Original Article
Running Title: Prognostic Value of LncRNA *XIST* and *TUG1* in Hepatocellular Carcinoma
Received: January 24, 2025; Accepted: October 20, 2025

Clinical Evaluation of LncRNA *XIST* and *TUG1* Expression Levels in Patients with Hepatocellular Carcinoma

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Abstract

Background: Among long non-coding RNAs, as key regulators in oncogenesis, X-inactive specific transcript (*XIST*) and Taurine upregulated 1 (*TUG1*) are frequently reported in various cancers. The aim of this study was to investigate the expression patterns and potential clinicopathological and prognostic relevance of *XIST* and *TUG1* in a cohort of Iranian patients with hepatocellular carcinoma (HCC).

Method: In this cross-sectional study, the expression levels of *XIST* and *TUG1* in paired tumor and adjacent non-tumor liver tissues from 22 HCC patients were assessed using real-time quantitative polymerase chain reaction. Patients were divided into two groups based on high and low expression levels for each gene. Associations with clinicopathological features and overall survival of HCC patients were evaluated using statistical methods including chi-square tests, paired t-tests, Kaplan-Meier analysis, and log-rank tests.

Results: The study results showed that the expression of *XIST* and *TUG1* was not significantly higher in tumor tissues than in adjacent normal tissues ($P = 0.369$, $P = 0.632$, respectively). *TUG1* expression showed a statistically significant association with tumor node metastasis stage ($P = 0.02$), but no other clinicopathological parameters were correlated with the expression levels of either gene. Kaplan-Meier analysis revealed no significant difference in overall survival based on high vs. low expression of *XIST* ($P = 0.735$) or *TUG1* ($P = 0.239$).
Conclusion: Our study found no significant differential expression of *XIST* and *TUG1* between tumor and adjacent non-tumor tissues in a cohort of Iranian HCC patients. Further studies with larger sample sizes are needed to validate these findings.

Keywords: Hepatocellular, Carcinoma, Long noncoding, RNA, Expression

Introduction

Hepatocellular carcinoma (HCC) is the most prevalent type of primary liver malignancy and a major cause of cancer-related deaths globally, accounting for approximately 90% of all primary liver cancers.[1] The etiology of HCC is multifactorial, involving chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, obesity, diabetes mellitus, alcohol abuse, chronic aflatoxin exposure, and various inherited conditions such as glycogen storage diseases, hemochromatosis, Wilson's disease, and alpha-1-antitrypsin deficiency.[2] Moreover, about 85% of HCC cases occurs in the setting of cirrhosis, highlighting the strong link between chronic liver damage and hepatic tumorigenesis.[1] Given the rising global burden of metabolic syndrome and lifestyle-related liver diseases, it is anticipated that the global mortality rate due to HCC may reach one million deaths annually by 2030.[3-5] Since the prognosis for HCC patients is heavily dependent on the stage at which the cancer is diagnosed, early detection is challenging due to the asymptomatic nature of the disease in its initial stages.[3, 6] This makes early prognostic evaluation and identification of reliable biomarkers critical for improving management strategies and overall survival in HCC patients.

In recent years, studies have increasingly focused on the development of molecular and genetic biomarkers for early cancer detection and prognosis prediction. Alpha-fetoprotein is one of the most recognized and commonly used biomarkers for diagnosing HCC; however, its sensitivity and specificity are limited, and numerous studies have reported inconsistent findings regarding the diagnostic utility of Alpha-fetoprotein.[7-9] With advancements in genomics, several nucleic acid-based biomarkers -particularly non-coding RNAs- have been explored in cancer research.[10, 11] In the human genome, although approximately 75% is actively transcribed into RNA, only a small fraction (~2%) encodes proteins. The remaining majority consists of non-coding RNAs, including long non-coding RNAs (lncRNAs), which are transcripts longer than 200 nucleotides and play key roles in regulating gene expression at epigenetic, transcriptional, and post-transcriptional levels.[12, 13] Evidence suggests that lncRNAs are involved in numerous physiological and pathological processes, such as cell growth, differentiation, apoptosis, immune responses, and oncogenic transformation.[14, 15] Aberrant expression of certain lncRNAs has been reported in several cancers, including HCC. These lncRNAs are increasingly recognized as potential diagnostic and prognostic biomarkers.

