Research Square

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Long Intergenic Non-Coding RNA— Linc00659— Expression Changes in Gastric Cancer

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Abstract

Purpose

Gastric cancer (GC) as a multifactorial disease is caused by environmental, infectious, and genetic factors. The aberrant expression of lncRNAs has been considered as a crucial feature of human cancer. In this research, we assessed the expression levels of a linc00659 in GC patients.

Methods

Expression of linc00659 in tumor and non-tumor tissues (a total of 82 samples) was evaluated using qRT-PCR in Iranian patients. The correlation between the linc00659 expression levels and clinicopathological features was assessed.

Results

Linc00659 was down-regulated in more GC samples compared to controls, but we found no significant association between the linc00659 expression levels and GC risk [expression ratio of linc00659 in tumor tissues versus non-tumor tissues was 0.57 ($p = 0.33$)]. After classifying patients into down−/up-regulation groups, a significant association was observed between the linc00659 expression and origin of the tumor ($p = 0.01$).

Conclusion

We found a significant association of the linc00659 expression with origin of the tumor. Further investigations with large sample size are required to assess the linc00659 function in tumor genesis.

Introduction

Gastric cancer (GC) as the fifth commonest cancer is the third leading cause of cancer-related death in the world. There were nearly 1.033 million new GC patients in 2018 and approximately 783000 of the affected cases died, based on GLOBOCAN estimates [1]. Four molecular subtypes of GC, including microsatellite instability (MSI), chromosomal instability (CIN), genomically stable (GS), and Epstein–Barr virus (EBV) has been indicated by the Cancer Genome Atlas (TCGA) project. CIN subtype is commonly found in the esophago-gastric junction/cardia and accounts for at least 50% of GCs [2, 3]. LncRNA molecules are involved in cancer and metastasis. Long noncoding RNAs (lncRNAs) are longer than 200 nt that do not translated into protein [4]. These molecules can be originated from another genomic position, like 3'- and 5' -UTR regions, enhancer sequences, exons, promoters, introns, and intergenic regions [5]. LncRNAs have diverse effect on regulating gene transcription, epigenetic modification translation, and post-transcription [6]. Aberrant expression or dysfunction of lncRNA causes many diseases [7, 8, 9]. Many lncRNAs using their tumor suppressor or carcinogenic effects in cancers showed irregular expression and could control cancer cell proliferation, angiogenesis, metastasis or invasion [10, 11]. Because of structural flexibility, such molecules are able to act as trap, signal, guide or molecular scaffolding affecting

metastasis. LncRNAs has tissue-specific expression patterns and can be used to classify tumor subtypes or predict the therapeutic effects of drugs [12, 13]. Recent genome-wide association studies (GWAS) and also molecular epidemiological evaluations investigated the correlation between ncRNA polymorphisms and disease risk.[14]. SNPs have been widely studied in cancer research. SNPs in lncRNAs influence the expression levels [15, 16, 17]. In the current study, the gene expression of linc00659 was assessed in GC patients of Iranian population.

Materials And Methods

Study cases

Forty-one tumor samples from GC patients as well as 41 samples from corresponding adjacent non tumor tissues (n = 82) were collected from GC patients who were subjected to endoscopy at Imam Khomeini Hospital of Ardabil, as a high-risk region in Northwestern Iran (age-standardized incidence rates (ASRs) = 51.8/100,000 for men and 24.9/100,000 for women) with one of the highest rates of gastric cardia cancer in the world [18]. Tissue specimens were assessed via standard histopathological assessments. The patients who had been subjected to radiation therapy, chemotherapy, or immunotherapy were not included. Informed consent signed by GC patients. The Ethics Committee of the DDRC confirmed this study. All tissues were stored at − 80°C. gastric cardia cancer rates

