RESEARCH ARTICLE

Editorial Process: Submission:08/17/2023 Acceptance:11/19/2023

Interplay of *LncRNA TUG1* and *TGF-β/P53* Expression in Colorectal Cancer

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Abstract

Background: Colorectal cancer (CRC) is one of the most prevalent and deadly cancers worldwide. It is still necessary to further define the mechanisms and explore the therapeutic targets of CRC. Long non-coding RNA taurine upregulated gene 1 (*LncRNA TUG1*) was initially discovered as a transcript upregulated by taurine and is observed to be expressed in numerous human cancers. **The Study Aim:** This article was to explore the correlation between transforming growth factor-beta (*TGF-β*)/tumor protein 53 (P53) signaling mechanisms as regulators for *LncRNA TUG1* in Egyptian patients with CRC. Subjects and Methods: Immunohistochemical (IHC) staining was achieved to study *TGF-β* and P53 expression in CRC specimens vs. normal colonic specimens and quantitative real-time PCR (qRT-PCR) was used to analyze *LncRNA TUG1*, *TGF-β*, and P53 relative gene expression in 96 tissue specimens (neoplastic specimens and the corresponding adjacent non-neoplastic specimens). **Results:** The expressions of *LncRNA TUG1*, *TGF-β*, and *P53* were overexpressed significantly in CRC specimens as opposed to the matched neighboring non-neoplastic specimens (P<0.001), also *LncRNA TUG1* was significantly positively correlated to the expression of *TGF-β* and *P53* (r=0.89, 0.91 respectively, P<0.001). **Conclusion:** These findings reveal that *LncRNA TUG1* may be a molecular component in the *TGF-β/P53* signaling pathway, and *LncRNA TUG1* could function as a CRC possible oncogene. *LncRNA TUG1* may serve as a potential oncogene for CRC. The *TGF-β/P53*/*LncRNA TUG1* interactions may be employed as potential targets for CRC diagnosis, prognostic evaluation, and cure.

Keywords: *LncRNA TUG1- TGF-β- P53*- expression- colorectal cancer

Asian Pac J Cancer Prev, **24 (11)**, 3957-3968

Introduction

Colorectal cancer (CRC) is a digestive tumor, ranks third in terms of cancer diagnoses, and is the second leading cause of cancer-related death globally (Sharma et al., 2022). Evidence indicated that the incidence rate of CRC has increased during the past few decades, particularly in emerging nations. Annually, around 1.85 million novel CRC cases are recorded, with a rising incidence of young persons under 50 (Siegel et al., 2020). The causes of the disease's heterogeneity are spread across the CRC etiology and include genetics, epigenetics, and environmental factors (Rawla et al., 2019).

Surgery, Radio- and chemotherapy are the key therapies in both early and metastatic CRC (Xie et al., 2020). The development of CRC from the normal colonic mucosa results from a cumulative process of genetic alterations (Zhai et al., 2016). Hence, the methods of genetic transformations and signal paths linked to the growth and progress of CRC have been further researched.

Long non-coding RNAs (LncRNAs), which are a subsection of RNAs extended than 200 nucleotides and lack protein-coding facility (Statello et al., 2021), are identified as critical controllers of cellular functions including gene expression and chromosomal alteration, which they employ vital outcomes on organizing cell growth, division, senescence, and DNA damage control (Oo et al., 2022). Moreover, altered LncRNAs expressions are strongly correlated with the pathogenesis of malignancies, such as breast, lung, pancreatic, hepatic, and colorectal carcinomas (Chi et al., 2019).

The cellular function of LncRNAs in tissues depends chiefly on their interplay with other biological molecules, involving protein, DNA, and RNA. Nuclear LncRNAs contribute to gene expression control through binding to transcription molecules also they can directly contribute to the nuclear structure organization, thereby organizing other genetic modifications (Kopp and Mendell, 2018). Cytoplasmic LncRNAs can attach to RNA biomolecules like mRNA or miRNA affecting the post-transcriptional control methods. Additionally, they can control signal transmission by directly interacting with proteins (Tang

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et al., 2020).

LncRNAs and their functions in CRC have drawn a lot of interest. According to reports, several of them, including CCAT1, UCA1, CCAL, and HOTAIR, are involved in the beginning and progress of CRC and alter the biological characteristics of cancerous tissue through involvement in many biological signal mechanisms including β-catenin, *P53*, JAK/STAT, AKT/mTOR, and NF-kB, among others, influence the course of the cell cycle (Tang et al., 2020).

Recently, LncRNA taurine upregulated gene 1 (*LncRNA TUG1*), a 7.1-kb extremely preserved nuclear LncRNA that was discovered on chromosome 22q12.2 in the human genetic code (Zhang et al., 2018). Deng et al. (2017) showed that *LncRNA TUG1* might quicken the G0/G1 cell cycle transition by concentrating on Cyclin D1 to encourage cell growth and have a cancerous role in various human carcinomas, including urothelial cancer (Han et al., 2013), osteosarcoma (Li et al., 2018a), and melanoma (Long et al., 2018). These searches expose that *LncRNA TUG1* could be an essential controlling genetic factor in malignancy. Nevertheless, the function of *LncRNA TUG1* in CRC development and metastasis remains blurred.

Transforming growth factor-β (*TGF-β*) is a 25-kDa multifunctional chemokine that communicates with intracellular SMAD family regulators and transmembrane serine-threonine kinase receptors (STKR) (Tzavlaki and Moustakas, 2020). The *TGF-β* signal path is essential for controlling cell division, growth, and apoptosis. *TGF-β* is dysregulated in CRC, and overexpression of it in the advanced stages of CRC increases the generation of numerous mitogenic growth elements and induces epithelial-mesenchymal transition (EMT) progression. Therefore, it promotes the growth of tumors in the advanced stages of CRC and is linked to a bad prognosis for those with CRC (Koveitypour et al., 2019). *P53* is a tumor suppressor protein that shows a crucial responsibility in preventing the development of cancer cells through the regulation of cell cycle progression, apoptosis, and DNA damage repair (Chen, 2016).

In CRC, *P53* gene mutations are among the most prevalent genetic changes identified in colorectal cancer cells. These mutations may cause loss of function or stabilization of the *P53* protein, resulting in decreased or absent expression of the gene. This nevertheless leads to the transformation of CRC but also increases its severity and invasion (Li et al., 2015). In the depletion of wild-type *P53* activity, Snail1-dependent EMT is stimulated in colon, breast, and lung cancers. Even though *P53* activity may influence cell cycle control, apoptosis, and DNA repair processes, the EMT and invasion programs begun by *P53* loss of function or mutation are fully dependent on Snail1 expression (Kim et al., 2011).

