RESEARCH ARTICLE

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SNHG1 Long Noncoding RNA is Potentially Up-Regulated in Colorectal Adenocarcinoma

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Abstract

Colorectal cancer (CRC) is one of the most common types of cancer worldwide. However, the molecular mechanisms involved in CRC initiation and progression is remained to be unknown. It seems that lncRNAs, as the main and lengthy functional transcripts of the genome, have important roles in different cancers such as CRC. CRC-related lncRNAs are reported to be involved in diverse molecular processes such as metastasis, invasion, cell proliferation, and apoptosis. This study was aimed to analyse the expression level of lncRNA *SNHG1* in colorectal adenocarcinoma and normal tissues. We performed an in silico analysis on a large cohort and confirmed the results by experimental analysis of clinical samples through real-time PCR. Our findings demonstrated that that *SNHG1* is potentially overexpressed in tumor tissues compared with adjacent normal tissues. The expression level of *SNHG1* was shown to be potentially associated with clinicopathological features of tumors. The current study suggests the potential role of *SNHG1* in colon cancer progression.

Keywords: *SNHG1*- RNA- long noncoding- gene expression- cancer- colorectal

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Introduction

Colorectal cancer (CRC) is one of the leading causes of cancer death in men and women totally. In spite of decline in incidence and improvement, there is an emergent need to early detection and treatment of CRC (Siegel et al., 2017; Torre et al., 2012). In Colorectal cancer, as a multi-step and multifactorial disease, it has been shown that gene expression plays a pivotal role in tumor progression (Birkenkamp-Demtroder et al., 2002).

Recent studies have shown that up to 90% of the genomic DNA are transcribed to a complicated network of transcripts. The major transcripts which are over 200 nt in length includes Long Non-coding RNAs (LncRNA) (Ponting et al., 2009). Also, it has been revealed that lncRNAs, as the major regulators of gene transcriptional system, are involved in the different biological process such as cell cycle regulation and cytoplasmic transport (Wang et al., 2011).

Much investigations have been focused on characterizing the role of lncRNAs in the prognosis of CRC (Han et al., 2015; Xie et al., 2016; Xu et al., 2014). For example Kogo et al., (2011) indicated that HOTAIR, as modulator of the cancer epigenome, promotes metastasis, recurrence, and prognosis of CRC.

In this study, we studied the expression level of *SNHG1* lncRNA (small nucleolar RNA host gene 1). Its gene is localized at 11q12.3 and has 11 exons. Aberrant expression of this lncRNA has been reported in some cancers such as non-small cell lung cancer (You et al., 2014), glioma (Wang et al., 2017), Lung squamous cell carcinoma (Zhang et al., 2017), neuroblastoma (Sahu et al., 2016) and etc. It seems that this lncRNA can function both in Cis and in trans to regulate transcription, promoting tumorigenesis and cancer progression (Sun et al., 2017). However, the role and mechanism of *SNHG1* in colorectal adenocarcinoma have not been completely understood.

In order to determine the potential clinical relevance of *SNHG1* in colorectal adenocarcinoma, we analyzed the expression pattern and dysregulation of this RNA by utilizing RNA-seq data and clinical information from a significant number of colorectal adenocarcinoma patients in The Cancer Genome Atlas (TCGA) and GEO datasets. The clinical validation demonstrated the potential role of *SNHG1* lncRNA in colon cancer. The data suggested that *SNHG1* could be a potential biomarker and potential therapeutic target for CRC.

Materials and Methods

In silico expression analysis of SNHG1 gene in large cohorts

In the current study, we considered differentially expression of *SNHG1* lncRNA using TCGA data from

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the Cancer Genome Browser database (https://genome-cancer.ucsc.edu/) in order to determine whether *SNHG1* lncRNA was associated with the progression of CRC. To evaluate this, we performed this type of analysis using two datasets including TCGA colon and rectum adenocarcinoma (COAD-READ) gene expression data assayed by RNA-seq (Illumina Hiseq). The expression level of *SNHG1* was compared between colorectal adenocarcinoma tissues (n=380) and adjacent normal tissues (n=50). Also, the Nexus expression database was checked for further analysis of *SNHG1* in colorectal tumors. Also, Kaplan-Meier survival analysis was used to explore the prognostic value of *SNHG1* through the TCGA database. The result shows that there was no significant difference in survival of CRC patients with higher and lower *SNHG1* expression. (P-value=0.9023) (Figure 4).

Validation study by clinical samples

To support the in silico analysis, we examined *SNHG1* expression level CRC tissues by qRT-PCR.