RNA extraction, cDNA synthesis and real-time reverse transcriptase PCR

TRIZOL Kit (Invitrogen) was used to separate total RNAs from the samples based on the manufacturer's protocol. The quantity and quality of the extracted RNAs were assessed using NanoDrop2000c spectrophotometer (Thermo Fisher). cDNA was synthesized with Takara kit (TaKaRa, Dalian, China). A total of 1µg of RNA was reverse transcribed in a final volume of 20 µL using random primers. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used to quantify lncRNA as the internal reference. Quantitative Real-time PCR reactions were done through the SYBR Green Master Mix (Takara). A rotor gene 6000 Corbett Real-Time PCR System was used to compare the relative lncRNA expression level between non-tumoral and tumoral samples. There was a non-template negative control for every run. The following gene-specific primers were used for linc00659 and GAPDH: linc00659 - forward /5' - ACCCTGAAGGACCATATCCA-3', linc00659- reverse /5' - GGCTCGGCTGTGTCTCAAG-3', GAPDH- forward /5' -TGCACCACCAACTGCTTA3', and GAPDH- reverse /5' -GGATGCAGGGATGATGTTC-3'. Relative fold change calculated using the $2^{-\Delta\Delta Ct}$ value. Experiments were repeated in triplicate.

Statistical analysis

Relative quantification of the target gene expression was calculated using two-sided Pair-Wise Fixed Reallocation Randomization Test by REST 2009 (Relative Quantification Software Tool) software. Fold change was determined to detect the differences in gene expression levels between tumor tissues compared to non-tumor tissues. Chi-square test ($\chi 2$) was employed for assessing the correlation of the

linc00659 gene expression and the clinicopathological characteristics of the samples. The p-values < 0.05 were considered as significant.

Results

Our study examined the expression levels of linc00659 in GC tissues compared with non-tumor tissues (n = 82) through real-time RT-PCR. The average age of cases was 66.02 ± 9.2 years (range, 42 to 83 years). Also, 74.3% of them were males. In addition, 63.4% of tumors were found in cardia subsite, 9.8% in cardia and non-cardia subsites, and 26.8% in non-cardia subsite. Histologically, 42.5% of them had diffuse type, 42.5% intestinal type, and 15% had other GC types. Linc00659 relative expression level was not significantly different between tumor samples compared to controls (Fold change: 0.57, p = 0.33; Fig. 1a). After classifying patients into up or down-regulation groups according to the expression levels of linc00659 in tumor tissues compared to non-tumor tissues, a significant association between the linc00659 expression levels and tumor site was seen (p = 0.01, Table 1). Such association was not significant (P > 0.05) for other clinicopathological characteristics, such as age, pathology, gender, and family history of GC.

Table 1
Results of association analysis between expression of linc00659 and clinicopathological characteristics (Up- and down-regulation of the gene have been defined based on the relative expression of the gene in malignant samples versus nonmalignant samples.

	Linc00659 up-regulation	*Linc00659* down-regulation	P-value
Age			0.09
60 >	1 (12.5%)	7 (87.5%)	
60 ≤	13 (44.8%)	16 (55.2%)	
Gender			1
Female	10 (38.5%)	16 (61.5%)	
Male	3 (33.3%)	6 (66.7%)	
Position			0.01
Cardia	10 (38.5%)	16 (61.5%)	
Cardia and non-cardia	4 (100%)	0 (0%)	
Non cardia	2 (18.2%)	9 (81.8%)	
Pathology			0.94
Intestinal	7 (41.2%)	10 (58.8%)	
Diffuse	7 (41.2%)	10 (58.8%)	
Other	2 (33.3%)	4 (66.7%)	
Family History			0.85
Positive	4 (40%)	6 (60%)	
Negative	11 (36.7%)	19 (63.3%)	

Discussion

The emergence of high-throughput RNAseq caused the identification of thousands of unknown lncRNAs that their improper expression has a close association with the development and initiation of cancers. Dysregulation of lncRNAs has a close relationship with tumorigenesis, metastasis, diagnosis, or prognosis of GC. Recently, several lncRNA-associated GCs have been detected. Nonetheless, the underlying mechanisms regulating the GC progression are largely unknown. LincRNAs are sequences of lncRNAs that do not overlap with the protein-coding genes. Long intergenic non-coding RNA-01296 can mediate tumorigenesis by sponging miR-122 in GC [19]. Chen et al. reported significantly reduced lincRNA-01317 levels in cancer tissue in comparison to para cancer tissue of GC patients. LincRNA-01317 expression levels showed a positive correlation with clinical survival rate. They also showed that lincRNA-

