Study of Long Non-Coding RNA Tug1 Expression in Egyptian Colorectal Adenocarcinoma Patients

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Abstract

Purpose Colorectal cancer (CRC) is one of the most fatal tumors worldwide. In Egypt, most CRC cases occur in individuals > 40 years old. TUG1 has been proved to be disrupted in different malignancies and may have a critical role in tumor progression, invasion, and metastasis. However, its role in CRC has not been adequately studied.

Materials / Methods Quantitative real-time polymerase chain reaction (PCR) was used to evaluate the expression levels of long non-coding RNA (LncRNA) taurine upregulated gene 1 (TUG1), in nonmetastatic and metastatic CRC tissues and adjacent noncancerous tissues as control.

Keywords colorectal adenocarcinoma CRC LncRNA TUG1 PCR

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Results LncRNA TUG1 expression was significantly upregulated in both nonmetastatic and metastatic CRC tissues, in comparison with the adjacent noncancerous tissue. It was found that TUG1 could have a possible prognostic role in CRC, by comparing the sensitivity and specificity of TUG1 with those of CEA and CA19–9.

Conclusion The results of the current study suggest that the LncRNA TUG1 participates in the malignant behaviors of CRC cells.

Introduction

Colorectal cancer (CRC) is ranked as the third most common cancer globally and second in cancer-related mortality. A total of 90% of CRC cases are diagnosed over the age of 55 years old.1 It is a multistep malignancy in which genetic and epigenetic alterations accumulate.2 Genetic diseases are familial adenomatous polyposis (FAP), hereditary non-polyposis CRC (HNPPC) and Gardner syndrome, while post-translational histone acetylation and methylation are epigenetic factors.3 Obesity, smoking, and chronic alcoholism are environmental risk factors.4

Noncoding RNAs have been widely studied as biomarkers in the context of many diseases with a focus on lncRNAs and miRNAs. LncRNAs are nonprotein coding transcripts > 200 nucleotides, that have an epigenetic effect in CRC.5 Taurine upregulated gene 1 (TUG1) is a LncRNA (~ 7.1 kb in length) located on chromosome 11. Previous studies expected TUG1 to be a new diagnostic biomarker and therapeutic target of certain cancers. TUG1 has been proved to act as a miRNA sponge (ceRNA) to regulate mRNA expression of the target

Keywords

► colorectal adenocarcinoma
► CRC
► LncRNA
► TUG1
► PCR

The results of the current study suggest that the LncRNA TUG1 participates in the malignant behaviors of CRC cells.
gene and govern the progression of different cancers such as breast, bladder, and renal cell carcinoma (RCC).6–9
Several studies demonstrated that TUG1 knockdown in different cancer tissues such as urinary bladder carcinoma and hepatocellular carcinoma (HCC) suppressed cell proliferation, invasion and EMT.10–12 Moreover, LncRNA TUG1 has been shown to potentiate cancer metastasis and tumor progression in gastric carcinoma in ovarian mucinous adenocarcinomas.10 The previous findings show the regulatory roles of the TUG1 cancer progression. Our work suggests that TUG1 could have a potential role in the epithelial to mesenchymal transition of cancer in CRC tissue samples.

Materials and Methods
The present study included 65 subjects divided into 3 groups. Group I: 25 nonmetastatic colorectal adenocarcinoma tissue samples; group II: 25 metastatic (locoregional or blood born) colorectal adenocarcinoma tissue samples; and group III: 15 matched adjacent noncancerous healthy tissues. Pathological diagnosis of CRC is performed by biopsy of the mass suspected of tumor development. Disease extent is determined by imaging. Staging is done based on the TNM system. All samples were obtained from the Department of General Surgery, colorectal surgery unit, Alexandria main university hospital.

Samples (~ 0.4 cm x 0.2 cm of tissue) were excised from cancer tissue and adjacent noncancerous tissue as control. Each sample was then divided into 2 sections; 1 was submerged in RNA later (Rnase inhibitor) (ThermoFisher Scientific)13; and was kept frozen at - 80°C until use. The samples were fixed in 10% formalin solution for 24 hours. They were processed for light microscopic study to obtain paraffin blocks (Formalin Fixed and Paraffin Embedded [FFPE]). Five microns of sample thickness were cut and mounted on glass slide then stained using Hematoxylin and eosin stain (►Fig. 1).

Each 30-mg tissue sample was homogenized in a 2 ml sterile tube with 700 ul Qiazol solution using an electric homogenizer at 3,000 rpm.14

Total RNA isolation from tissue samples was conducted by using the Qiagen miRNeasy Mini Kit (Qiagen, CA) according to the instructions of the manufacturer. (ID: 217004).14 Single-stranded cDNA was synthesized from purified samples of RNA using High-Capacity cDNA Reverse Transcription Kit. (Applied Biosystems, USA) (Cat. No. 4368814 Archive).15 The purity and the concentration of RNA were measured at 260, 280 and 230 nm by using the Thermo Scientific, NanoDrop 2000/2000c Spectrophotometer (USA) Ratios of A260/A280 and A260/A230 = 1.8–2.1 indicates the high purity of RNA.

