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Original Article

Expression and Bioinformatics Analysis of Taurine-Upregulated Gene 1 (TUG1) in Esophageal Squamous Cell Carcinoma (ESCC) Tissue Samples Collected from Patients in Golestan Province, Northeast of Iran

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Abstract

Background and objectives: Esophageal squamous cell carcinoma (ESCC) is the most widespread type of esophageal cancer. LncRNA TUG1 was first identified in a genomic screening study for the treatment of taurine in retinal cells. This study aimed at analyzing TUG1 expression in ESCC tissues collected from an Iranian population of patients. Bioinformatics study was also conducted for better understanding the function of this lnc-RNA.

Methods: We examined the expression of TUG1 in 31 pairs of ESCC tissues and adjacent non-cancerous tissues by qRT-PCR. Bioinformatics analysis was conducted using various databases. Student's t-test was performed using SPSS software (version 16.0) to evaluate the difference in TUG1 expression between ESCC and adjacent non-cancerous tissues.

Results: TUG1 expression level in ESCC tissues was significantly higher than that in the adjacent non-cancerous tissues (P=0.04).

Conclusion: TUG1 is upregulated in ESCC, which may be related to history of smoking.

Keywords: ESCC; EZH2; Gene expression; Long non-coding RNA; TUG1; SUZ12

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INTRODUCTION

After cardiovascular diseases and accidents, cancer is the most common cause of death worldwide (1) .Esophageal cancer (EC) is the sixth main cause of cancer-related mortality and the eighth most common malignancy in the world (2, 3). In spite of many treatments like surgery and chemotherapy, EC has a poor prognosis with a five-year survival rate (4). Histologically, EC is classified into two general types: esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (4, 5). ESCC is the most widespread type of EC mainly in low- or medium-income countries, such as Iran (4). In the Golestan Province located in the northeastern part of Iran, EC has an age-standardized incidence rate of 43.4 per 100,000 men and 36.3 per 100,000 women (6) .It has been suggested that EC development may be dependent on several risk factors, including genetic susceptibility, poor oral health, hot tea consumption and poor economic and social status (7). In addition, smoking increases the risk of ESCC development by 1.4 fold (8, 9).

Long non-coding RNAs (lncRNAs) are molecules of longer than 200 nucleotides that do not encode proteins. These molecules are associated with some cellular progression processes such as parental imprinting, cell proliferation, apoptosis and metastasis via epigenetic modification, chromatin remodeling and sponging miRNAs (10, 11). Evidence suggest that lncRNAs are involved in the onset and progression of cancer and can act as a tumor suppressor or an oncogene (12). Recent investigations reveal that there may be a relationship between upregulation of some lncRNAs such as HOTAIR, POU3F3, FOXCUT and MALAT1 and development of ESCC (13, 14). Taurine up-regulated 1 (*TUG1*), a newly discovered lncRNA, with the length of 7.1 kb, was first identified in a genomic screening study for the treatment of taurine in retinal cells (15). TUG1 often associates with polycomb repressive complex 2 (PRC2) to silence target genes.

Based on the reports from LncRNADisease v2.0 database (http://www.rnanut.net/lncrnadisease/), TUG1 has a role in the pathogenesis of diseases such as B-cell lymphoma, malignant glioma, lung squamous cell carcinoma, renal cell carcinoma, ESCC, intrahepatic cholangiocarcinoma and oral squamous cell carcinoma. Moreover, lncRNA TUG1 has been recently found to contribute to tumor progression via regulation of different cellular processes, including migration, invasion, proliferation, differentiation and apoptosis (16). Considering the unknown factors affecting ESCC progression, identifying biomarkers that detect ESCC is of great importance for clinical surveillance and clinical decision making (13).

In a previous study by Yang et al., it has been reported that alcohol drinking and tobacco smoking together significantly enhance the risk of ESCC among Chinese men (9). Pakzad et al. also reported the high incidence rate of EC in less developed countries because of lifestyle habits, smoking, alcohol use and poor nutrition (17). In ESCC, heavy smokers have been proved to have a two-times higher odds of p53 mutation than non-smokers (18) . Dysregulation of TUG1 is probably associated with p53 mutation during ESCC development (5); therefore, TUG1 may be a therapeutic option for ESCC.

In this study, we evaluate overexpression of lncRNA TUG1 in the tumor tissue and adjacent non-cancerous tissue of ESCC patients in the Golestan Province, northeastern Iran. Moreover, we investigated the relationship between the regulation of lncRNA TUG1 and ESCC clinical parameters.

MATERIALS AND METHODS

Tissue samples

A total of 31 pairs of ESCC and adjacent normal tissues were collected from ESCC patients (15 men and 16 women). The mean

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age of cases was 60.70 ± 13 years. Written informed consent was obtained from all patients prior to participation in the study.