Taurine upregulated 1 (*TUG1*) is a novel lncRNA located on chromosome 22q12 that has been shown to be dysregulated in various cancers. *TUG1* has been implicated in promoting tumorigenesis and tumor

progression by modulating key cellular processes such as proliferation, invasion, metastasis, apoptosis, epithelial-mesenchymal transition, and therapeutic resistance.[16] Another lncRNA of interest is the X-inactive specific transcript (*XIST*), located on the X chromosome. Originally recognized for its role in X-chromosome inactivation, recent studies have identified *XIST* as an oncogenic lncRNA that contributes to inflammation, tumor growth, and metastasis.[17, 18] Dysregulated *XIST* expression has been observed in multiple cancer types, including colorectal, pancreatic, and thyroid cancers, where it has been associated with poor clinical outcomes. Although prior studies from different countries have reported the clinical significance of *TUG1* and *XIST* expression in HCC, population-specific variations may influence gene expression patterns and their prognostic impact. In this context, the present study aimed to evaluate the expression levels of *XIST* and *TUG1* in a cohort of Iranian HCC patients and to assess their potential associations with key clinicopathological features, including tumor node metastasis (TNM) stage and overall survival.

Materials and Methods

Patients and clinical tissue samples

This cross-sectional study was conducted at Imam Reza and Montaseriyeh hospitals affiliated with Mashhad University of Medical Sciences in Mashhad, Iran. A total of 22 paired tissue samples, including tumor tissue and adjacent non-tumorous liver tissue were obtained from patients diagnosed with HCC, based on radiological imaging and histopathological confirmation. Patients who had received any form of prior oncologic treatment, such as chemotherapy, radiotherapy, transarterial chemoembolization, radiofrequency ablation, or other locoregional therapies, were excluded from the study. Additionally, individuals with concurrent malignancies in organs other than the liver were not included. Tissue specimens were sectioned during surgical procedures such as liver resection (hepatectomy) or tru-cut liver biopsy. Freshly excised tissues were immediately immersed in RNAlater solution (Thermo Fisher Scientific, Waltham, MA, USA) and kept at 4°C overnight to stabilize the RNA. Samples were then stored at -80°C until RNA extraction was performed.

The study protocol was approved by the Ethics Committee of the Faculty of Medicine at Mashhad University of Medical Sciences (Approval ID: IR.MUMS.Medical.REC.1400.294) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants prior to their enrollment in the study.

Quantitative real-time reverse transcriptase polymerase chain reaction (PCR)

Total RNA was extracted from tissue samples using the Trizol reagent kit (Sangon Biotech Co., Ltd., Shanghai, China), following the manufacturer's standard protocol. The concentration and purity of the extracted RNA were assessed using a NanoDrop spectrophotometer (Thermo-Fisher Scientific, Waltham, MA, USA) at an absorbance of 260 nm. Subsequently, 2 µl of total RNA (20–200 ng) was reverse-transcribed into cDNA using a commercially available cDNA synthesis kit (Wizbiosolutions, Seongnam, Gyeonggi, Korea). Quantitative real-time PCR was carried out using the Roche LightCycler® 96 System with SYBR Green master mix, gene-specific primers (Table 1), and nuclease-free water. All experiments were conducted in triplicate, and gene expression levels of *TUG1* and *XIST* were calculated relative to the housekeeping gene *GAPDH*, using the $2^{-\Delta\Delta Ct}$ method. *GAPDH* was selected as the internal control due to its well-established stability and uniform expression across a

variety of biological and pathological conditions, including liver tissues and samples. The expected amplicon lengths were 90 bp for *GAPDH*, 114 bp for *TUG1*, and 80 bp for *XIST*, all of which were confirmed using 2% agarose gel electrophoresis to verify product size specificity.

Statistical analysis

Data were analyzed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were presented as mean and standard deviation values for continuous variables and as frequency and percentage values for categorical variables. Comparisons of categorical variables were performed using the chi-square test and paired t-tests were used to analyze differences in gene expression between tumor and non-tumor tissues. When data did not follow normal distribution criteria, the Wilcoxon signed-rank test was applied. Survival analysis was conducted using the Kaplan-Meier method and compared with the log-rank test. A two-sided *P* value of less than 0.05 was set as statistically significant.

