Evaluation of lncRNA FOXD2-AS1 expression as a diagnostic biomarker in CRC

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Abstract

Background

Colorectal cancer (CRC) is still considered one of the prevalent cancers worldwide. Investigation of potential biomarkers for early detection of CRC is essential for the effective management of patients using therapeutic strategies. Considering that, this study was aimed to examine the changes in lncRNA FOXD2-AS1 expression through colorectal tumorigenesis.

Material and Methods

Fifty CRC tumor tissues and fifty adjacent normal tissue samples were prepared and involved in the current study. Total RNA was extracted from the samples and then reverse transcribed to complementary DNA. Next, the expression levels of lncRNA FOXD2-AS1 were evaluated using real-time PCR in CRC samples compared to normal ones. Also, receiver operating characteristic curve (ROC) analysis was used to evaluate the diagnostic value of FOXD2-AS1 for CRC.

Results

The obtained results showed that the expression level of FOXD2-AS1 gene was significantly ($p < 0.0001$) up-regulated in tumor tissues compared to normal marginal tissues. Also, a significant correlation was observed between higher the expression of FOXD2-AS1 and the differentiation of tumor cells. Furthermore, ROC curve analysis estimated an AUC value of 0.59 for FOXD2-AS1, suggesting its potential as a diagnostic target.

Conclusion

Taken together, the current study implied that tissue-specific upregulation of lncRNA FOXD2-AS1 might be appropriate diagnostic biomarkers for CRC. Nonetheless, m studies are needed to validate these results and further illustrate FOXD2-AS1 function through colorectal tumorigenesis.

Introduction

Colorectal cancer (CRC) is the third most prevalent malignancy in both sexes, worldwide, with the 5-year survival rate near to 10–15% for patients suffering a metastatic form of CRC. Therefore, it is necessary to clarify the potential mechanism involved in CRC metastasis, which presents diagnostic and potential goals for better management of CRC patients using current therapeutic approaches [1]. Early detection of CRC has been shown to increase the 5-year survival rates to 90% after removal of the local tumor, while detection in advanced and metastatic stages lowers it to 10% [2, 3]. Therefore, there is a constant need to is necessary to explore new therapeutic and diagnostic targets with high sensitivity and specificity [4].

Long non-coding RNAs (lncRNAs) which are defined as non-protein-coding transcripts with more than 200 nucleotides in length, play imperative roles in the regulation of multiple biological and pathological processes, including cancer development and metastasis [5, 6]. Several studies have shown that dysfunction/dysregulation of various lncRNAs is associated with abnormal cell proliferation, apoptosis, invasion, migration, and chemosensitivity [7–10].

In particular, recent studies have established the dysregulation of lncRNA FOXD2-AS1 through the development of multiple human cancers, including nasopharyngeal carcinoma (NC) [11], hepatocellular carcinoma (HCC) [12–15], gastric cancer (GC) [16], colorectal cancer (CRC) [1, 17], esophageal squamous cell carcinoma (ESCC) [18, 19], non-small cell lung cancer (NSCLC) [20, 21], breast cancer [22], glioma [23, 24] and so on. This lncRNA, with a transcript length of 2527 nucleotides, is located on chromosome 1p33 and functions as an oncogene through tumorigenesis [25]. It was shown that FOXD2-AS1 upregulation could lead to provoked tumor cell proliferation, apoptosis inhibition, and induction of migration and invasion through regulating the Wnt signaling [19], Notch [1], and PI3K / Akt pathways [26].

In addition, other studies have confirmed that increased expression of FOXD2-AS1 shows a significant correlation with poor outcomes and clinicopathologic characteristics in cancer patients. Nevertheless, the prognostic potential of FOXD2-AS1 expression in various human cancers, including CRC has not been clearly defined due to limited sample sizes and/or methodology. Then, characterization of FOXD2-AS1 expression patterns in larger sample sizes is important to completely understand its value as a therapeutic and diagnostic target for CRC therapy.