LncRNA TUG1 gene has a polyadenylation tail, which is critical for the control of the growth during tumor growth. Also, the *LncRNA TUG1* promoter has a conserved *P53*-binding area, and some researchers reported that *P53*-controlled *LncRNA TUG1* expression controls progression during tumor growth (Zhang et al., 2014b).

In the current research, we intended to explore the genetic responsibility of *LncRNA TUG1* as an oncogenic regulator in colorectal tumorigenesis and its interplay with two key signal paths, *TGF-β* and *P53*. We hypothesized that *LncRNA TUG1*/*TGF-β/P53* interplay may be involved in the growth and progress of CRC and studying these three genetic elements might aid in the discovery of new therapeutic strategies for the management of CRC and improve patient outcomes.

Materials and Methods

Subjects selection

This research was carried out by the Medical Biochemistry and Molecular Biology, Pathology, and General Surgery Departments at Zagazig University Hospitals in Egypt between October 2022 and August 2023. Ninety-six human CRC and para-neoplastic (adjacent normal) specimens were included in this retrospective case-control analysis and were obtained from the Department of General Surgery during tumor removal surgery, (Figure 1). Epi-Tools Epidemiological calculators were used to calculate the sample size and the results revealed a statistical significance of at least 87.1%. (Humphry et al., 2004). The analysis was agreed upon via the Institutional Review Board (IRB), Faculty of Medicine, Zagazig University (approval number is ZU-IRB #9992/11-10-2022) and all patients provided written, fully informed approval. The criteria for being included were: 1) CRC was clinically and pathologically diagnosed in all cases; 2) Pathology validated the condition following the American Joint Committee on Cancer (AJCC) staging guideline following extensive surgical debulking (Edge and Compton, 2010), tumor typing and grading were done using 2019 WHO stated standards (Nagtegaal et al., 2020); 3) Preoperative didn't receive neoadjuvant therapy; 4) Hepatic, renal, cardiac, and respiratory functioning in the patients who underwent colonoscopy were determined to be satisfactory with no distant metastasis. The omission standards were: 1) Cases aged 73 or older; 2) Cases with inflammatory bowel diseases or numerous primary tumors; 3) The clinicopathological information was incomplete. The data of the neoplastic specimens encompassing site, grade, stage, differentiation, depth, lymph node state, and liver metastasis were got from the pathology and hospital reports of the patients.

The Helsinki Declaration, a code of ethics for human research, was followed when conducting the study. Every technique employed in this investigation was carried out in conformity with the applicable rules and instructions.

Assay of LncRNA and mRNAs Expression by qRT-PCR

Whole cellular RNA was isolated from homogenized colon tissue samples using the easy-REDTM Total RNA extraction kit as directed by the manufacturer (iNtRON Biotechnology, Seongnam, Korea). Total RNA was reverse transcribed into complementary DNA (cDNA) using Maxime Reverse Transcriptase PreMix Kit (iNtRON Biotechnology, Seongnam, Korea) in line with the instructions provided by the manufacturer. Gene expression analysis was achieved by qRT-PCR via TOPreal™ qPCR 2X PreMIX (SYBR Green with low

ROX) Kit. The qRT-PCR was carried out as follows: 10 µl of SYBR Green, 1 µl of each forward and reverse primer, 4 µl of cDNA, and 4 µl of RNAase-free water in 20 µl final volume. LncRNA and mRNAs expressions were normalized by employing β-actin as a positive control gene. Forty-five cycles; initial denaturation phase of 10 min at 95°C, then 15 s at 95°C, 30 s at 60°C, and 30 s at 72°C. The thermal protocol for the replication of the four genes was carried out under similar circumstances. The forward and reverse primers used for each gene (*LncRNA TUG1*, *TGF-β*, *P53*, and β-actin) are listed in (Table 1). *LncRNA TUG1*, *TGF-β*, and *P53* relative gene expression were internally normalized to β-actin as a housekeeping gene. The relative quantitative values were expressed using the threshold cycle (CT) technique ($2^{-\Delta\Delta CT}$ procedure) (Livak and Schmittgen, 2001).

Immunohistochemical Staining

IHC staining was achieved on 5-µm sections cut from formalin-fixed, paraffin-embedded tissue utilizing mouse monoclonal anti-*TGF-β* (1:100) (Novus Biologicals, Littleton, CO, USA) & *P53* (DO-1 Ab, 1:20; Immunotech, Marseille, France). Sections were exposed to primary antibodies for 32 min at 37°C. The ultra-View Universal DAB detection kit (Ventana) was used to find the staining.

Immunohistochemical Assessment

TGF-β cytoplasmic IHC content was graded considering the amount and strength of staining. A 4-tiered scale of 0 to 3 was used to rate the staining intensity, with 3 denoting the most extreme staining. Depending on the proportion of stained cells in the area of interest, the degree of positive immunoreactivity was assessed as follows: 0%, <20%, 20–50%, and >50% received a score of 0, 1, 2, and 3, respectively. The intensity and extent of the positive staining were added up to produce an overall score. Cases classified as positive had final scores of more than 3 (Kim et al., 2014).

The *P53* expression was scored semi-quantitatively, and A positive percentage was used to split the five grades into 0 (zero), 1 (≤10%), 2 (≤50%), 3 (≤80%), and 4 (>80%). Staining of more than 10% of tumor cells was considered to be *P53*-positive (Wang et al., 2017). A blind process was used to score each stain independently via a single Pathologist.

Statistical analysis

The statistical data analysis was conducted by applying the GraphPad Prism 8.0 statistical software package. The data got from the cases were represented as mean ± standard deviation (SD) and analyzed statistically by applying the Mann-Whitney test to compare 2 groupings, one-way analysis of variance (ANOVA) after that the t-test was applied when needed. Qualitative results were expressed as numbers and percentages. The Chi-square test (X^2) was used to determine the relationship between qualitative data. Pearson's correlation coefficient was employed to assess the association between the studied biomarkers. Graphs were drawn using GraphPad Prism 8.0 software. Findings were deemed significant when P-value was <0.05, while P-values of <0.001 were deemed highly significant, and P≥0.05 was deemed non-statistically significant.

Results

This analysis was conducted on 96 colonic specimens categorized into two groups (48 colonic neoplastic specimens and 48 neighboring non-neoplastic colonic specimens). The clinicopathological features of the cases were reported in (Table 2), thirty-two patients (66.7%) were males, and 16 patients (33.3%) were females. Their age varied from 35 to 72 years, with a mean value of 44.48±10.25 years.

