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Autumn 2021, Volume 6, Issue 23 (1-4)

Evaluation of LBX-2AS1 gene expression in patients with colorectal cancer

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DOI: 10.22034/pmj.2021.249034

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Submitted: 2021-08-01
Accepted: 2021-09-23

Keywords:
Colorectal Cancer
Long Non Coding RNA
Gene Expression
LBX2-AS1gene

Abstract:

Colorectal Cancer (CRC) is the third most common cancer in Iranian men and the fourth most common cancer in women. Recent studies have shown that lncRNAs may also engage in remodeling the tumor microenvironment and tumor metastasis. A lncRNA called LBX2 antisense RNA 1 (*LBX2-AS1*) has been reported to exert crucial regulatory actions in various cancer. In this study, we evaluate the expression of LBX2-AS1gene in 30 colorectal cancer tumor samples. Gene expression was assessed by Real-time PCR method and the results were analyzed by 2^-ΔΔct. relative expression of this gene in tumor samples compared to healthy samples showed a 1.4-fold increase in tumor samples. According to our findings in this study and the results of other studies, it can be concluded that this gene can be used as a therapeutic target.

INTRODUCTION

Colorectal cancer (CRC) is already the third leading cause of cancer death in the world, and its incidence is steadily rising in developing nations (1). Besides an ageing population and dietary habits of high-income countries, unfavorable risk factors such as obesity, lack of physical exercise, and smoking increase the risk of colorectal cancer. Excluding skin cancers, colorectal cancer is the third most common cancer diagnosed in both men and women in the United States (2). The American Cancer Society's estimates for the number of colorectal cancer cases in the United States for 2021 are: 104,270 new cases of colon cancer and 45,230 new cases of rectal cancer. Overall, the lifetime risk of developing colorectal cancer is: about 1 in 23 (4.3%) for men and 1 in 25 (4.0%) for women (3). A number of other factors (described in Colorectal Cancer Risk Factors) can also affect your risk for developing colorectal cancer. In the United States, colorectal cancer is the third leading cause of cancer-related deaths in men and in women, and the second most common cause of cancer deaths when men and women are combined. It's expected to cause about 52,980 deaths during 2021 (4).The National Cancer Registry Program in Iran (INCRS) was started in 2000, and its results have provided an important source of information for cancer studies in the country. This is pathology-based program with >85 % of the expected number of cancers being registered (5). According to the information obtained from this source, there is a significant difference in the incidence rates of CRC in different parts of Iran. According to the annual report of INCRS, CRC is the fourth common cancer in men after stomach, bladder, and prostate cancer and the second among women after breast cancer (besides melanoma) (6). Recent studies have shown a rapid rise in the incidence of colorectal cancer in Iran (7). In a study done about 35 years ago in Iran, the estimated annual incidence rate of CRC was 1.5–5.5/100,000 individuals. A research study conducted in 2008 reported an age-standardized incidence rates (ASR) of 7–8 (per 100,000) for both males and females and further stated that 5000 new cases of CRC (7 per 100,000 individuals) are annually reported from Iran . Of these 5000 cases, 2262 die annually because of CRC (8).

Approximately 5 to 10 percent of colon cancer is hereditary. The major hereditary colon cancer syndromes are Lynch syndrome (previously known as Hereditary Non-Polyposis Colorectal Cancer or HNPCC) and Familial Adenomatous Polyposis (FAP) (9). Other genes have also been implicated in hereditary colon cancer risk. The numerically most prominent group of genes that change expression during cancer progression of the colon encode proteins related to metabolism (22%), in particular, mitochondrial metabolism, followed by genes encoding proteins related to transcription and translation (11%), cellular

processes (9%) including cell cycle proteins and proteins involved in growth and differentiation, cell adhesion (8%), protein folding and degradation (7%), transport (6%), immune system (6%), and nucleic acid interaction (6%) (10). Remarkably, proteins related to apoptosis or signaling and signal transduction were only rarely altered. For some groups, it was remarkable that the alterations detected were mainly either up-regulation or down-regulation (11). In recent years, noncoding RNAs (ncRNAs) have been demonstrated to be involved in colon cancer development and progression. It is well known that ncRNAs belong to a class of transcripts that are mostly translated into proteins, but they also play important roles in a variety of cellular and physiologic processes (12). Long non-coding RNAs (LncRNAs) with a length longer than 200 nucleotides participates in multiple biological processes, including cell proliferation, differentiation, development, apoptosis and metastasis, often by serving as a competing endogenous RNA (ceRNA) to regulate the expression of specific miRNAs, and then target molecules downstream of these miRNAs (13). In fact, lncRNAs can interact with RNA, DNA and protein, and form RNA-RNA, RNA-DNA, RNA-protein complexes, leading to regulation of gene expression via multiple mechanisms, including modulation of transcription, mRNA stability and translation (14). LncRNAs can act as a guide, scaffolds or decoy molecule of proteins to recruit proteins or RNAs.

LBX2 antisense RNA 1 (LBX2-AS1), a novel lncRNA, exerts an oncogenic role in different malignancies. For instance, LBX2-AS1 is up-regulated in gastric cancer and silencing LBX2-AS1 restrains proliferative, migratory, and invasive capacities of gastric cancer cells by miR-491-5p/ZNF703, miR-219a-2-3p/FUS or miR-4766-5p/CXCL5 axis (15). High expression of LBX2-AS1 is also reported in ovarian cancer and its up-regulation is in relation to undesirable survival outcome (16). LBX2-AS1 may promote ovarian cancer progression through miR-455-5p/E2F2, miR-491-5p/E2F2 (17). and miR-4784/KDM5C axis. Furthermore, LBX2-AS1 that can be activated through ZEB1, accelerates migration and epithelial-mesenchymal transition in esophageal squamous cell carcinoma *via* interaction with HNRNPC, thereby stabilizing ZEB1 as well as ZEB2 (18). LBX2-AS1 up-regulation exhibits correlation to the staging, metastasis, and prognosis of hepatocellular carcinoma patients. It may drive hepatocellular carcinoma progression *via* miR-384/IRS1 pathway. Also, this lncRNA accelerates proliferation as well as metastases *via* Notch pathway in non-small cell lung cancer (19). Given the previous findings, LBX2-AS1 plays critical roles in carcinogenesis. In this study, we evaluate the expression of LBX2-AS1gene in 30 colorectal cancer tumor samples.