Materials And Methods

Study population:

In the present study, fifty samples of colorectal cancer tissues along with marginal normal tissue samples were prepared from patients diagnosed with a primary colorectal tumor in the Imam Reza Hospital (Tabriz University of Medical Sciences, Tabriz, Iran) without receiving any treatment during 2016 to 2018. The samples were immediately transferred into Qiagen RNAase inhibitor solution (Germany) and stored at -80°C. Table 1 represents the pathological information of included patients. All patients had given written informed consent and the study was approved by the local ethical committee of Tabriz University of Medical Sciences (TBZMED.REC.1394.356).

Table 1
General Characteristic of Study patients

Parameters	Classification	Number of Patients
Age	≤55	20
	>55	20
Sex	Female	18
	Male	22
Lymph node metastasis	positive	24
	negative	16
Tumor histology	Good	23
	Medium	13
	poor	4
Tumor depth	T2	3
	T3	9
	T4	28
Intravenous invasion	Positive	29
	negative	11
Stage (AJCC)	I, II, III	14
	IV, V	26
Hepatic metastasis	Positive	5
	negative	35
Tumor size	>3	3
	≤3	37

RNA extraction and cDNA synthesis:

Total RNA extraction from tissue samples was done using BioFACT (BioFACT TM, South Korea) following supplied instructions by the manufacture. After assessment of integrity, quality, and quantity of extracted RNA using by gel electrophoresis and Nanodrop OneC Spectrophotometer (Thermo Scientific™, USA), respectively, the reverse transcription of RNA (1000 ng) to complementary DNA (cDNA) was performed by using BioFACT cDNA synthesis kit (Korea) in T100 thermal cycler system (BioRAD).

qRT-PCR:

The quantitative real-time PCR (qRT-PCR) was used to assess the changes in FOXD2-AS1 gene expression in biological samples. The reactions were performed in the Applied Biosystems Step One™ qRT-PCR system (Foster City, CA, USA) using 2X Real-Time PCR Master Mix (BioFACT™, Seoul, South Korea). Each reaction was repeated three times in a total volume of 20 µl containing 10 µl master mix, 1 µl cDNA, 0.5 µl each of forward and reverse primers (4 pmol/µl) and 8 µl ddH₂O). To normalize FOXD2-AS1 expression levels, GAPDH was used as the internal control. The primer sequences and annealing temperatures are shown in Table 2. The condition for reactions was as follows: 15 min initial incubation at 95°C, followed by 45 amplification cycles of denaturation at 94°C for 10 sec, annealing at 60°C for 30 sec, and extension at 72°C for 20 sec.

Table 2
Characteristics of the primers used in the study

Target	Forward Primer (5'-3')	Reverse Primer (5'-3')	Amplicon Size (bp)	T _m (°C)
FOXD2-AS1	TGGACCTAGCTGCAGCTCCA	AGTTGAAGGTGCACACACTG	173	60

Statistical analysis:

All data were expressed as the mean ± standard errors and statistical analysis was done using Graph Pad Prism 6 (Graph Pad Software Inc. CA, USA). To evaluate the statistical significance of differences in FOXD2-AS1 expression levels between malignant and normal samples, Kolmogorov-Smirnov’s test was carried out. Also, the prognostic value of the target gene was evaluated using Receiver operating characteristic (ROC) curve analysis. Only p values less than 0.05 were regarded as statistically significant through this study.

Results

Up-regulation of FOXD2-AS1 in tumor tissues:

The obtained results from qRT-PCR showed that the expression level of FOXD2-AS1 gene was significantly ($p < 0.0001$) up-regulated in colorectal tumor tissues compared to normal marginal tissues, as illustrated in Fig. 1. Furthermore, FOXD2-AS1 upregulation was shown to be significantly correlated with tumor cell differentiation. As seen in Fig. 2, the expression levels of FOXD2-AS1 were higher in well and moderately differentiated tumors compared to poorly differentiated ones ($p < 0.0001$).

FOXD2-AS1 potential as a diagnostic biomarker

Considering the upregulation of FOXD2-AS1 in CRC samples compared to normal samples, its diagnostic potential was also evaluated. As shown in Fig. 3, ROC curve analysis estimated the area under the curve (AUC) near 0.59 ($p < 0.089$), which suggested that FOXD2-AS1 could be considered as a diagnostic target for screening of CRC patients.