Clinical samples

A total of 40 samples comprising 20 colorectal adenoccarcinoma tumors and their normal 20 adjacent adenocarcinoma normal tissues were obtained from Iran National Tumor Bank. All of the tissue specimens were frozen in liquid nitrogen after collection and then stored in -80^{o}C until the extraction of Total RNA. The clinical and pathological parameters of these samples which were confirmed by pathologists are given in Table 1. The process of the study was approved by Shahid Chamran University of Ahvaz, Iran.

RNA Isolation and cDNA synthesis

Total RNA was extracted from tissue samples using the RNX™-plus reagent (Cinnagen, Iran) following manufacturer's instructions. The quality and quantity of extracted RNA were checked by gel electrophoresis and spectrophotometry respectively. The cDNA was generated using the reverse transcription Kit (Takara, Japan) according to the manufacturer's instructions. One microgram of Total RNA in each sample was reversely transcribed into cDNA by Reverse transcription Kit (Takara, Japan) in a total volume of 10 µl according to the manufacturer's protocols.

Real-time quantitative PCR

Real-time PCR analysis was performed in order to determine the relative expression level of target genes with the SYBR Premix ExTaq Kit (Takara, Japan) by the ABI Prism7500 system (Applied Biosystems, Foster City, CA, USA). All reactions were run in duplicate using *SNHG1* specific primer as target gene and *GAPDH* as the internal control to normalize the RNA levels for different sample. The reaction mixture consisted of cDNA (1µl), 5 µl of 2X SYBR Green I master mix (Takara, Japan) and 200 nM of each primer in a 10 µl reaction according to the manufacturer's instructions. The cycling conditions included an initial denaturation at 95^{o}C for 30s followed by 40 cycles of denaturation at 95^{o}C for 5sec, annealing/ extension at 60^{o}C for 34s. The specificity of the PCR products was examined by melting curve analysis, followed by gel electrophoresis. The Livak method (ΔΔCT) was used to analyze differential gene expression (Livak et al., 2001).Serially diluted cDNA was used to obtain a standard curve for each gene transcript. The primers sequence were as follows: for SNHG1 Forward: 5′- ACGTTGGAACCGAAGAGAGC -3′ and Reverse: 5′- GCAGCTGAATTCCCCAGGAT -3′and for *GAPDH* Forward: 5′- GTGAACCATGAGAAGTATGA-3′ Reverse: CATGAGTCCTTCCACGATAC-5′.The sequence of the *SNHG1* PCR product was confirmed by Sanger sequencing.

Statistical analysis

For in silico analysis of *SNHG1* expression in TCGA derived tissues, the statistical analysis was done by t-test through R-software integrated in cancer genome browser. the clinical study, Statistical analysis was done using the GraphPad Prism v6 software. Differences in RNA levels between the normal and tumor tissues of patients groups was assessed by paired Student's t-test. A P value < 0.05 was considered as significant.

Results

SNHG1 is up-regulated in TCGA colorectal tumor tissues compared to normal tissues

Normalized expression (Z score) derived from TCGA tissues of cancer genome browser was compared between groups of study. In TCGA (The Cancer Genome Atlas) data which applies RNA sequencing of large cohorts, we found that *SNHG1* is significantly up-regulated in CRC tissues compared with adjacent normal tissues (Figure 1).

Experimental study of SNHG1 expression in colorectal adenocarcinoma tumor and adjacent non-cancer tissues

We further examined *SNHG1* expression level in 40 tissues including 20 pairs of human CRC tissues and normal adjacent samples in order to investigate the

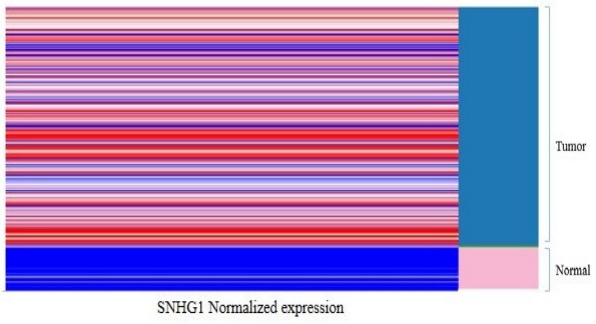


Figure 1. Heatmap Shows *SNHG1* Expression Profile in TCGA Database. TCGA colon and rectum adenocarcinoma (COADREAD) gene expression data grouped in primary tumors (380) and normal solid tissues (50) and represents a heatmap .The data heatmap shows up-regulation in Colorectal cancer tissues (shown a red subgroup in the column) in comparison to normal tissues (shown a green subgroup the right column).The normalized gene expression data assayed by RNA-seq (Illumina Hiseq) has been shown in red (Z>0) and blue color (Z<0) in this heatmap. Data is derived from the Cancer genome browser database.