Results

Baseline characteristics of patients

The demographic and clinical profiles of the 22 HCC patients included in this study are summarized in Table 2. The mean age of the patients was 52.95 ± 15.37 years, with an equal distribution across two age categories: < 60 years (n = 11, 50.0%) and ≥ 60 years (n = 11, 50.0%). The majority of the participants were male (19 patients, 86.4%), while females accounted for only 13.6% (n = 3). The average tumor size was 5.18 ± 2.60 cm. Liver cirrhosis was observed in 63.6% of the patients (n = 14), and HBV infection was present in 45.5% (n = 10). Only 2 patients (9.1%) tested positive for HCV. A majority of the patients (68.2%, n = 15) had a single hepatic nodule. Additional clinical data, including tumor encapsulation, vascular invasion, differentiation grade, TNM staging, surgical intervention, and patient survival status, are detailed in Table 2.

Expression levels of XIST and TUG1 in HCC tissues

Quantitative PCR analysis revealed that although the mean expression levels of *TUG1* and *XIST* genes appeared higher in tumor tissues compared with adjacent non-tumorous tissues, these differences were not statistically significant (P_{XIST}= 0.369, P_{TUG1}= 0.632). The comparative expression patterns are depicted in Figure 1.

Association between XIST and TUG1 expression and clinicopathological features

The patients were classified into high and low expression groups for each gene based on the median expression value. For *XIST*, 13 patients were classified in the high-expression group and 9 in the low-expression group. For *TUG1*, each group included 12 patients. The associations between lncRNA expression levels and clinicopathological variables are summarized in Table 3. No significant association were found between *XIST* expression and clinical or pathological parameters. In contrast, *TUG1* expression demonstrated a statistically significant association with TNM stage (*P* = 0.02), with patients in the high-expression group more frequently presenting with advanced stages (T3–T4). Other characteristics such as tumor size, liver cirrhosis, viral status (HBV, HCV), and vascular invasion did not show significant correlations with the expression levels of either gene.

Regarding the relationship between gene expression levels and disease stage, the mean expression values of *TUG1* and *XIST* were compared across TNM stages (T1–T4). As shown in Figure 2, patients in the T4 group exhibited the highest mean expression for both genes (*TUG1*: 25.50 ± 3.19; *XIST*: 24.10 ± 4.03). Despite these numerical differences, statistical analysis did not reveal significant variation in gene expression between stages

for *XIST*, whereas *TUG1* expression demonstrated a trend toward increased levels in more advanced stages, with the highest mean in T4. This is in alignment with the data presented in Table 2, showing the association between *TUG1* expression and TNM stage ($P = 0.02$).

Prognostic analysis of XIST and TUG1 expression

The association between lncRNA expression and overall survival was evaluated using Kaplan-Meier survival analysis (Figure 3). The mean survival time for patients with high *XIST* expression was 38.49 ± 5.06 months, compared with 31.25 ± 4.54 months in the low-expression group. For *TUG1*, the corresponding survival times were 41.05 ± 4.05 months (high-expression group) and 32.54 ± 6.26 months (low-expression group). However, differences in survival outcomes were not statistically significant for each gene based on the log-rank test ($P = 0.239$ and $P = 0.735$, respectively).

Discussion

In the present study, no statistically significant differences were observed in the expression levels of *XIST* and *TUG1* between HCC tumor tissues and adjacent non-tumor tissues. Furthermore, no substantial associations were found between the expression levels of *XIST* or *TUG1* and most clinicopathological features or overall survival, except for TNM stage in the *TUG1* group.

Several studies have previously reported upregulation of *TUG1* in HCC and its association with tumor size, TNM stage, and poor prognosis. For example, Farzaneh et al. highlighted *TUG1* as a functional regulator of epithelial-mesenchymal transition and metastasis in HCC.[19] Similarly, Huang et al. reported significantly higher *TUG1* expression in HCC tissues and showed that its upregulation promoted tumor cell proliferation and apoptosis resistance via epigenetic silencing of KLF2.[20] In contrast, our study did not observe a significant difference in *TUG1* expression between tumor and adjacent non-tumorous tissues. However, we did find that higher *TUG1* expression was significantly associated with more advanced TNM stages, suggesting a possible role in tumor progression.