01317 can target KCNQ1, because KCNQ1 showed down-regulation following transfection of the cells with lincRNA-01317 [20]. Zou et al. indicated the association between LINC00324 overexpression and the poor prognosis of GC patients [21]. Liu et al. suggested that LINC00941 has a crucial oncogenic function in GC and can be served as a diagnosis and prognosis biomarker for GC [22]. Luo reported an increase in LINC00483 and MAPK1 levels in GC cells and tissues. Overexpression of MAPK1 reduced the LINC00483 knockdown effect on GC development. LINC00483 increased MAPK1 expression through competitively sponging miR-490-3p. Up-regulation of miR-490-3p inhibited GC development, which was decreased by LINC00483. In addition, inhibition of LINC00483 caused a decrease in xenograft tumor growth through the regulation of MAPK1/miR-490-3p axis [23]. Yang et al. reported a significant up-regulation of LINC00265 expression in tumor tissue compared to controls. LINC00265 possibly increases GC cell proliferation through the CBX4/miR-144-3p axis [24]. Chen et al. demonstrated that the expression level of lincRNA-p21 was markedly reduced in GC samples than that in control samples and this lower lincRNA-p21 level showed a significant correlation with more distant metastasis, higher invasion depth grade, and advanced TNM stage. Knockdown of lincRNA-p21 promoted malignant behavior of GC cells and induced epithelial to mesenchymal transition (EMT). LincRNA-p21 overexpression demonstrated opposite effects. Also, lincRNA-p21 knock down elevated the expression of yes associated protein (YAP) as the core effector of Hippo signaling, through an increase in mRNA levels as well as its nucleus translocation instead of the canonical Hippo pathway. [25]. Gong et al. indicated that linc00659 expression levels were markedly up-regulated in GC patients. Elevated levels of linc00659 showed an association with advanced tumor stage as well as unfavorable prognosis of GC patients. Also, up-regulated linc00659 expression could promote the invasion of GC cells. Bioinformatics studies indicated IQ motifcontaining GTPase activating protein 3 and matrix metalloproteinase 15 as possible downstream targets of linc00659 associated with tumor metastasis, however, the exact underlying mechanism needs further exploration. Up-regulation of linc00659 expression could predict a poor prognosis and promoted invasion and migration of GC cells. Sheng et al. reported an expression level of linc00659 in GC, which can be due to the promotion of cell invasion and regulation of cell proliferation. SUZ12 is a Transcription factor and regulates linc00659. Also, linc00659 regulated cell cycle and invasion of GC through an increase in the expression of SUZ12 [26]. Tsai et al. demonstrated that the linc00659 up-regulated in colon cancer. Up-regulation of linc00659 was linked with poor survival in colorectal cancer (CRC) patients. Linc00659 was found with a significant co-expression with cycle-related genes in CRC. Silencing of Linc00659 expression caused cell growth inhibition and could induce apoptosis, probably through the suppression of AKT-PI3K signaling in colon cancer [27]. In this article, we examined the linc00659 expression levels in Iranian GC patients. The expression level of linc00659 in tumor samples compared to non-tumor samples was 0.57. In contrary to recent studies, in our research, although linc00659 was down-regulated in more GC samples compared to controls, the difference did not reach significant statistical level. After classifying patients into up- or down-regulation groups according to the expression levels of linc00659 in tumor tissues compared to non-tumor tissues, a significant association between the linc00659 expression levels and tumor site was seen ($p < 0.05$). No significant association between linc00659 expression levels and GC risk is probably due to the sample size, maybe was not enough for the assessment. The differences between our findings and two recent studies, can be due to the fact that many lncRNAs display both

tumor suppressive and oncogenic functions [28]. Also, the human populations are genetically diverse and SNPs in lncRNAs largely influence the expression level [17, 29]. Thus, it can be expected that the lncRNA functional mechanisms in the process of carcinogenesis be different. Thus, more investigations are needed for illuminating the functional mechanisms of linc00659 in GC and revealing its influence on cellular pathways and tumorigenesis.

Abbreviations

NcRNAs: noncoding RNAs; LncRNAs: long noncoding RNAs; cDNA: complementary DNA; GAPDH: glyceraldehyde3-phosphate; SNP: Single nucleotide polymorphisms

Declarations

Ethics approval

The study was approved by the ethics committee of the DDRC, based on the ethical principles of human research and experimentation expressed in the 1964 Declaration of Helsinki and its later amendments.