Table 1. Clinical Characteristics of Colorectal Cancer Patients

Clinical parameters	Numbers
Samples	20
Gender	
Male	10
Female	10
Age	
<64	11
≥64	9
Tumor size	
<5	10
≥5	10
Tumor site	
Colon	12
Rectum	8
Grade of tumor	
Low Grade	19
High Grade	1
TNM stage	
Stage1-2	9
Stage3-4	11
Lymphatic invasion	
Yes	8
No	11
Weight Loss	
Yes	16
No	4

TNM, Tumor-Node-Metastasis

potential roles of *SNHG1* in human CRC development. As shown in the Figure 2, *SNHG1* was up-regulated in tumor tissues compared to their match normal tissues (P-Value<0.0064). The results potentially validated the results derived from our in silico analysis through cancer genome browser.

Correlation between SNHG1 expressions and patient's clinicopathological characteristics

We examined the correlation between *SNHG1* expression and clinicopathological characteristics in CRC samples to find out whether *SNHG1* was associated

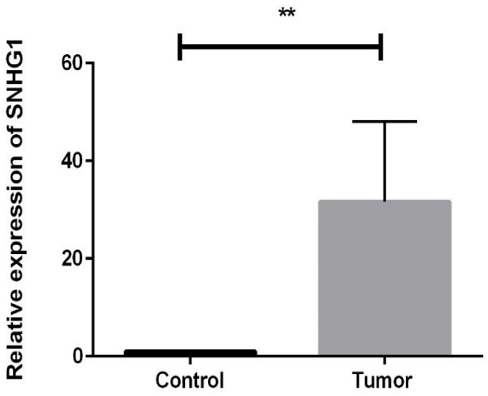


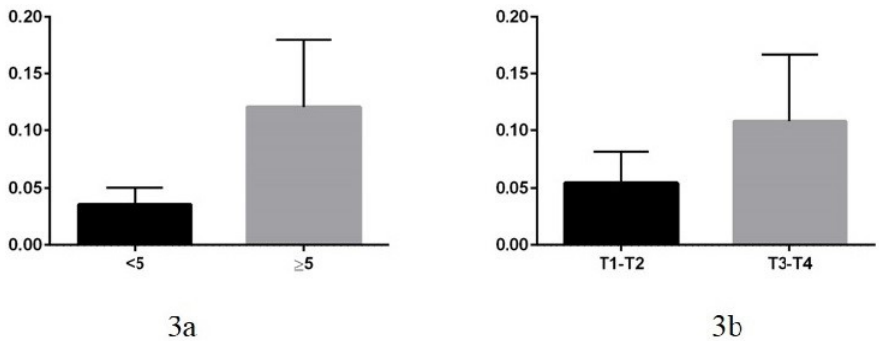
Figure 2. *SNHG1* Expression Analysis in Colorectal Cancer Tissues and Corresponding Adjacent Normal Tissues. *SNHG1* Real time-PCR expression data normalized to *GAPDH* expression as internal control. Paired t-test result showed the higher an internal of the *SNHG1* in colorectal cancer a higher tissues (P-value =0.0064). reported Data are presented as mean ± SEM.

with the progression of CRC. Our analysis showed up-regulation in tumors with TNM stages III-IV and larger tumors compared with tumors with TNM stage I-II and small tumors respectively(P-value=0.0429) (Figure 3). Despite non-significant difference, the data showed the potential role of *SNHG1* in cancer progression. Moreover, the *SNHG1* expression in tumor tissue samples with lymphatic invasion was compared to non-invasive ones that it shows there is a significant increase in gene expression in lymphatic invasive tissue samples (P-value=0.0054) (Figure 3c).

However, this hypothesis needs to be confirmed by more clinical and experimental studies.

Discussion

Although the incidence and mortality of the colorectal cancer declined for the last decades, it remains one of the most common type of malignant tumors (Siegel et al., 2017). High recurrence rate of CRC urged the identification of novel biomarkers for prognosis and therapies. Recent investigations have revealed that the expression of lncRNAs are dysregulated in a variety of cancers (Huarte et al., 2015; Hajjari et al., 2014; Qiu et al., 2013). Many recent and important discoveries showed a wide involvement of lncRNAs in gene-regulation,