We determined *LncRNA TUG1*, *TGF-β*, and *P53* relative expression in organic tissue samples gotten from the 48 cases with CRC incorporating core neoplastic specimens and nearby non-neoplastic healthy specimens. We noticed a strongly significant variance in *LncRNA TUG1*, *TGF-β*, and *P53* expression between neoplastic (4.11±0.59, 3.11±0.55, and 2.30±0.43-fold-change, respectively) and their matching adjacent non-neoplastic tissue samples (1.02±0.18, 0.99±0.15, and 0.87±0.14-fold-change, respectively) (P<0.001) (Table 3) and (Figure 2). Also,

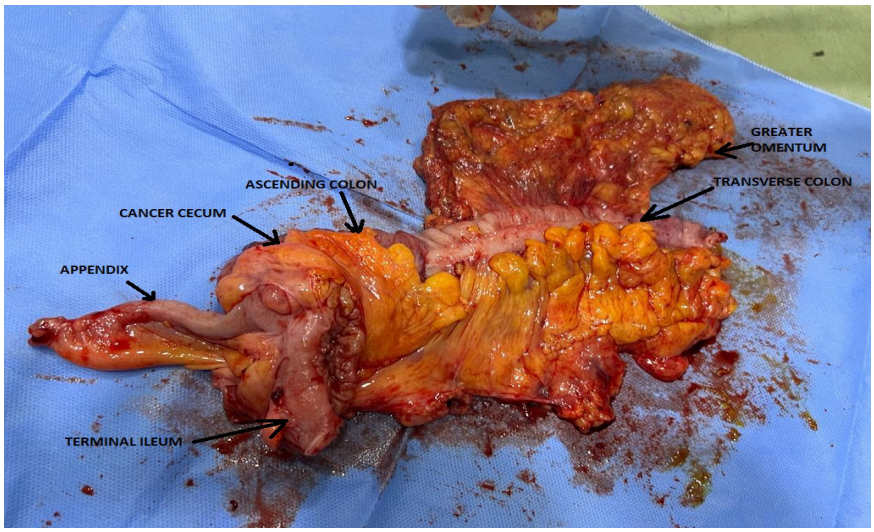

Figure 1. Cancer Cecum Operated by Rright Hemicolectomy

Table 1. The qRT-PCR Primers

Genes	Forward Primers (5'-3')	Reverse Primers (5'-3')
LncRNA TUG1	TAGCAGTTCCCCAATCCTTG	CACAAATTCCCATCATTCCC
TGF-β	AACCCACAACGAAATCTATG	CTTTTAACTTGAGCCTCAGC
P53	AGAGTCTATAGGCCCACCCC	GCTCGACGCTAGGATCTGAC
β-actin	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT

Table 2. Clinical Characteristics of the Studied Groups

Characteristics	Patients No. 48 (%)
Gender	
Male	32 (66.7)
Female	16 (33.3)
Age (Years)	
Range	35-72
Mean±SD	44.48±10.25
Location	
Colon	29 (60.4)
Rectum	19 (39.6)
Grade	
G1+G2	34 (70.8)
G3+G4	14 (29.2)
TNM Stage	
I&II	26 (54.2)
III&IV	22 (45.8)
Histological type	
Adenocarcinoma (differentiated)	37 (77.1)
Mucinous or undifferentiated	11 (22.9)
Tumor invasion depth	
T1+T2	19 (39.6)
T3+T4	29 (60.4)
Lymph node metastasis	
Positive	28 (58.3)
Negative	20 (41.7)
Liver metastasis	
Positive	18 (37.5)
Negative	30 (62.5)

the IHC assessment of both *TGF-β* and P35 revealed that both showed significantly positive higher expression in CRC (77.00%, 41.67%) in comparison to the adjacent non-neoplastic colonic tissue (4.20%, 10.42%), (P<0.00001, 0.000486) respectively as in (Table 4) and (Figures 5 and 6).

When we examine the correlation of *LncRNA TUG1* expression with the clinic-pathological criteria of the CRC patients, we noticed that its expression in tissues exposed no association with age, sex, cancer site, and grade (P:0.204, 0.065, 0.303, and 0.719, respectively); however, it was correlated with the cancer staging, differentiation degree, tumor depth, lymph node, and liver metastasis with a significantly elevated expression in stages III and IV (4.38±0.58) (P:0.0049), poorly differentiated neoplastic tissues (4.53±0.61) (P:0.012), T3&T4 invasion depth (4.49±0.41) (P<0.001), positively metastatic LN (4.31±0.60) (P:0.0073), and liver metastatic cases (4.64±0.36) (P<0.001) (Table 5).

The associations of *TGF-β* mRNA expression with the clinicopathological data indicated that *TGF-β* overexpression was significantly correlated with the pathological stage (P:0.028), invasion depth (P<0.001), LN metastasis (P:0.025), and liver metastasis (P<0.001) of CRC. Besides, we detected a significant link between *P53* over-expression and the neoplastic staging (P:0.005), cancer differentiation (P:0.01), depth (P<0.001), metastatic LN (P:0.023), and metastatic liver (P<0.001) (Table 5).

Both *TGF-β* and *P53* IHC markers positive expression showed significant association with higher tumor stage (III+IV), adenocarcinoma histopathological subtype, tumor depth (T3+T4), positive LN metastasis and positive liver metastasis (P=0.005, 0.0428, 0.000073, 0.0020, 0.02664), (P=0.00006, 0.017314, 0.000397, 0.010072, 0.041097), respectively, as in (Table 6) and (Figures 5 and 6). *P53* IHC marker also showed significantly elevated expression in tumor grade (III+IV) (65.00%) compared to (35.00%) in tumor grade (I+II), (P<0.00001), as in (Table 6) and (Figure 6).

Conversely, no significant relationship was detected between *TGF-β* and *P53* IHC markers expression and age, gender and location (P=0.549, 0.224, 0.247), (P=0.591431, 0.147299, 0.212309), respectively. Additionally, the *TGF-β* IHC marker showed no significant relationship with tumor grade (P=0.361), as in (Table 6). Pearson's correlation coefficient testing was done to investigate the relationship between the expression of *LncRNA TUG1* and both *TGF-β* and *P53* in the affected CRC tissues. As (Figure 3) shows a strong positive correlation between *LncRNA TUG1* and *TGF-β*, with a correlation coefficient of 0.89 (P<0.001), also, (Figure 4) shows a strong positive correlation between *LncRNA TUG1* and *P53*, with a correlation coefficient of 0.91, (P<0.001), proving

Table 3. Comparison of *LncRNA TUG1, TGF-β*, and *P53* Relative Gene Expression in the Study Specimens

Gene	Non-neoplastic specimens	Neoplastic specimens	P-value
LncRNA TUG1	1.02±0.18	4.11±0.59	<0.001**
TGF-β	0.99±0.15	3.11±0.55	<0.001**
P53	0.87±0.14	2.30±0.43	<0.001**

The data expressed as mean±SD, **statistically highly significant difference (P<0.001), P-value for Mann-Whitney U test.