Relative quantification of tissue lncRNAs TUG1 genetic expression: real-time polymerase chain reaction (PCR) was performed using Thermo Scientific Maxima SYBR Green qPCR Master Mix (2X) (Thermo Scientific), and specific primers for lncRNA TUG1. Sequences used are TUG1 forward primer (5′- CTGAAGAAAGCCAACATC-3′) and reverse (5′-GTAGGCTACTCAGGATTG-3′).16 Primers were revised using primer blast system. GAPDH was used as internal control to normalize the expression of TUG1. The used GAPDH forward primer is 5′-GTCTCCTCTGACTTCAACAGCG -3′ and reverse primer is 5′-ACCACCCGTGTGCTTAGCCA -3′.17

Real time PCR was done using Applied Biosystems StepOne Real-time PCR System. (Cat. No. 4376357).18 Reagents were purchased from Applied Biosystems, USA. The calculation of RNAs’ expression was done using the comparative cycle threshold (CT) method (2^-ΔΔCT).

Results
For statistical analysis, SPSS Statistics for Windows, Version 17.0 (SPSS Inc, Chicago, IL, USA) was used. The significance of the differences between the two groups was estimated with the Student t-test. Multiple group comparisons were analyzed by one-way analysis of variance (ANOVA). The age of the patients ranged from 40 to 83 years old. Thirty patients were males with a percentage of 46.15% and 35 were females with percentage 53.85% (► Table 1).

The correlation between sex and tissue TUG1 expression in patients of groups I and II was statistically insignificant (► Table 2).

Analysis of LncRNA TUG1
To discover the role of TUG1, its level was measured in CRC tissues and corresponding non-cancerous tissues and results showed that TUG1 was upregulated in CRC tissues with a

Fig. 1 Pathological examination (formalin fixed and paraffin embedded [FFPE]).
strong significant difference between patients of group I and II and the control group ($p < 0.001$) (– Table 3 – Fig. 2A). Also, LncRNA TUG1 expression was significantly higher in patients of group I in comparison with samples of group II. ($p_1 = 0.002$). LncRNA TUG1 expression was significantly higher in patients of stage I in comparison with the control group (Group III) ($p_2 < 0.001$). A meaningful change was found when comparing the TUG1 expressions in cases of stage II and the control group. ($p_3 < 0.001$).

**Correlation Studies**

1. – Correlation between tissue TUG1 expression and age, hemoglobin, CEA, and CA19.9 serum levels in patients of group I were statistically insignificant ($p = 0.760$, 0.473, 0.507, 0.493, respectively).

**Discussion**

Colorectal cancer has become a main health problem and one of the most fatal malignancies, and its incidence is increasing worldwide. Colorectal cancer is usually accompanied by distal metastasis (liver or lung), and it is associated with increased mortality rate.¹⁹ Therefore, there is an urgent necessity to discover the molecular mechanisms of CRC progression.²⁰

Long noncoding RNAs (lncRNAs) are RNAs with a length $>200$ nucleotides. They participate in various biological

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**Table 1** Sex distribution of the 3 studied groups

<table>
<thead>
<tr>
<th></th>
<th>Group I (n = 25)</th>
<th>Group II (n = 25)</th>
<th>Group III (n = 15)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
<td>No %</td>
</tr>
<tr>
<td>Male</td>
<td>14 56.0</td>
<td>10 40.0</td>
<td>6 40.0</td>
<td>30 46.15</td>
</tr>
<tr>
<td>Female</td>
<td>11 44.0</td>
<td>15 60.0</td>
<td>9 60.0</td>
<td>35 53.85</td>
</tr>
</tbody>
</table>

**Table 2** Relation between Sex and different measurements in group I and II

<table>
<thead>
<tr>
<th></th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Male (n = 14)</td>
</tr>
<tr>
<td>TUG1</td>
<td>2.83 (1.62–4.40)</td>
</tr>
<tr>
<td>Group II</td>
<td>Male (n = 10)</td>
</tr>
<tr>
<td>TUG1</td>
<td>5.34 (2.09–8.03)</td>
</tr>
</tbody>
</table>

$p$-value for comparing between Male and Female.

1. – Correlation between tissue TUG1 expression and age, hemoglobin, CEA, and CA19.9 serum levels in patients of group II was statistically insignificant ($p = 0.322$, 0.719, 0.565, 0.685, respectively) (– Table 4).