3a 3b

Figure 3. Comparison of the *SNHG1* Expression between Colorectal Adenocarcinoma Tissues. (a), *SNHG1* expression in larger and small tumors (P-value=0.0092); (b), *SNHG1* expression in tumors with different TNM stages (P-value=0.0429)

transport, differentiation, dosage-compensation, and protein synthesis (Chen et al., 2010). The key regulatory function of lncRNAs proposed them as therapeutic targets or biomarkers. So, they could be used a new strategy in cancer early diagnosis. Different studies have reported that the expression of these RNAs are significantly changed in various types of human malignancies especially colon cancer (Gutschner et al., 2012; Prensner et al., 2011).Therefore, exploration of CRC-related lncRNAs and detection of their clinical importance and biological properties may accelerate the development of lncRNA-based diagnostic methods for CRC. Here, we sought the expression of the lncRNA termed small nucleolar RNA host gene1 (*SNHG1*) to find its potential role in colorectal cancer. For this purpose, we focused on the TCGA database and found a significant *SNHG1* overexpression in colorectal cancer tissues derived from TCGA large cohort database. Moreover, in order to validate in silico data, we quantified the expression levels of *SNHG1* lncRNA by Real-time PCR in tissue samples. Our results for *SNHG1* in CRC were consistent with our in silico data. In summary, this study suggests that *SNHG1* might play a key potential oncogenic role in colorectal cancer. We also showed that SNHG1 overexpression is potentially associated with advanced TNM stage. Our findings are consistent with the recent study showing the up-regulation of *SNHG1* expression in colorectal carcinoma (Sun et al., 2017). However, this is the first study highlighting the up-regulation of *SNHG1* in a large cohort of samples validated by experimental analysis on colorectal adenocarcinoma type tissues.

The association of aberrant expression of *SNHG1* in human tissues and cell lines with the pathogenesis of different cancers clarifies that it might be implicated in the complex process of tumorigenesis. Researches have shown that overexpression of *SNHG1* can promote progression of cancers (Liu et al., 2017; Wan et al., 2016). Recent studies show that Wnt/β-catenin signaling pathway is mediated by *SNHG1* overexpression in cancers (Cui et al., 2017; Wang et al., 2017). Recently, Sun et al., (2017) found that the expression levels of *SNHG1* in human lung and colorectal cancers were higher in patients and the expression level is positively correlated with the promotion of tumor cell growth. Interestingly, this study uncovered that *SNHG1* can act in cis and trans through diverse mechanisms to regulate gene expression .Their findings revealed that MYC is a key transcriptional factor that has been regulated in trans. It supports Sahu et al., (2016) finding which indicated that *SNHG1* is up-regulated by MYCN amplification in neuroblastoma. Also, *SNHG1* promotes the expression of its neighboring gene by cis-acting and induces the formation of chromatin looping of the *SNHG1-SLC3A2*. So, *SNHG1* interferes with the inactivation of the PI3K/AKT pathway by regulating *SLC3A2* gene (Sun et al., 2017). Sun et al., (2017) and Zhou et al., (2017) examined the expression level of *SNHG1* upon knockdown in different cell lines. They showed the importance of *SNHG1* expression in CRC. In this study, we studied the expression level of *SNHG1* lncRNA to reveal the importance of this gene in CRC patients and this is just as an experimental validation study in our country.

Some other studies on different types of cancers have proposed other mechanisms such as inhibiting apoptosis through *P53* target genes (Shen et al., 2017) and TAp63/ZEB1 pathway (Zhang et al., 2017), as well as cell cycle progression through suppression of miR-195 expression (Zhang et al., 2016). This microRNA blocks the G (1)/S transition through targeting cyclin D1, CDK6, and E2F3 (Xu et al., 2009). Also, some studies show that *SNHG1* may act through regulating of epigenetic state (Hu et al., 2017). Besides that, Tian et al., (2017) and Xu et al., (2018) reported *SNHG1* up-regulated in colorectal cell lines and the differential expression of *SNHG1* may indicate the potential role of this lncRNA in cancer initiation and progression. This study was aimed to highlight the potential role of *SNHG*1 in colorectal cancer. To our knowledge, this is the first experimental validation study highlighting the over-expression of *SNHG1* in colorectal cancer.

In conclusion, enhanced *SNHG1* expression was observed in colorectal adenocarcinoma tissues. These results indicate that *SNHG1* lncRNA is likely to be involved in the development of colorectal cancer. Thus, our data suggest *SNHG1* as a potential novel prognostic biomarker or therapeutic target for patients with colorectal adenocarcinoma. However, there is a need to examine this lncRNA a larger scale and long term follow up in order to confirm it. So, the current study can be a basis for further experiments on the exact functions of *SNHG1* a potential biomarker or therapeutic target in cancer-related pathways.

Acknowledgments

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Conflict of interest

None.

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