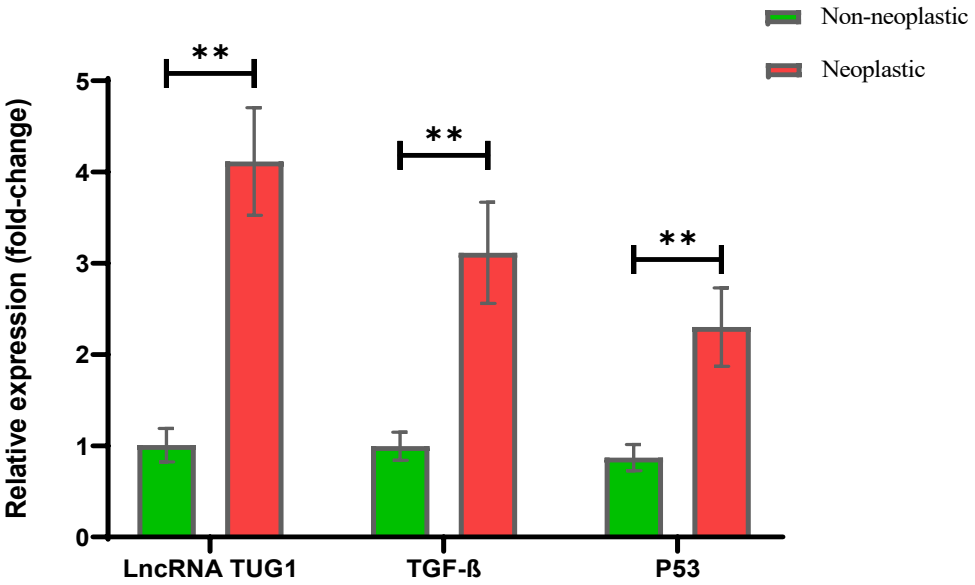

Figure 2. *LncRNA TUG1, TGF-β*, and *P53* Relative Expression Analysis in the Examined Groups Represented as mean±SD, **means P<0.001 in comparison between healthy and neoplastic specimens using Unpaired t-test.

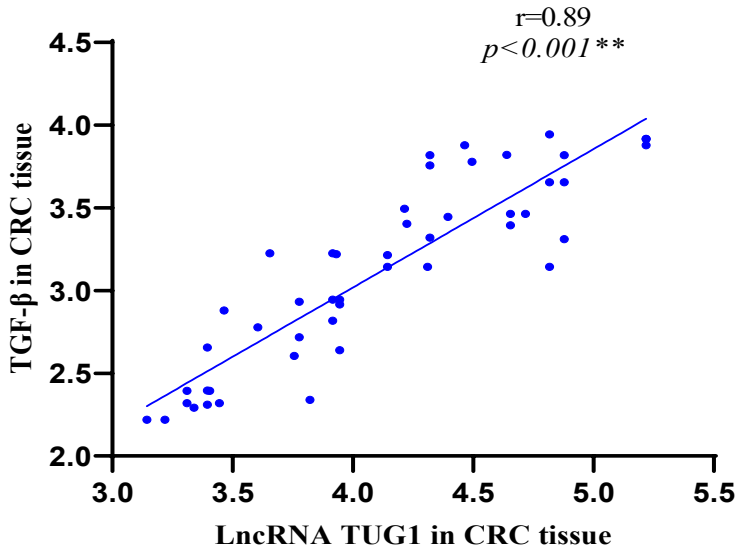

Figure 3. Correlation between Tissue Expression of *LncRNA TUG1* and *TGF-β* assessed in Fold-Change. The curve indicates a strong positive correlation with a Pearson's correlation coefficient (r) of 0.89, P<0.001.

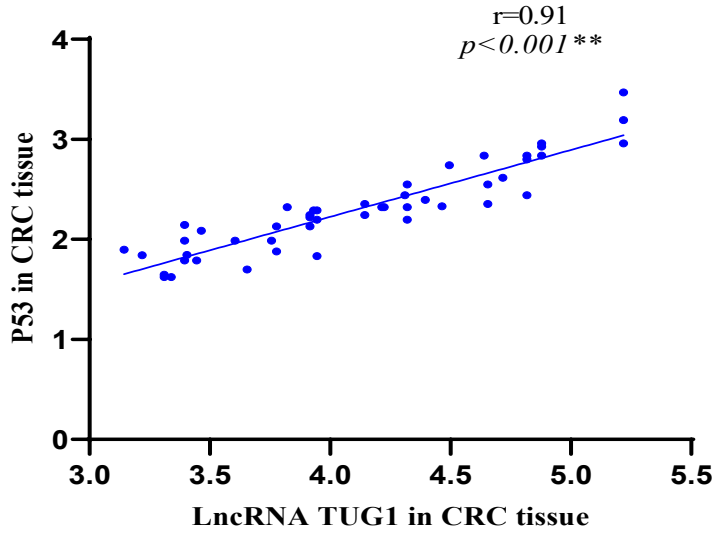

Figure 4. Correlation between Tissue Expression of *LncRNA TUG1* and *P53* assessed in Fold-Change. The curve indicates a strong positive correlation with a Pearson's correlation coefficient (r) of 0.91, P<0.001.

Table 4. Comparison of *TGF-β* and *P53 IHC* Markers in the Study Specimens

	Non-neoplastic specimens	Neoplastic specimens	X^2	P-value
TGF-β				
Positive	2 (4.20%)	37 (77.00%)		<0.00001**
Negative	46 (95.83%)	11 (23.00%)	52.9015	
P53				
Positive	5 (10.42%)	20 (41.67%)		
Negative	43 (89.58%)	28 (58.33%)	12.169	<0.000486**

that increased *LncRNA TUG1* correlated with *TGF-β/P53* upregulation in CRC tissues. Overall, these findings suggest that *LncRNA TUG1* might be a component of the *TGF-β/P53*-regulatory network in CRC.

Discussion

CRC is a conventional deadly cancer variety in both males and females that affects the colon and rectum, with millions of novel cancer cases and fatalities assumed to have appeared (Rawla et al., 2019). The study of the expressed genes involved in CRC pathogenesis may aid in the discovery of biological indicators for the assessment of malignancy identification and overture unique molecular focuses for anti-cancer treatment (Sarhadi and Armengol, 2022). The involvement of LncRNAs in tumorigenesis mediated through different intracellular factors, such as Wnt/β-catenin, phosphatidylinositol-3-kinase (PI3K), EMT, *TGF-β*, and *P53* signals paths may offer additional knowledge into cancers pathogenesis (Amaral et al., 2011).

