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

Yasmine Amin[1] Amel Ketat[1] Hanan Kamal[1] Inass Zaki[2] Ahmed Moaz[3] Rasha Abdelmawla Ghazala[1]

[1] Medical Biochemistry Department, Faculty of Medicine, Alexandria University, Alexandria, Egypt
[2] Pathology Department, Faculty of Medicine, Alexandria University, Alexandria, Egypt
[3] Colorectal Surgery Department, General Surgery Unit, Faculty of Medicine, Alexandria University, Alexandria, Egypt

Address for correspondence Yasmine Mohamed Nageeb Amin, Medical Biochemistry Department, Faculty of Medicine, Alexandria University, Alexandria, Egypt (e-mail: Yassmen.nagib@alexmed.edu.eg).

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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.

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.

Keywords
- colorectal adenocarcinoma
- CRC
- LncRNA
- TUG1
- PCR

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 (HNPCC) 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

[+] *these authors contributed equally to the present work.*

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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 ($\sim 0.4\,cm \times 0.2\,cm$ 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 blots (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 A_{260}/A_{280} and $A_{260}/A_{230} = 1.8–2.1$ 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′-CTGAAGAAAGGCAACATC-3′) and reverse (5′-GTAGGCTACTACAGGATTTG-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′-ACCACCCTGTTGCTGTAGCCAA – 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 ($2^{-\Delta\Delta CT}$).

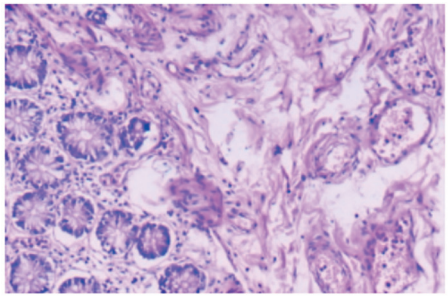
Results

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**).

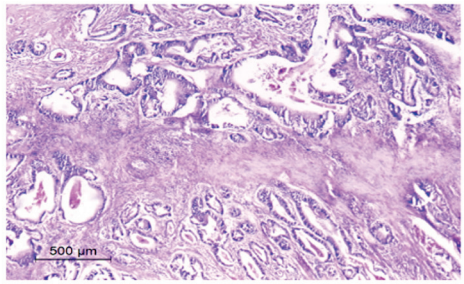
The correlation between sex and tissue TUG1 expression in patients of groups I and II was statistically insignificant (►**Table 2**).

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



Normal colonic mucosa (H&Ex10).



A case of moderately differentiated adenocarcinoma, Grade 2 invading the muscularis propia pT2 (H&Ex40).

Fig. 1 Pathological examination (formalin fixed and paraffin embedded [FFPE]).

Table 1 Sex distribution of the 3 studied groups

	Group I ($n=25$)		Group II ($n=25$)		Group III ($n=15$)		Total	
	No.	%	No.	%	No.	%	No	%
Sex								
Male	14	56.0	10	40.0	6	40.0	30	46.15
Female	11	44.0	15	60.0	9	60.0	35	53.85

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

	Sex		
Group I	**Male ($n=14$)**	**Female ($n=11$)**	***p-value***
TUG1			
Median (Min – Max.)	2.83 (1.62–4.40)	2.04 (1.74–4.88)	0.344
	Sex		
Group II	**Male ($n=10$)**	**Female ($n=15$)**	***p-value***
TUG1			
			0.605
Median (Min. – Max.)	5.34 (2.09–8.03)	4.46 (2.04–7.98)	

p-value for comparing between Male and Female.

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 of 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).
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**).

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

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

	Group I ($n=25$)	Group II ($n=25$)	Group III ($n=15$)	*p-value*
TUG1				
Min. – Max.	1.62–4.88	2.04–8.03	0.04–3.33	$<0.001^*$
Median (IQR)	2.71 (1.97–3.38)	4.46 (2.96–6.44)	0.73 (0.49–2.28)	
Sig. bet. groups.	$P_1=0.002^*$, $P_2=0.010^*$, $P_3<0.001^*$			

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.

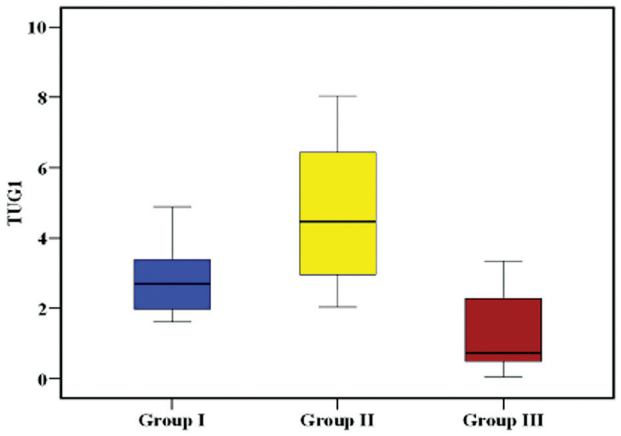


Fig. 2 Expression levels of *TUG1*, *TUG1* was upregulated in CRC tissues with strong significant difference between patients of group I and II and control group. *TUG1* expression was significantly higher in patients of group I in comparison with samples of group II.

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 *TUG 1* 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 carcinogenesis 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.

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

	TUG1			
	Group I		Group II	
	r_s	p-value	r_s	p-value
Age (years old)	- 0.064	0.760	0.206	0.322
HB	0.150	0.473	- 0.076	0.719
CA19–9	0.139	0.507	0.121	0.565
CEA	0.144	0.493	- 0.085	0.685

Abbreviations: HB, hemoglobin.
r_s: Spearman coefficient.
*: Statistically significant at $p \leq 0.05$.
Group I: Nonmetastatic colorectal adenocarcinoma.
Group II: Metastatic (locoregional and blood born) colorectal adenocarcinoma.

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Conflict of Interests
The authors have no conflict of interests to declare.

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