Materials and method

30 tumoral and Adjacent healthy tissue were recruited for the study where their paraffin embedded tissue blocks were retrieved from the repository of the Department of Pathology. RNA was extracted after deparaffinizing the samples using PureLink™ FFPE RNA Isolation Kit (Thermo scientific) and according to the product instructions. After examining the quality and quantity of the extracted RNA After examining the quality and quantity of the extracted RNA, cDNA was synthesized. Gene expression was assessed by Real-time PCR method and specific primer: F-AGTTTGTCCCAGGTTTGGCA and R-CATGCCAGG GTCCTTGTTCT. The results were analyzed by 2^-ΔΔct.

RESULTS

Data from genomic analysis obtained by high throughput methods show that a group of non-coding RNAs is associated with colorectal cancer. One of these is LBX2-AS1 lncRNA.

We evaluated the expression of LBX2-AS1 in colorectal cancer patients by qRT-PCR and found that LBX2-AS1 expression was higher in the CRC patients than in the normal colon samples. Relative expression of this gene in tumor samples compared to healthy samples showed a 1.4-fold increase in tumor samples.

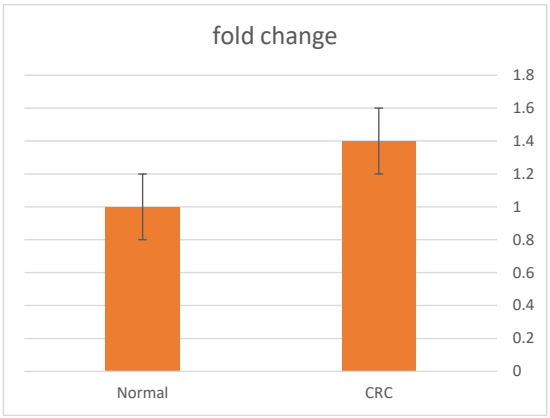

Fig.1 Relative expression of this gene in tumor samples compared to healthy samples showed a 1.4-fold increase in tumor samples.

DISCUSSION

Cancer is among the most life-threatening diseases, and its morbidity and mortality rank first or second among non-communicable diseases (1). According to global cancer data from 2018, cancer morbidity and mortality are increasing annually with the rapid growth of the population and the problem of the aging population. There are estimated 1.93 million new CRC cases diagnosed, and 0.94 million CRC caused deaths in 2020 worldwide, representing 10% of the global cancer incidence (total 19.29 million new cases) and 9.4% of all cancer caused deaths (total 9.96 million deaths). This cancer is the third most common cancer in Iranian men (standardized incidence: 8.1-8.3 per 100,000) and the fourth most common cancer in

women with a standardized incidence of 6.5 to 7.5 per 100,000. Cancer genes are defined operationally by their altered expression, leading to an abnormal phenotype in a significant subset of cancers. The altered expression may facilitate initiation or progression of a neoplasm, as oncogenes do, or may inhibit it, as do tumor suppressor genes. RNA-based therapeutics against cancer has gradually changed from concept to reality (20). Among these therapeutics, non-coding RNA (ncRNA), which refers to a class of RNA that does not encode protein, exerts clinical therapeutic effects against tumors by inhibiting the transcription of mRNA and binding to protein to block its function (21). According to the molecular size of ncRNA, it can be classified as either small non-coding RNA (sncRNA), measuring under 200 nucleotides in length, or long non-coding RNA (lncRNA), measuring over 200 nucleotides in length (22).

An increasing number of studies have documented that lncRNAs play diverse roles in regulating gene transcription, post-transcription, translation, and epigenetic modification. Aberrant expression or dysfunction of lncRNA is closely associated with various diseases (23). LncRNAs may regulate cell proliferation, apoptosis, migration, invasion and maintenance of stemness during cancer development (24). Recent studies have shown that lncRNAs may also engage in remodeling the tumor microenvironment and tumor metastasis. A lncRNA called LBX2 antisense RNA 1 (LBX2-AS1) has been reported to exert crucial regulatory actions in non–small cell lung cancer (25), esophageal squamous cell carcinoma [40], and gastric cancer. In this study, we evaluate the expression of LBX2-AS1gene in 30 colorectal cancer tumor samples. Relative expression of this gene in tumor samples compared to healthy samples showed a 1.4-fold increase in tumor samples. In another similar study, Qing Li et al. looked at the expression of LBX2-AS1 gene in colorectal cancer cell lines. Their results were similar to the results of our study(26). They reported that the expression of this gene in colorectal cancer cell lines was higher than in healthy cell lines. Hangzhi Gu et al. reported that the expression of LBX2-AS1 gene increases significantly in cell lines and tissue samples of ovarian cancer (27). Haitao Wen et al. showed that lncRNA LBX2-AS1 depletion inhibits the proliferation, migration, and invasion of glioma cells and increases apoptosis through the Akt/GSK3β pathway they reported lncRNA LBX2-AS1 is expected to become a new target for glioma therapy (28). It is suggested that this study be performed on a larger population, as well as changes in expression at the protein level by Western blotting. According to our findings in this study and the results of other studies, it can be concluded that this gene can be used as a therapeutic target.

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