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

Total RNA was extracted from the tissues using Trizol reagent (Invitrogen, CA, USA). The quantity and quality of the extracted RNA were evaluated using a spectrophotometer (Picodrop, UK). Complementary DNA (cDNA) was synthesised using PrimeScript™ RT Reagent Kit (Takara, Shiga, Japan) and DNase I, RNase-free Kit (SinaClon, Iran) to remove additional DNA. Next, qRT-PCR was performed using the SYBR RealQ Plus Master Mix Green (Ampliqon, Denmark) and 2 µl of cDNA in an ABI 7300 thermocycler (Applied Biosystems, CA, USA). All the above-mentioned procedures were conducted based on the instructions provided by the manufacturers. The forward and reverse primers used in this study were TUG1 F: 5′-CTGAAGAAAGGCAACATC-3′, TUG1 R: 5′-GTAGGCTACTACAGGATTTG-3, GAPDH F: 5′-GAAGGTGAAGGTCGGAGT-3′, and GAPDH R: 5′-GAAGATGGTGATGGGATTTC-3′. The relative expression of TUG1 was calculated and normalised using the $2^{-\Delta\Delta Ct}$ method relative to GAPDH, as the internal control. The cycling conditions for the qRT-PCR experiment are listed in table 1.

Table 1. The cycling conditions for the qRT-PCR experiment

Stage	Temperature (°C)	Duration
First stage (one cycle)	95.0	15 sec
Second stage (40 cycles)	95.0	15 sec
	59.0	45 sec
	72.0	40 sec
Third stage (one cycle)	95.0	15 sec
	60.0	1 sec
	95.0	15 sec

Statistical analysis

All statistical analyses were performed using SPSS software (version 16.0). Student's t-test was used to evaluate the difference in TUG1 expression between ESCC and adjacent non-cancerous tissues.

RESULTS

lncRNA TUG1 expression in ESCC

The relative expression of lncRNA TUG1 was measured by qRT-PCR in 31 pairs of cancerous and adjacent non-cancerous tissues. As shown in figure 1, the expression level of TUG1 in ESCC tissues was significantly higher (3.98-fold) than that in the adjacent non-cancerous tissues (P=0.04).

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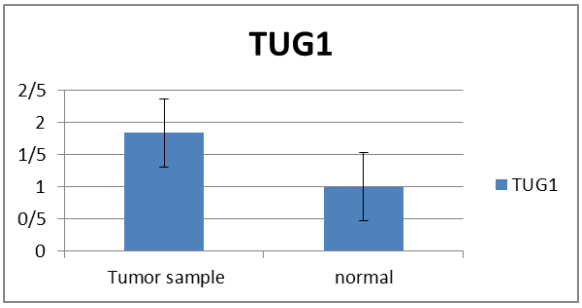

Figure 1. TUG1 relative expression level in ESCC and adjacent non-cancerous tissues

Bioinformatics analysis

LncRNADisease v2.0 (http://www.rnanut.net/lncrnadisease/) database confirmed the involvement of TUG1 in ESCC. The mutation of these variants resulted in a change in TUG1 expression. Bioinformatics studies also indicated a strong association of EZH2, SUZ12 and HOXB7 with TUG1 products with EC.

TUG1 expression and clinical parameters

By comparing the expression level of TUG1 among clinicopathological parameters, we found a significant relationship between smoking and TUG1 expression level (P=0.038; Table 2). However, other clinical parameters had no significant correlation with TUG1 expression level.

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Table 2. Clinicopathological factors and the expression of TUG1

Variables	Number of patients (%)	Fold change	p-value
Age (years)			
<50	6 (19.35)	1.985815	0.764
≥50	25 (80.64)	4.460171	
Sex			
Male	15 (48.38)	2.984263	0.105
Female	16 (51.61)	4.915951	
Histologic grade			
1+2	24 (77.41)	3.933252	0.277
3+4	7 (22.58)	4.145873	
Tumor size			
≤5mm	17 (54.83)	4.743343	0.937
>5mm	14 (45.16)	3.055881	
Stage			
T1-T2	9 (29.03)	0.710888	0.141
T3-T4	21 (67.74)	5.566401	
Metastasis			
M_O	15 (48.38)	2.000325	0.788
M_1	9 (29.03)	0.727055	
Smoking			
No	24(77.41)	4.202844	0.038*
Yes	7(22.58)	3.221559	
Lymph node metastasis			
No	16(51.61)	0.962504	0.155
Yes	15(48.38)	7.201274	
Necrosis			
No	17(54.83)	2.138108	0.812
Yes	14(45.16)	6.21938	

