		
Gene	Primer sequence	Annealing temperature (°C)
TMPO AS-1	Forward: 5′-AGACGCCGATAAGGGACAG-3′ Reverse: 5′-AGCCAAGGGTCCTCACA-3′	59
GAPDH	Forward: 5′-CAAGATCATCAGCAATGCCTCC-3′ Reverse: 5′-GCCATCACGCCACAGTTTCC-3′	59

TMPO AS-1: TMPO antisense RNA 1; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase

Table 3. The statistical analysis of receiver operating characteristic (ROC) curve for diagnostic evaluation	
ROC curve data	Values
Area	0.7
95% confidence interval	0.6252 to 0.9547
Std. Error	0.08403
P-value	0.005101
The number of margin samples	40
The number of tumor samples	40

Roc: Receiver operating characteristic; Std: Standard

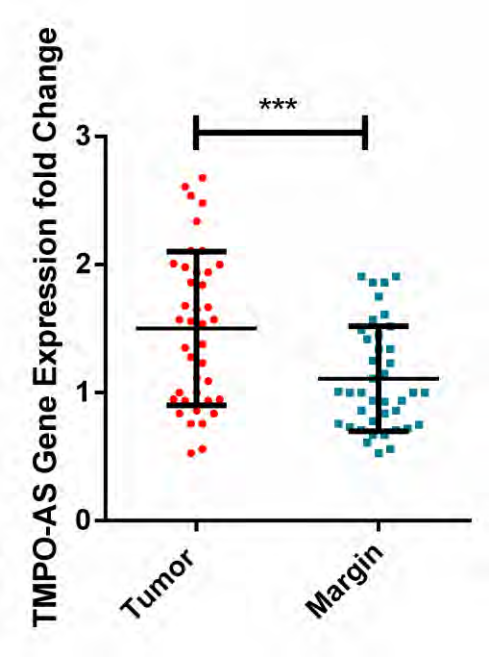

Figure 1. The expression level of TMPO AS-1 gene was significantly upregulated in the melanoma tissues as compared with the marginal region. We evaluated the expression of the TMPO AS-1 expression level by Real-time PCR in the tumor tissues and healthy margin tissues.
TMPOAS1: TMPO antisense RNA 1; PCR: Polymerase chain reaction

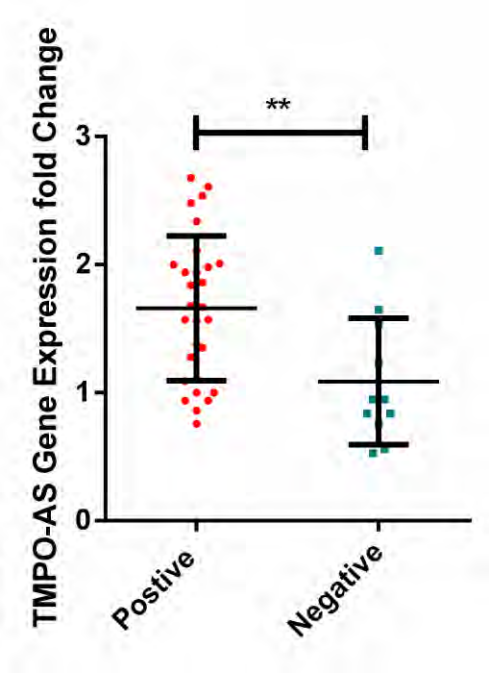

Figure 2. The expression level of TIMPO AS-1 gene was significantly higher in the melanoma tissues with metastasis as compared with those without metastasis. We evaluated the expression of the TMPO AS-1 expression level by Real-time PCR in the melanoma tissues with positive metastasis and negative metastasis.

TMPO AS-1: TMPO Antisense RNA 1

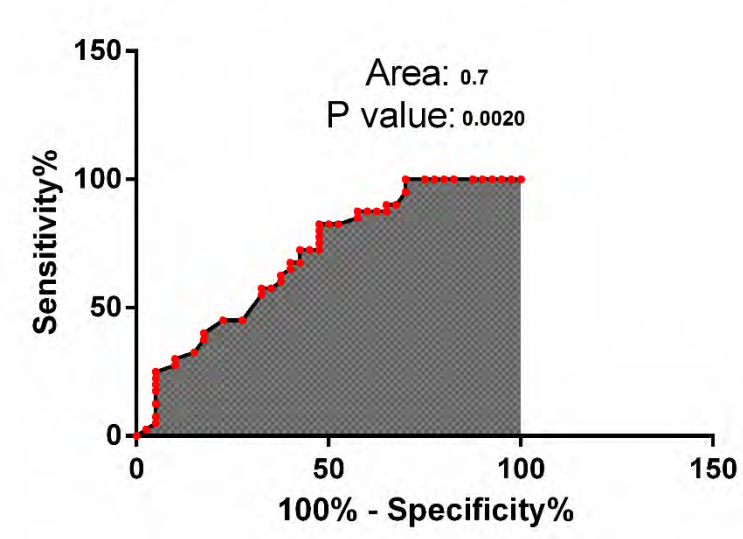

Figure 3. ROC curve was plotted for TMPO-AS1 to differentiate the melanoma cases from the normal controls. In this analysis the area under plot was 0.7 and *P*-value was 0.002 (Area: 0.7, *P*-value: 0.0020).

ROC: Receiver operating characteristic; TMPOAS1: TMPO Antisense RNA 1

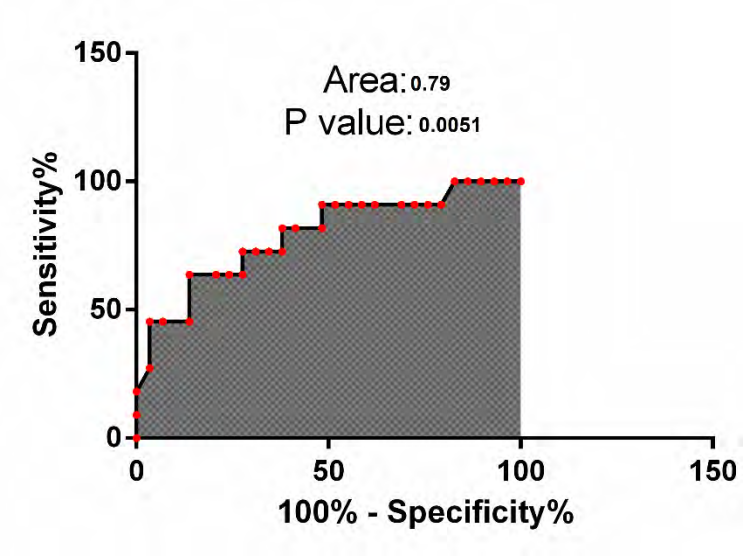

Figure 4. ROC curve was plotted for TMPO AS-1 expression level to differentiate metastasis in the melanoma tissues from those without metastasis. (Area: 0.79, *P*-value: 0.0051). In this analysis, the area under plot was 0.79 and *P*-value was 0.0051.
ROC: Receiver operating characteristic; TMPOAS1: TMPO antisense RNA 1