Consent to participate: Informed consent for participation in the study was given by each subject in writing.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

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CRediT authorship contribution statement

S.L.N. provided direction in the preparation of the manuscript; E.Abdolzadeh performed the experiments; E.A. and E.Abdolzadeh. wrote the first draft of manuscript; V.K.O. and E.A. analyzed data; S.L.N., A.Y., and S.Z. discussed and revised the manuscript; A.Y., and E.A. managed the references; S.L.N. approved the version to be published.

Declaration of competing interest

No conflict of interest to be declared

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Figures

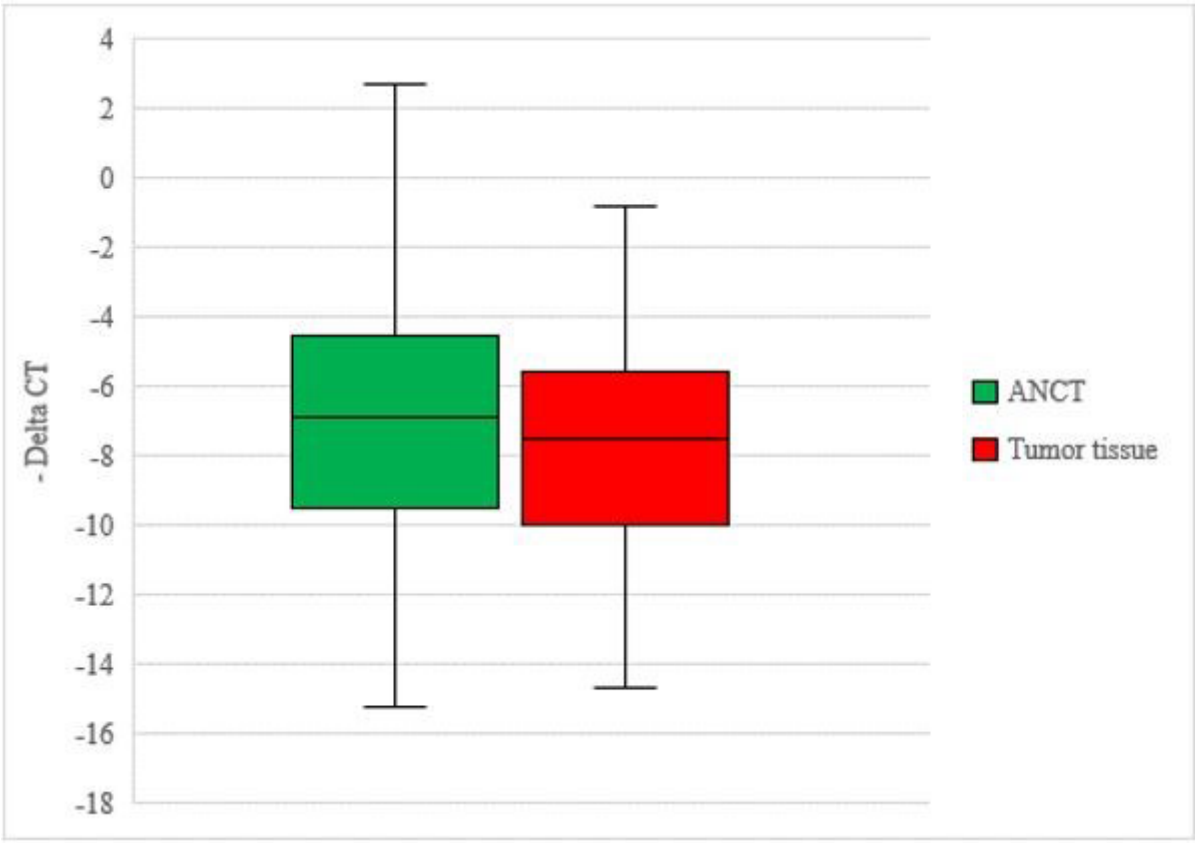

Figure 1

A) Expression level of linc00659 in malignant and nonmalignant tissues as demonstrated by − ΔCt = Ct GAPDH− Ct target gene.