Regarding *XIST*, several reports have shown that it functions as an oncogenic lncRNA in HCC and other malignancies. For instance, Dong et al. demonstrated that *XIST* expression was elevated in HCC and promoted cell proliferation by sponging miR-488.[21] Conversely, Ma et al. found that plasma *XIST* levels were reduced in HCC patients, and lower expression was correlated with higher tumor grade and stage.[22] These inconsistencies in the literature may stem from differences in patient ethnicity, sample source, disease stage at sampling, or even methodological variations in RNA quantification.

The divergence between the findings of recent studies as well as our study may also reflect population-specific genetic or epigenetic factors, particularly since this is one of the few investigations focusing on an Iranian HCC cohort. Additionally, lncRNA expression is known to be influenced by numerous biological and environmental conditions, including viral infections such as HBV and HCV. While we documented the infection status of patients, no significant relationship between HBV/HCV status and lncRNA expression levels was observed. However, future studies with larger sample sizes could better elucidate these potential interactions.

Another important consideration is that our analysis was confined to mRNA-level quantification, which may not fully reflect functional activity. While lncRNAs do not code for proteins, they exert regulatory functions via interactions with RNA, DNA, or proteins. Thus, integrating functional

assays (e.g., knockdown/overexpression experiments), in situ hybridization, or subcellular localization studies would provide deeper insight into their mechanistic roles in HCC.

Our study has several strengths, including the use of matched tumor and adjacent normal tissues, and the application of rigorous RNA extraction and qPCR protocols. Nonetheless, there are notable limitations that may impact the accuracy of the results, including the small sample size and the absence of a control group. Additionally, selection bias might have occurred, as it is not feasible to evaluate microvascular invasion or histological grade across the entire sample prior to a liver biopsy.

Conclusion

Our study provides preliminary data that may help refine hypotheses for future research in this field. The lack of consistent findings across populations highlights the importance of replication studies in diverse ethnic cohorts and emphasizes the need for functional characterization of lncRNAs beyond mRNA quantification. Although we did not find strong evidence for a prognostic role of *XIST* and *TUG1* in this cohort, the association between *TUG1* and TNM stage suggests its potential utility as a progression marker that warrants further investigation.

Acknowledgments

The authors would like to express their gratitude to the Clinical Research Development Unit of Ghaem Hospital, Mashhad University of Medical Sciences, for their assistance. This study was supported by research project No. 992279 as a residency thesis at Mashhad University of Medical Sciences.

Authors' Contributions

H.MM: Study design, data gathering, drafting and reviewing the manuscript; S.R: Study design, and reviewing the manuscript; Z.G, S.F, and MH.T: Study design, and reviewing the manuscript; S.J: Data gathering, drafting; M.A: Data gathering, drafting, reviewing the manuscript.

All authors have read and approved the final manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Funding

None declared.

Conflict of Interest

None declared.

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Table 1. Primer sequences and amplicon lengths used for quantitative RT-PCR analysis of *GAPDH*, *TUG1*, and *XIST* genes

Gene	Forward primer	Reverse primer	Length
GAPDH	5'-TGCACCACCAACTGCTTA-3'	5'-GATGGCATGGACTGTGGTCAT-3'	90 bp
TUG1	5′-CTCTCTTTACTGAGGGTGCTTTAGCT-3'	5'-TCTCTCCATATTTTGGCTCTGCTT-3'	114 bp
XIST	5'-TCAGCCCATCAGTCCAAGATC-3'	5'-CCTAGTTCAGGCCTGCTTTTCAT-3'	80 bp

RT-PCR: Reverse transcription polymerase chain reaction; *GAPDH*: Glyceraldehyde 3-phosphate dehydrogenase; *TUG1*: Taurine upregulated 1; *XIST*: X-inactive specific transcript; bp: Base pair;