LncRNAs in CRC are hypothesized to be involved in the *TGF-β* pathway to regulate tumor development, however, the mechanism by which LncRNAs regulate *TGF-β* expression in CRC is not completely comprehended. Some researchers have demonstrated that *TGF-β*/SMAD signaling network contribution with LncRNAs obliges an influential responsibility in CRC progress, opening

Table 5. Association between *LncRNA TUG1, TGF-β,* and *P53* Expression and Clinic-Pathological Features in CRC Cases

Features	No.	LncRNA TUG1	U	P	TGF-β	U	P	P53	U	P
Age										
≤50	34	4.19±0.63	181.5	0.204	3.19±0.56	168	0.114	2.37±0.46	176.5	0.167
>50	14	3.93±0.44			2.92±0.51			2.13±0.30		
Gender										
Male	32	0.27±0.20	171.5	0.065	3.02±0.55	176	0.081	2.23±0.39	190	0.153
Female	16	0.27±0.21			3.29±0.52			2.44±0.49		
Location										
Colon	29	4.04±0.58	226	0.303	3.06±0.60	237.5	0.429	2.25±0.45	222.5	0.267
Rectum	19	4.23±0.60			3.20±0.48			2.38±0.38		
Grade										
G1+G2	34	4.11±0.61	221.5	0.719	3.14±0.58	214.5	0.603	2.31±0.45	235	0.476
G3+G4	14	4.13±0.55			3.05±0.51			2.29±0.39		
TNM Stage										
I&II	26	3.88±0.51	149.5	0.0049*	2.95±0.59	191.5	0.028*	2.14±0.36	151.5	0.005*
III&IV	22	4.38±0.58			3.30±0.44			2.49±0.43		
Histological type										
Adenocarcinoma (differentiated)	37	3.99±0.53	100.5	0.012*	3.05±0.57	143	0.07	2.22±0.38	104.5	0.01*
Mucinous or undifferentiated	11	4.53±0.61			3.34±0.45			2.58±0.49		
Tumor depth										
T1+T2	19	3.54±0.25	7	<0.001**	2.62±0.33	40.5	<0.001**	1.93±0.21	25.5	<0.001**
T3+T4	29	4.49±0.41			3.43±0.41			2.54±0.36		
LN metastasis										
Positive	28	4.31±0.60	180	0.0073*	3.27±0.51	173	0.025*	2.42±0.44	171.5	0.023*
Negative	20	3.84±0.46			2.90±0.56			2.13±0.36		
Liver metastasis										
Positive	18	4.64±0.36	45	<0.001**	3.54±0.31	82	<0.001**	2.64±0.32	51.5	<0.001**
Negative	30	3.79±0.45			2.86±0.51			2.09±0.35		

CRC, colorectal cancer; LN, lymph node; The data expressed as mean±SD *statistically significant difference (P<0.05), **statistically highly significant difference (P<0.001), P-value for Mann-Whitney U test.

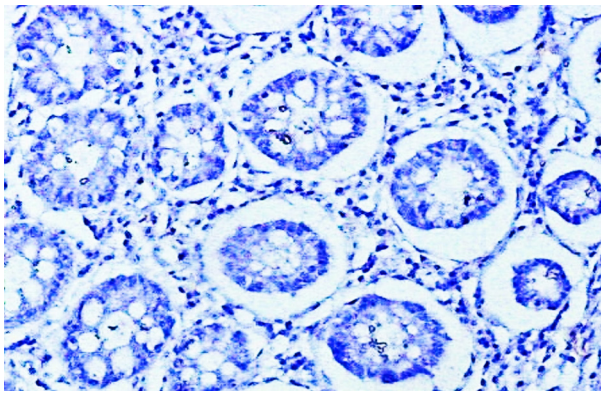

Figure 5A. Negative Expression of *TGF-β* in Normal Colon, (×400, TGF-β IHC).

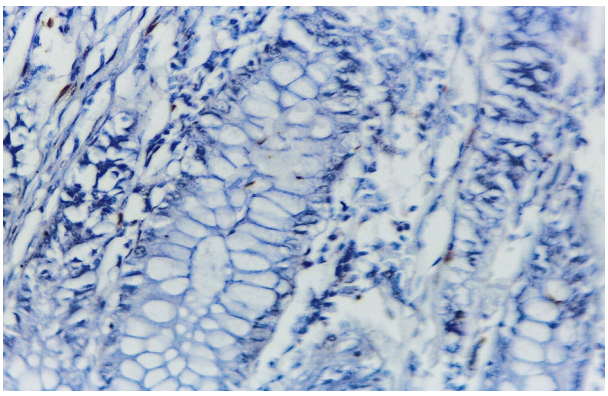

Figure 6A. Negative Expression of *P53* in Normal Colonic Tissue (×400, P53 IHC).

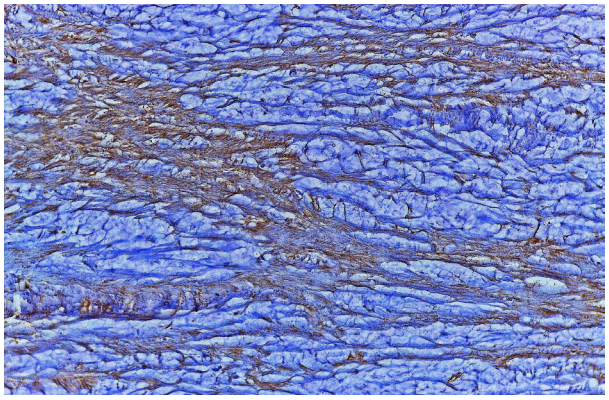

Figure 5B. Positive Cytoplasmic Expression of *TGF-β* in a Case of Mucinous Colonic Adenocarcinoma (×400, *TGF-β IHC).*

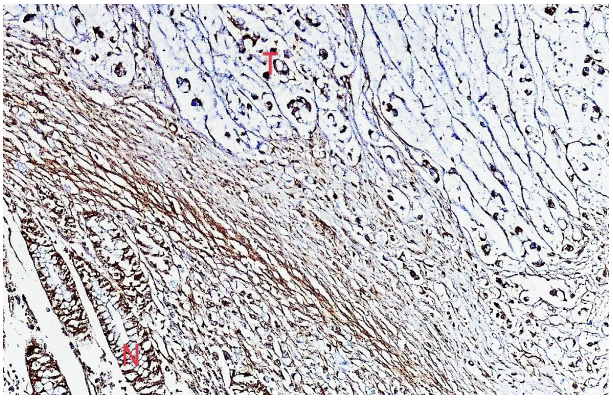

Figure 6B. Positive Nuclear Expression of *P53* (T) in a Case of Signet Ring Adenocarcinoma of the Colon with Adjacent Normal Tissue (N) Showing Positive *P53* Expression (×200, P53 IHC).