Discussion

Considering that CRC is one of the leading causes of cancer-related death in the world, mostly due to poor prognosis and metastasis [27], identifying new diagnostic targets applicable to non-invasive methods, including liquid biopsy, is a constant need for more effective treatment of this disease [28]. Numerous findings have shown that lncRNAs acting as oncogenes or tumor suppressors through tumorigenesis of multiple human cancers possess great potential as the diagnostic and therapeutic targets [29]. Particularly, lncRNA FOXD2-AS1 has been recently identified as an oncogene that is up-regulated in various types of solid tumors, showing a negative correlation with the survival of patients [23, 26, 30]. More importantly, FOXD2-AS1 was previously reported to be upregulated in human CRC tissues and to be correlated with patients' poor survival and cancer progression. However, to confirm the reliability of these studies and further validate the significance of FOXD2-AS1 through colorectal tumorigenesis, there was a need for more investigation on a larger size of samples with diverse geogenetical patterns.

Subsequently, in the present study, the changes in expression levels of FOXD2-AS1 through colorectal tumorigenesis were investigated using qRT-PCR in Iranian patients, as a potential diagnostic target for CRC. The obtained results, in consistence with previous findings, showed that CRC tumors have increased expression levels of FOXD2-AS1 in comparison with normal tissue samples. Moreover, upregulated levels of FOXD2-AS1 were observed to be significantly and positively correlated with tumor differentiation in CRC patients; the higher the expression of this gene, the greater the differentiation of tumor cells. These findings proposed that FOXD2-AS1 may act as a potential oncogene that induces CRC progression. Also, ROC curve analysis illustrated that FOXD2-AS1 expression patterns could be a considerable target for the differentiation of CRC patients from healthy cases.

In line with the findings of this study showing the oncogenic role of FOXD2-AS1. Recently, Li et al reported that FOXD2-AS1 was also overexpressed in gastric tumors compared to normal gastric tissue samples, which showed a remarkable relationship with patients' prognosis [31]. Besides, Rong et al established that FOXD2-AS1 which functions as a tumor inducer via modulating WNT signaling pathway was also significantly up-regulated through lung cancer progression [19]. Furthermore, Ziwei Yang and colleagues showed that plasma levels of FOXD2-AS1 might be appropriate diagnostic biomarkers for gastric cancer [4]; further indicating the value of FOXD2-AS1 as a diagnostic and prognostic target for human cancers, including CRC.

Conclusion

In conclusion, the current study illustrated that FOXD2-AS1 is upregulated in CRC patients and its expression levels may be considered as a predictive target for cancer incidence and progression. Nonetheless, more investigations are needed to validate these results and further clarify FOXD2-AS1 significance in human cancers, including CRC.

Declarations

Ethics approval and consent to participate

This study was confirmed by Ethical Committee of Tabriz University of Medical Sciences (TBZMED.REC.1394.356) and written informed consent was obtained from all patients.

Consent for publication

Written informed consent was obtained from all patients.

Availability of data and material

All data in this article have been sent to the journal.

Conflict of interests

The authors declare that they have no competing interests.

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Authors' contributions

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(II) Administrative support: Sahar Safaie

(III) Provision of study materials or patients: Dariush Shanehbandi

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(V) Data analysis and interpretation: Milad Asadi

(VI) Manuscript writing: Hooman Shalmashi and Abdolreza Mehdi Navaz

(VII) Final approval of manuscript: All Author

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Figures

Figure 1

qRT-PCR results showing Increased mRNA expression levels of FOXD2-AS1 in CRC tissue samples compared to normal tissue samples; ****p<0.0001.

Figure 2

The correlation between FOXD2-AS1 expression and tumor differentiation in CRC; ****p<0.0001.

ROC of Data 1

Figure 3

ROC curve analysis; the accuracy of FOXD2-AS1 expression pattern as a diagnostic biomarker was evaluated.