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DISCUSSION

According to recent studies, lncRNAs are critical for many biological processes, such as cellular development and differentiation (19-21). Dysregulation of lncRNA often leads to malignancy (22-24). Jiang et al. reported the upregulation of TUG1 in ESCC tissues, mainly in older cases or chemotherapy-resistant tumors (5). Xu et al. observed TUG1 upregulation in ESCC and suggested a possible involvement in proliferation and migration of ESCC cells (25). Some studies suggested that *TUG1* overexpression may also be involved in osteosarcoma (26), human breast cancer (15) and bladder urothelial carcinoma (27). However, Li et al. claimed that TUG1 is downregulated in human glioma tissues, and dysregulation of TUG1 can influence apoptosis and cell proliferation in glioma cells, suggesting that TUG1 may have different roles in various cancers (28). In our study, smoking was significantly associated with upregulation of TUG1 in ESCC samples (P=0.038). According to Khalil et al., TUG1 is induced by p53 wild type and is able to regulate the cell cycle by connecting to PRC2 (29). They have also indicated that p53 is the activator and suppressor of a large number of genes, and TUG1 acts as a downstream suppressor in transcriptional pathways. In silico studies have revealed that TUG1, as a competing endogenous RNA, can regulate mRNA expression. Therefore, TUG1 takes a vital part in tumor development and progression through RNA-RNA interactions. Data extracted from the UniProt database (https://www.uniprot.org/) reveals TUG1 interactions with mRNAs and proteins (Table 3).

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Table 3. Data extracted from the UniProt database on the interaction of TUG1 with mRNAs and proteins

Gene	Protein	Function	Biological process
HOXB7	Homeobox protein Hox-B7	DNA-binding transcription factor activity, proximal promoter DNA-binding transcription activator activity, RNA polymerase II-specific	Embryonic skeletal system morphogenesis, multicellular organism development, regulation of transcription, DNA-templated
EZH2	Histone-lysine N-methyltransferase EZH2	Chromatin DNA binding, DNA-binding transcription factor activity, RNA polymerase II-specific, histone methyltransferase activity, primary miRNA binding,	Chromatin organization, G1 to G0 transition, histone methylation, negative regulation of gene expression, epigenetic, positive regulation of cell proliferation
SUZ12	Polycomb protein SUZ12	Chromatin DNA binding, methylated histon binding, RNA bindind	Negative regulation of cell differentiation, histone ubiquitination, negative regulation of gene expression, epigenetic, positive regulation of cell proliferation
MORC2	MORC family CW-type zinc finger protein 2	Transcriptional repressor, cytosolic function in lipogenesis, Zinc ion binding	Fatty acid metabolic process
SMTN	Smoothelin	Actin binding, structural constituent of muscle	Actin cytoskeleton organization, muscle organ development, smooth muscle contraction
OSBP2	Oxysterol-binding protein 2	Cholesterol binding, lipid binding, sterol transporter activity	Spermatid development

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TUG1 may bind to PRC2 and epigenetically regulate gene expression. HOXB7 regulates cell growth, mainly by activating the MAPK and PI3K/AKT pathways, and the destruction of TUG1 can elevate p-ERK, p-AKT and p-GSK3β levels. Regulation of cell growth under TUG1 occurs partly through HOXB7 regulation (30). Chen et al. have also indicated that HOXB7 is one of the HOX genes expressed only in the ESCC tissue and not in normal esophageal mucosa (31) .On the other hand, Huang et al. reported that lncRNA expression can be regulated by some transcription factors such as p53 and SP1 (32). Based on RAID v2.0 database (www.rna-society.org/raid/), TUG1 may interact with EZH2 and SUZ12 proteins. Hence, evaluating of the function and interaction of these proteins with TUG1 in ESCC in future studies can be beneficial for finding novel strategies to control ESCC progression.

In our study, the qRT-PCR analysis showed lncRNA TUG1 overexpression in an Iranian population of ESCC patients. However, further investigations with a larger study population are needed to clarify the exact role of TUG1 in ESCC. In this survey, due to the high cost of testing, the clinical function of TUG1 was evaluated only with qRT-PCR and bioinformatics analysis. More studies are required to understand the exact function of TUG1 in EC and its association with the mentioned proteins.

CONCLUSION

Our findings reveal that TUG1 is overexpressed in ESCC tissues, which may be associated with history of smoking. Our results also illustrate the importance of lifestyle and epigenetic factors in the development and progression of ESCC. Therefore, TUG1 can be considered as a potential therapeutic target in ESCC. The bioinformatics analysis also revealed a strong association between the proteins EZH2, SUZ12, and HOXB7 and TUG1, which may be associated with EC development .

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DECLARATIONS

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Ethics approvals and consent to participate

Written informed consent was obtained from all patients prior to participation in the study.

Conflict of interest

The author declares that there is no conflict of interest regarding publication of this article.

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