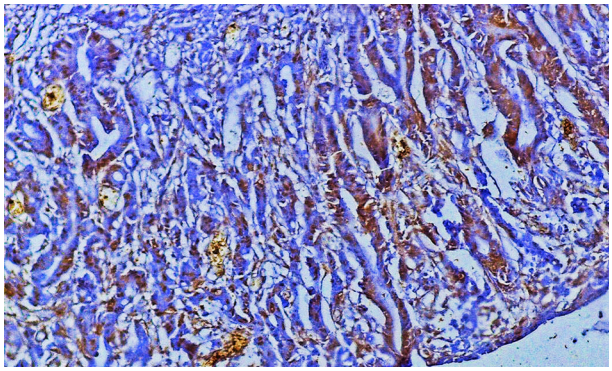

Figure 5C. Positive Cytoplasmic Expression of *TGF-β* in a Case of Colonic Adenocarcinoma (×100, *TGF-β IHC*).

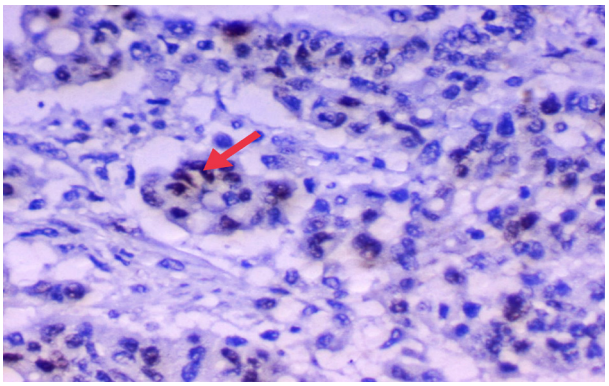

Figure 6C. Positive Nuclear Expression of *P53* (arrow) in a Case of Adenocarcinoma of the Colon (×400, *P53* IHC).

a new window for early detection and therapy (Hu et al., 2023). Research by Wang et al., (2019b) instructed that LncRNA-SNHG6 promotes CRC cell growth and invasiveness by triggering the *TGF-β*/SMAD signals cascade. Oppositely, LncRNAs can additionally be triggered via *TGF-β*. For instance, Yuan et al., (2014) exposed a new *TGF-β*-persuaded LncRNA named LncRNA-activated by *TGF-β* (LncRNA-ATB) that endorses the progress of hepatic malignancy via SMAD-self-regulating signals triggering STAT3 axis. Taken together, the previous studies intimate the reality of an intricate feedback cycle involving the TGF- β signaling route and LncRNAs.

LncRNAs may be essential controlling systems that act together with *P53* to negotiate their functions in causing several significant malignancy phenotypes (Lin et al., 2019). Many studies have explored the connection between *P53* and the LncRNAs interplay. Various LncRNAs, like maternally expressed gene 3 (MEG3), metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), *P53*-eRNAs, and wild-type *P53* (Wra*P53*), act as *P53* controllers, whereas further LncRNAs like LncRNA-P21, H19, *LncRNA TUG1*, and P21-associated-

Table 6. Association between *TGF-β* and *P53* IHC Expression and Clinicopathological Characteristics in CRC Patients

Characteristics	No.	*TGF-β* IHC (+) (No=37, %)	*TGF-β* IHC (-) (No=11, %)	X^2	P	*P53* IHC (+) (No=20, %)	*P53* IHC (-) (No=28, %)	X^2	P
Age									
≤50	34	27 (73.00%)	7 (63.64%)	0.3578	0.549	15 (75.00%)	19 (67.86%)	0.2881	0.591431
>50	14	10 (27.00%)	4 (36.36%)			5 (25.00%)	9 (32.14%)		
Gender									
Male	32	23 (62.16%)	9 (81.82%)	1.4742	0.224	11 (55.00%)	21 (75.00 %)	2.1	0.147299
Female	16	14 (37.84%)	2 (18.18%)			9 (45.00%)	7 (25.00%)		
Location									
Colon	29	24 (64.86%)	5 (45.45%)	1.3358	0.247	10 (50.00%)	19 (67.86%)	1.5556	0.212309
Rectum	19	13 (35.13%)	6 (54.55%)			10 (50.00%)	9 (32.14%)		
Grade									
G1+G2	34	25 (67.57%)	9 (81.82%)	0.8335	0.361	7 (35.00%)	27 (96.33%)	21.309	<0.00001**
G3+G4	14	12 (32.43%)	2 (18.18%)			13 (65.00%)	1 (3.67%)		
TNM Stage									
I&II	26	16 (43.24%)	10 (90.91%)	7.7599	0.005**	4 (20.00%)	22 (78.57%)	16.1215	0.00006**
III&IV	22	21 (56.76%)	1 (9.09%)			16 (80.00%)	6 (21.43%)		
Histological type									
Adenocarcinoma (differentiated)	37	31 (83.78%)	6 (54.54%)	4.1034	0.0428*	12 (60.00%)	25 (88.00%)	5.6643	0.017314*
Mucinous or undifferentiated	11	6 (16.22%)	5 (45.45%)			8 (40.00%)	3 (12.00%)		
Tumor depth									
T1+T2	19	9 (24.32%)	10 (90.91%)	15.7193	0.000073**	2 (10.00%)	17 (60.71%)	12.547	0.000397**
T3+T4	29	28 (75.68%)	1 (9.09%)			18 (90.00%)	11 (39.29%)		
LN metastasis									
Positive	28	26 (70.27%)	2 (18.18%)	9.4652	0.0020*	16 (80.00%)	12 (42.86%)	6.622	0.010072*
Negative	20	11 (29.73%)	9 (81.82%)			4 (20.00%)	16 (57.14%)		
Liver metastasis									
Positive	18	17 (45.95%)	1 (9.09%)	4.914	0.02664*	16 (80.00%)	2 (7.14%)	4.172	0.041097*
Negative	30	20 (54.05%)	10 (90.91%)			4 (20.00%)	26 (92.86%)		

IHC, immunohistochemical; CRC, colorectal cancer; LN, lymph node. *Statistically significant difference (P<0.05), **statistically highly significant difference (P<0.001), P-value for Chi-Square test (X^2).

ncRNA-DNA-damage-activated (PANDA), serve as *P53* effectors (Zhang et al., 2014a; Pal et al., 2020). Also, Lai et al. (2022) considered that *P53* might be a transcript regulator of *LncRNA TUG1* and has a potential connecting area in the promoter of *LncRNA TUG1*.

Recent studies have shown that the *LncRNA TUG1* expression is dysregulated in cell lines and tissues of CRC. Specifically, its overexpression has been linked to the proliferation and invasion of CRC cells, while downregulation of *LncRNA TUG1* has been demonstrated to stop the progress and metastasis of CRC (Xiao et al., 2018).