Table 2. Demographic and clinical characteristics of patients with HCC

Variables		Mean/ Frequency	% / SD
Age		52.95	15.37
Age distribution	<60	11	50.0
	≥60	11	50.0
Gender	Female	3	13.6
	Male	19	86.4
Tumor size		5.18	2.60
Liver cirrhosis	Yes	14	63.6
	No	8	36.4
HBV	Yes	10	45.5
	No	12	54.5
HCV	Yes	2	9.1
	No	20	90.4
Single nodule	Single	15	68.2
	Multiple	7	31.8
Tumor differentiation	Well	10	45.5
	Moderate	8	36.4
	Poor	4	18.2
Tumor encapsulation	complete	15	68.2
	perforated	7	31.8
Vascular invasion	Yes	5	22.7
	No	17	77.3
Tumor staging	T1	8	36.4
	T2	3	13.6
	T3	4	18.2
	T4	7	31.8
Liver transplant status	Recipient	15	68.2
	Lobectomy	7	31.8
Condition of patients	Alive	16	72.7
	Expired	6	27.3

HCC: Hepatocellular carcinoma; SD: standard deviation; HBV: Hepatitis B virus; HCV: Hepatitis C virus

Table 3. The relationship between *XIST* and *TUG1* expression levels and clinicopathological parameters in HCC patients

Variables		*XIST* expression			*TUG1* expression		
		Low (n=9)	High (n=13)	*P*-Value	Low (n=10)	High (n=12)	*P*-value
Age (mean ±SD)		57.22 ± 13.62	50.0 ± 16.30	0.29	52.30 ± 17.58	53.50 ± 14.01	0.86
Tumor size		5.67 ± 1.95	4.84 ± 2.99	0.48	5.38 ± 2.99	5.01 ± 2.34	0.75
Gender	Male (%)	7 (77.8)	12 (92.3)	0.54	8 (80.0)	11 (91.7)	0.57
	Female (%)	2 (22.2)	1 (7.7)		2 (20.0)	1 (8.3)	
Cirrhosis	Yes	4(44.4)	10 (76.9)	0.19	6 (60.0)	8 (66.7)	0.55
	No	5 (55.6)	3 (23.1)		4 (40.0)	4 (33.3)	
HBV	Yes	5 (55.6)	5 (38.5)	0.43	7 (70.0)	3 (25.0)	0.08
	No	4 (44.4)	8 (61.5)		3 (30.0)	9 (75.0)	
HCV	Yes	1 (11.1)	1 (7.7)	0.09	0 (50.0)	2 (16.7)	0.48
	No	8 (88.9)	12 (92.3)		10 (100.0)	10 (83.3)	
Tumor differentiation	Well	5 (55.6)	5 (38.5)	0.52	6 (60.0)	4 (33.3)	0.19
	Moderate	2 (22.2)	6 (46.2)		4 (40.0)	4 (33.3)	
	Poor	2(22.2)	2 (15.4)		0 (0.0)	4 (33.3)	
TNM stage	T1	3 (33.3)	5 (38.5)	0.54	6 (60.0)	2 (16.7)	0.02
	T2	1 (11.1)	2 (15.4)		0 (0.3)	3 (25.0)	
	T3	3 (33.3)	1 (7.7)		3 (30.0)	1 (8.3)	
	T4	2 (22.2)	5 (38.5)		1 (10.0)	6 (50.0)	
Tumor nodule	Single	5 (55.6)	10 (76.9)	0.38	6 (60.0)	9 (75.0)	0.65
	Multiple	4 (44.4)	3 (23.1)		4 (40.0)	3 (25.0)	
Liver status	Recipient	5 (55.6)	10 (76.9)	0.38	7 (70.0)	8 (66.7)	0.99
	Lobectomy	4 (44.4)	3 (23.1)		3 (30.0)	4 (33.3)	
Tumor encapsulation	Complete	7 (77.8)	8 (61.5)	0.65	9 (90.0)	6 (50.0)	0.07
	Perforated	2 (22.2)	5 (38.5)		1 (10.0)	6 (50.0)	
Vascular invasion	Yes	2 (22.2)	3 (23.1)	0.99	1 (10.0)	4 (33.3)	0.32
	No	7 (77.8)	10 (76.9)		9 (90.0)	8 (66.7)	
Patients condition	Alive	6 (66.7)	10 (76.9)	0.65	6 (60.0)	10 (83.3)	0.35
	Expired	3 (33.3)	3 (23.1)		4 (40.0)	2 (16.7)	