**Table 3** Comparison between the 3 studied groups according to TUG1 expression

<table>
<thead>
<tr>
<th></th>
<th>Group I (n = 25)</th>
<th>Group II (n = 25)</th>
<th>Group III (n = 15)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TUG1</td>
<td>1.62–4.88</td>
<td>2.04–8.03</td>
<td>0.04–3.33</td>
<td>$&lt; 0.001^*$</td>
</tr>
<tr>
<td></td>
<td>Median (IQR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.71 (1.97–3.38)</td>
<td>4.46 (2.96–6.44)</td>
<td>0.73 (0.49–2.28)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: IQR, interquartile range.

*: Statistically significant at $P \leq 0.05$.

Group I: Nonmetastatic colorectal adenocarcinoma.

Group II: Metastatic (locoregional and blood born) colorectal adenocarcinoma.

Group III: Noncancerous control colon tissue.
processes, such as chromatin remodeling, transcriptional activation or interference, and they are involved in the occurrence of CRC by acting as tumor suppressor genes and oncogenes.21

Several studies have documented a tumor-promotive function of TUG1 in different malignancies, especially CRC. However, the mechanisms by which it exerts its role in CRC remain undefined.22

Our study revealed that TUG1 is upregulated in CRC. Wang et al.23 reported that the LncRNA TUG1 upregulated in CRC promoted CRC progression and 5-fluorouracil (5-FU) resistance by sponging miR-197–3p. Also, Shen et al.24 found that the decrease in LncRNA TUG1 inhibited CRC tumor cell migration, invasion, and EMT, and has a major role in reducing lung metastasis.

TUG1 plays a main role in regulating different cancer types by functioning as a ceRNA as in oral squamous cell carcinoma by sponging mir-593–3p as reported by Jiang et al.25 and, in case of osteosarcoma, Farzaneh et al.26 expressed that MALAT1 LncRNA has been found to regulate CDK9 expression through sponging miR-206 and it can also interact with miR-202 and promote lung metastasis.

Tian et al. declared that the increase in TUG1 expression has been shown to enhance CRC cell proliferation, invasion, and EMT in vitro, through promoting SW620 cell motility by decreasing miR-26a-5p activity and upregulating MMP-14. Moreover, TUG1 promoted carcino genesis and EMT in colon cancer by stimulating the P38MAPK/Hsp27 axis.27

However, Barbagallo. et al analyzed via RT-PCR the expression of 17 LncRNAs in 20 CRC tissues compared with noncancerous adjacent tissues, and in serum exosomes of these 20 CRC patients compared with 20 healthy individuals identified 8 ncRNAs (including TUG1) differentially expressed in tissues while in serum exosomes of CRC patients was downregulated.28

### Conclusion

TUG1 was upregulated in CRC tissues and cells. Its effects are on proliferation and apoptosis of cancer cells. Collectively, the present study demonstrated that TUG1 overexpression induces proliferation and inhibits apoptosis in CRC. This possible molecular mechanism provides a theoretical basis for the research on LncRNA-directed therapeutics in CRC.

### Recommendations

The diagnostic and prognostic impact of TUG1 in CRC is an interesting area for future studies on a large cohort of patients with a long-term follow-up. In addition, targeting the downstream TUG1 targets could be an innovative approach toward molecularly based adjuvant therapies of CRC.

### Consent for Publication

Not applicable.

### Availability of Data and Materials

The data supporting the conclusions are included within the article.

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### Table 4 Correlation between LncRNA TUG1 and age, hemoglobin, CEA and CA19.9 in Group I & II

<table>
<thead>
<tr>
<th></th>
<th>TUG1 Group I</th>
<th></th>
<th></th>
<th>TUG1 Group II</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rs</td>
<td>p-value</td>
<td>rs</td>
<td>p-value</td>
<td></td>
</tr>
<tr>
<td>Age (years old)</td>
<td>-0.064</td>
<td>0.760</td>
<td>0.206</td>
<td>0.322</td>
<td></td>
</tr>
<tr>
<td>HB</td>
<td>0.150</td>
<td>0.473</td>
<td>-0.076</td>
<td>0.719</td>
<td></td>
</tr>
<tr>
<td>CA19–9</td>
<td>0.139</td>
<td>0.507</td>
<td>0.121</td>
<td>0.565</td>
<td></td>
</tr>
<tr>
<td>CEA</td>
<td>0.144</td>
<td>0.493</td>
<td>-0.085</td>
<td>0.685</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: HB, hemoglobin. 
rs: Spearman coefficient.
*: Statistically significant at p ≤ 0.05.
Group I: Nonmetastatic colorectal adenocarcinoma.
Group II: Metastatic (locoregional and blood born) colorectal adenocarcinoma.
References


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