The mechanism by which *LncRNA TUG1* mediates its effects on CRC cells is through its facility to cooperate with different cellular factors and control the expression of diverse genes implicated in the cell cycle and apoptosis. For example, it has been found to attach to and control the effectiveness of the tumor suppressor protein P21 and the oncogenic miRNA-9-5p (Zhou et al., 2019). Based on the function of *LncRNA TUG1* in promoting tumor proliferation by interacting with several genes affecting apoptosis and cell cycle progression (Li et al., 2018b), in our present research, we studied the conduct of *LncRNA TUG1*/*TGF-β*/*P53* interaction in CRC. QRT-PCR and immunohistochemistry were used to measure *LncRNA TUG1*, *TGF-β*, and *P53* expressions in CRC specimens and neighboring normal specimens. Based on the findings of qRT-PCR, we found that the relative genetic expressions of *LncRNA TUG1*, *TGF-β*, and *P53* were significantly elevated in CRC specimens compared with the neighboring non-neoplastic specimens (P<0.001). This outcome indicates that *LncRNA TUG1*/*TGF-β*/*P53* interplay might be implicated in the advancement of CRC. Also, for clinicopathological variables, *LncRNA TUG1* is supposed to be participated in colorectal carcinogenesis, as a significantly elevated expression level was found in stages III and IV, poorly differentiated neoplastic tissues, highly invasive CRC, positively metastatic LNs, and cases with liver metastasis. Furthermore, overexpressed-*TGF-β*/*P53* were significantly correlated with the neoplastic staging, cancer differentiation, invasion depth, LN, and liver metastasis as shown in (Table 5). Overall, these outcomes suggested that *LncRNA TUG1*/*TGF-β*/*P53* signaling might be genetic risk factors for CRC development and progress. Our study showed a strong positive correlation between *LncRNA TUG1* and both *TGF-β* & *P53* (r=0.89 and 0.91, respectively, (P<0.001)), exploring that the regulatory mechanism of *LncRNA*

TUG1 in CRC pathogenesis mediated through its potential interplay with *TGF-β/P53* signaling pathway. Therefore, the *LncRNA TUG1*/*TGF-β/P53* axis has great potential as a medicinal objective for CRC therapy.

Our results came in line with what Zhai et al., (2016) observed that *LncRNA TUG1* expression was significantly overexpressed in CRC specimens compared to adjacent non-neoplastic specimens, and the capacity of colon cancer cells to migrate was greatly inhibited by the suppression of *LncRNA TUG1* expression.

In addition, Xiao et al., (2018) and Wang et al. (2019a) informed that *LncRNA TUG1* was upregulated in colon cancer cells compared to healthy colon specimens, and that extreme *LncRNA TUG1* expression was positively associated with tumor stage, depth, and LN metastasis. Sun et al., (2016) also reported that *LncRNA TUG1* expression was meaningfully upregulated in cell lines of CRC and primary CRC clinical samples, and that high *LncRNA TUG1* expression was associated with cancer depth, advanced cancer stage, and liver metastasis and patients with CRC who expressed higher *LncRNA TUG1* had worse rates of long-term survival and disease-free longevity. Contrasting the finding of our research, Zhang et al., (2014b) showed that *LncRNA TUG1* was extensively downregulated in non-small cell lung cancer (NSCLC) specimens compared to lung specimens free of tumors and might act as a standalone predictor of life expectancy in NSCLC patients.

Isin et al. (2014) showed significant downregulation of *LncRNA TUG1* in multiple myeloma cases as compared to healthy individuals when measuring its expression level in the patient's plasma using qRT-PCR. Also, Liu et al., (2015) assessed *LncRNA TUG1* expression throughout DNA damage-induced apoptosis after treating glioma cell lines U87 and U251 with resveratrol and doxorubicin and found that it was decreased upon necrosis development in both cell lines but remained unaltered throughout apoptosis. This finding is probably because *LncRNA TUG1* displays unusually tissue-specific expression outlines and demonstrates an essential role as an oncogene or tumor suppressor in different malignancies. In this research, regarding IHC findings, we observed that positive *TGF-β* expression was significantly linked to higher tumor stage (III+IV), adenocarcinoma histopathological subtype, tumor depth (T3+T4), positive LN metastasis and positive liver metastasis (P=0.005, 0.0428, 0.000073, 0.0020, 0.02664), respectively, as in (Table 6), (Figures (5A, B and C)). Kubiczkova et al., (2012) agreed that *TGF-β* IHC expression is profoundly positive in CRC specimens especially in metastatic cases and higher node stage compared to normal tissue. They confirmed as well that high levels of *TGF-β* correlate with tumor progression. Meanwhile, Simms et al., (2012) disagreed as they concluded that *TGF-β* inhibits metastasis in a portion of human CRC cells.

Our study results also revealed a positive significant correlation between *LncRNA TUG1* and *TGF-β* which provides important insights that the *LncRNA TUG1*/*TGF-β* axis may have a probable role in CRC, this comes in harmony with Qin and Zhao (2017) who reported that *TGF-β* was increased after the overexpression of *LncRNA TUG1* enhancing the growth and invasion of pancreatic malignancy cells through *TGF-β*/SMAD path regulating EMT process, but the tendency was opposite after the interference with *LncRNA TUG1* expression. Also, Guo et al., (2021) assumed that *LncRNA TUG1* is linked to the progress of atrial fibrosis (AF) in AF patients via controlling the miR-29b-3p/*TGF-β* path.

Shen et al., (2020) suggested that *LncRNA TUG1* is located downstream of *TGF-β* signals and *TGF-β* advances migration, invasion, and lung metastasis in CRC via a *LncRNA TUG1*/TWIST1/EMT interplay. Hence, *LncRNA TUG1* is necessary for *TGF-β*-indorsed pathophysiological aspects of CRC.

While Yue et al., (2016) revealed that LncRNA-ATB promotes the EMT process by *TGF-β*/E-cadherin signaling and is engaged in the progress and prospects of CRC. In contrast with our results, Jung et al., (2017) found that the *TGF-β* signal reduces the growth of colon cells and advocates apoptosis. In addition to being a characteristic of CRC cells, altered intestinal epithelial cells also exhibit loss of *TGF-β* signals and their antiproliferative actions. Also, Wiener et al., (2014) and Zhao et al., (2015) reported that Bcl-2-like protein 11 (BIM) was a key factor of *TGF-β*-tempted apoptosis in colonic adenoma and hepatic malignancy. The differences between the previous studies may be because the TGF-β signal limits the progress of hyperplastic, pre-neoplastic injuries in various organs via stimulating cell cycle stop and apoptosis in the early phase of tumorigenesis. Once carcinogenesis has advanced, TGF-β collaborates with a variety of oncogenic processes to promote the growth of aggressive, poorly differentiated, and invasive malignancies (Hao et al., 2019). Extreme studies are required to completely understand the underlying molecular processes by which *LncRNA TUG1*/TGF-β interact in CRC and to explore the prospective scientific consequences of using this pathway for CRC treatment.