HCC: Hepatocellular carcinoma; *TUG1*: Taurine upregulated 1; *XIST*: X-inactive specific transcript; SD: Standard deviation; HBV: Hepatitis B virus; HCV: Hepatitis C virus; TNM: Tumor node metastasis

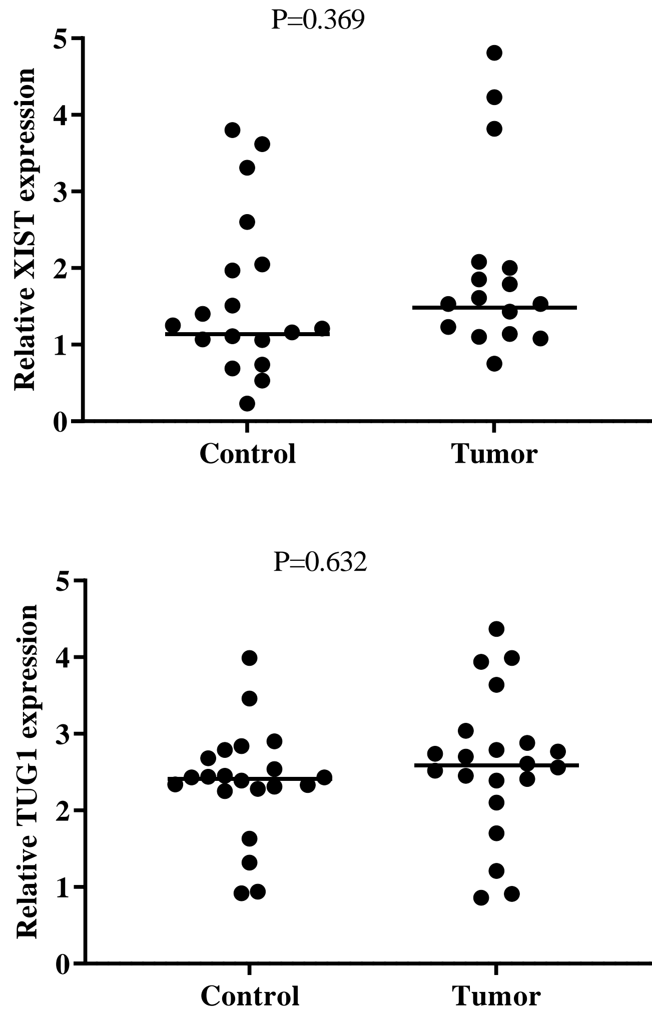

Figure 1. This figure shows the comparison of *XIST* and *TUG1* expression levels in HCC tumor tissues and adjacent non-tumorous tissues. No significant differences were observed between tumor and non-tumor samples for either lncRNA.

HCC: Hepatocellular carcinoma; *TUG1*: Taurine upregulated 1; *XIST*: X-inactive specific transcript

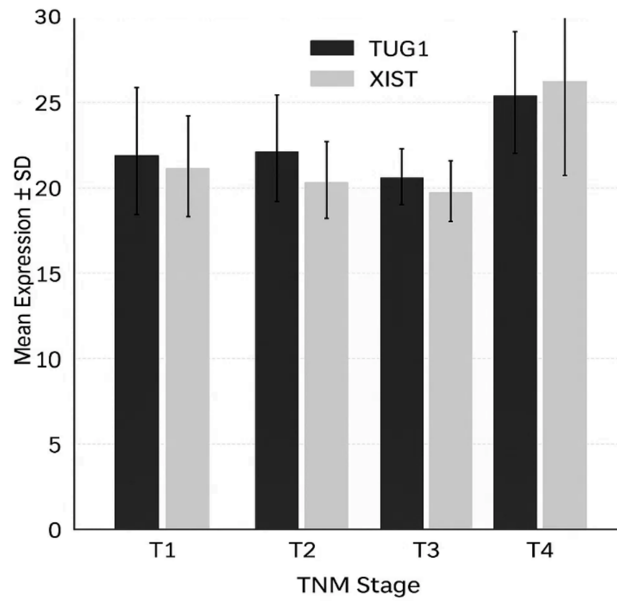

Figure 2. This figure shows the comparison of mean expression levels of lncRNAs *TUG1* and *XIST* across different TNM stages in HCC patients.

HCC: Hepatocellular carcinoma; *TUG1*: Taurine upregulated 1; *XIST*: X-inactive specific transcript; SD: Standard deviation; TNM: Tumor node metastasis

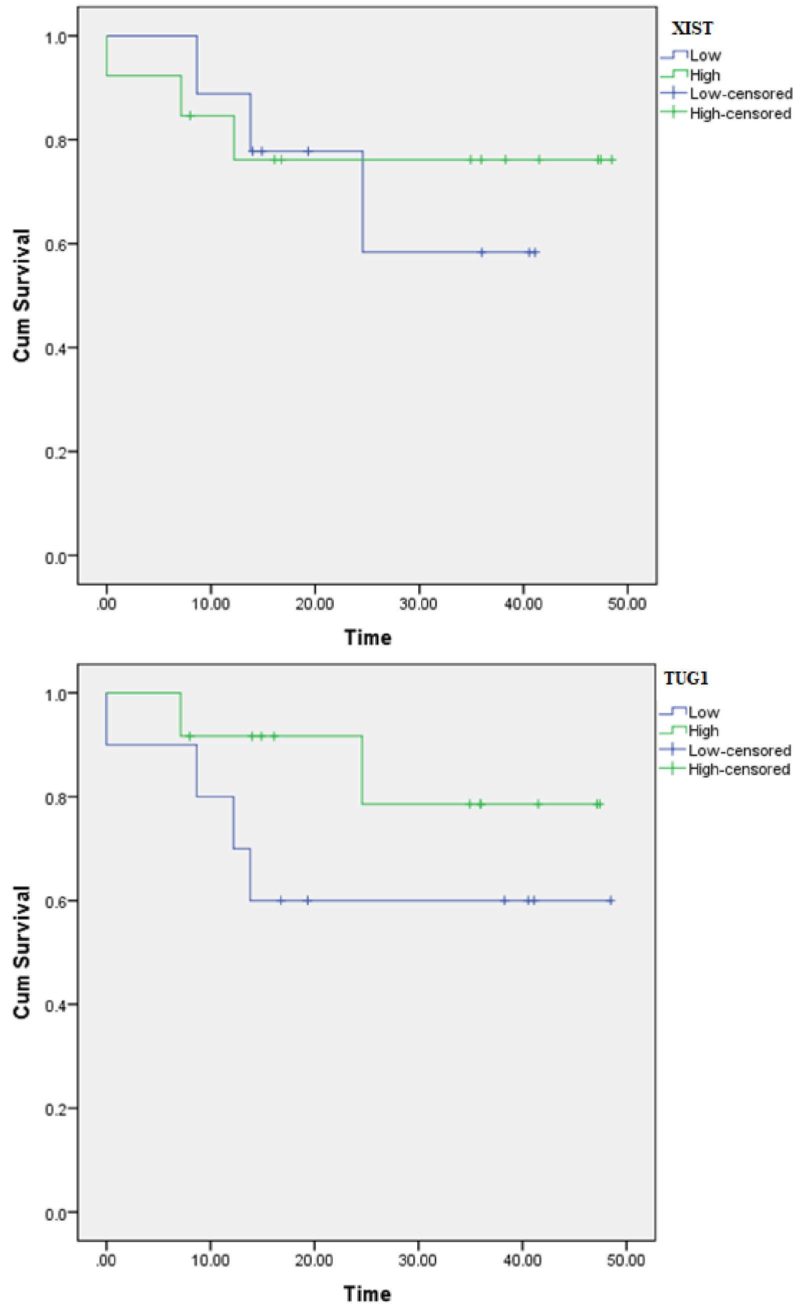

Figure 3. This figure shows the Kaplan-Meier curves comparing overall survival between HCC patients with high and low expression of *XIST* and *TUG1*. No statistically significant differences were observed (Log-rank test, P_{TUG1}= 0.239, P_{XIST}= 0.735).

HCC: Hepatocellular carcinoma; *TUG1*: Taurine upregulated 1; *XIST*: X-inactive specific transcript