In our study, *P53* IHC marker expression exhibited significantly remarkable correlation with higher tumor grade (III+IV), higher stage (III+IV), adenocarcinoma histopathological subtype, positive LN metastasis, tumor depth (T3+T4) and positive liver metastasis (P<0.00001, 0.00006, 0.017314, 0.000397, 0.010072, 0.041097) respectively, as in (Table 6) and (Figures (6A, B and C)). In concordance with our study, Rambau et al., (2008) and Kim et al., (2022) revealed that CRC cases with *P53* positive frequently had more advanced tumor-node-metastasis stages, greater levels of malignancy, and shorter disease-free and overall survival times. They also stated that *P53* positivity was unofficially a critical prognostic factor for CRC individuals.

However, Komura et al., (2022) conducted that complete loss of *P53* IHC expression predicts a worse prognosis in CRC. And they added that CRC cases with wild-type (100%) *P53* IHC expression seemed to have higher existence than cases with overexpression (80%) or total loss following chemotherapy. This disagreement with our results might be due to the fact they worked on different molecular subtypes of colon cancer expressing different subtypes of *P53* mutations and that they also included large numbers of CRC cases. Based on the

available evidence suggests that dysregulation of *P53* expression might influence the growth and advance of CRC, the results of our present study hypothesized that *LncRNA TUG1* may function through a muted *P53*-dependant route as proved by the reality that *P53* is a renowned tumor suppressor in CRC. Hence, the processes beneath *LncRNA TUG1*-*P53*-mediated cell growth in CRC may be dependent, in portion, on the regulatory interaction between each other.

Lai et al., (2022) considered that *P53* may act as a transcription element for *LncRNA TUG1*, and *LncRNA TUG1* displayed the same pattern of expression as the *P53* protein. When the *P53* mRNA expression was decreased, *LncRNA TUG1* expression was also decreased. Silencing both *P53* mRNA and *LncRNA TUG1* expression increased DNA damage and apoptosis after the bup-induced dorsal root ganglion injury reaction was triggered. Additionally, Zhang et al., (2014b) showed that *LncRNA TUG1* contributed to the AKT/mitogen-activated protein kinase signal route and was a directly transcribed objective of the tumor suppressor *P53*, they revealed that the lack of *P53* expression may be attributed to the reduced expression of *LncRNA TUG1* in NSCLC. Also, other investigators hypothesized that *P53* can stimulate *LncRNA TUG1* production, which then attaches to polycomb repressive complex 2 (PRC2) and inhibits the expression of certain genes that regulate cell-cycle (Khalil et al., 2009).

These results gave us a basis for further research into the role of *LncRNA TUG1*/*P53* in the pathogenesis of CRC. However, Zhang et al., (2018) provided that overexpressed *LncRNA TUG1* might partially action via negatively controlling *P53* expression inhibiting apoptosis and promoting oncogenic effects in laryngeal carcinoma as they showed that *LncRNA TUG1* knockdown apparently elevated *P53* mRNA expression as well as that of *P53* protein. Furthermore, Liang et al., (2017) informed that *LncRNA TUG1* could suppress cell death and persuade epithelial-mesenchymal modification via the Wnt/β-catenin route in cancers. Huang et al., (2015) proved that *LncRNA TUG1* promotes cell growth of hepatocellular carcinoma. *LncRNA TUG1* epigenetically repressed Krüppel-like factor 2 (KLF2) transcription by attaching to PRC2 and employing it to KLF2 promoter site and *LncRNA TUG1* silencing resulted in reduced propagation, colony growth, carcinogenesis, and cell-death induction.

We guessed that the contrast between our findings and others was since malignancies were histologically altered and that the pathological routes implicated in these malignancies were wholly complicated.

In this research, we have reported that the overexpressed level of *LncRNA TUG1* may promote CRC pathogenesis and progress through *LncRNA TUG1*/*TGF-β/P53* interplay. Thus, we expected that *LncRNA TUG1* may be an oncogenic molecular regulator in CRC. However, we cautiously contemplated that an alternate controlling process might underlie *TGF-β*, *P53*, and *LncRNA TUG1* interaction, demanding further testing. Hence, In the future we intend to explore further the molecular pathway of *LncRNA TUG1*'s concern in the CRC pathogenesis, involving its influence on the *TGF-β/P53*-dependent signal route, and investigate for the protein or miRNA substrate of *LncRNA TUG1* employing western blotting and RNA-pull-down technique or dual luciferase reporter system, to understand its function completely.

Also, our study encountered other restrictions that require to be considered. Firstly, the research used a relatively small sample size, which may not be typical of the entire population. Secondly, the research did not consider other elements, such as genetic mutations, environmental factors, and lifestyle, which might affect the expression levels of the genes. Lastly, the study did not use other techniques, such as western blotting and RNA sequencing, to validate the results obtained by qRT-PCR and immunohistochemistry.

In conclusion, finally, we established for the first time the clinical importance of *LncRNA TUG1*, *TGF-β*, and *P53* by showing both their overexpression in CRC tissues and how it relates to the clinical characteristics of CRC cases. Furthermore, significant correlations were detected between *LncRNA TUG1* and both *TGF-β* and *P53*. The analysis suggested that *LncRNA TUG1* could be an essential oncogenic modulator in the pathogenesis of CRC, involved in several key pathways, including apoptosis, cell differentiation, and cell migration. Therefore, targeting the *LncRNA TUG1*/*TGF-β/P53* axis could potentially be an effective diagnostic, prognostic, and therapeutic approach for the treatment of CRC. Future studies with larger sample sizes, more comprehensive analysis, and consideration of other potential factors are needed to fully elucidate the role of *LncRNA TUG1*/*TGF-β/P53* signaling in CRC, which could significantly aid the management of CRC patients.

Author Contribution Statement

BAI and WEO created the idea and designed the research. BAI and MAG conducted the statistical analysis of the data. AMT, BAI, and WEO accomplished all the laboratory tests and interpreted the patients' data. AMM and WAA chose the patients. All authors wrote, read, and approved the final manuscript.

Acknowledgements

We thank all participants involved in our work.

Ethical issue

This research has been approved by the Institutional Review Board (IRB), Faculty of Medicine, Zagazig University (approval number is ZU-IRB #9992/11-10-2022).

Availability of data

Data is available upon request.

Conflict of interest

The authors provide that they do not have any competing interests in this research.

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