Expression Analysis of Circulating miR-21 in Iranian Patients with Hereditary Colorectal Cancer

Original Article

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ABSTRACT

Background and objectives: Colorectal cancer (CRC) is one of the leading causes of cancer-related mortality in the world. MicroRNAs (miRNAs) have potential as diagnostic biomarkers for various diseases including cancer. This study was undertaken to investigate expression of miR-21 before and after surgery in patients with hereditary CRC.

Methods: After collecting blood samples from 39 patients and 39 healthy controls, total RNA was extracted by the TRIzol method. Following cDNA synthesis, expression of *miR-21* in serum of subjects was evaluated using real-time PCR, along with two reference genes, *let-7d* and *let-7g*. The real-time expression results and Ct values were collected and analyzed based on the 2-ΔΔct method.

Results: In spite of tumor removal, serum miR-21 expression levels was significantly higher in hereditary CRC patients compared with controls (P=0.022).

Conclusion: Our results confirmed that samples from hereditary cases of CRC must not be included in experiments on the diagnostic potential of miRNAs.

Keywords: Colorectal cancer, Gene Expression, Real-Time Polymerase Chain Reaction.

INTRODUCTION

Colorectal cancer (CRC) is characterized as a tumor growth on the inner lining of the rectum or colon (1,2). It is the third most common cancer among both men and women (3). Growing westernized diet have led to an increase in the incidence and mortality of CRC (4). Indeed, CRC is complicated by the difficulty of early diagnosis, due to the lack of reliable cancer-specific diagnostic biomarkers (5). MicroRNAs (miRNAs) are a class of non-coding RNAs of nearly 22 bases in length that play crucial roles in both physiological and pathological processes. In addition, they are involved in cancer pathogenesis from initiation to metastasis, primarily through interaction with the 3' of target mRNA, which leads to post-transcriptional inhibition or mRNA degradation (6). In this respect, growing body of evidence have indicated that certain miRNAs released from tumor cells are chemically stable and can be detected in a broad range of body fluids, including plasma, which makes quick diagnosis feasible (7,8). Therefore, miRNAs have emerged as promising novel biomarkers for CRC diagnosis. So far, hundreds of miRNAs have been reported to be associated with CRC progression and metastasis (9). A large number of studies have been performed to investigate the expression level of circulating miR-21 in blood samples of cancer patients and healthy individuals. However, results of the studies on the diagnostic value of circulating miR-21 have been inconsistent (10,11). Here, we intended to investigate the expression level of mir-21 in Iranian patients with hereditary CRC and healthy individuals.
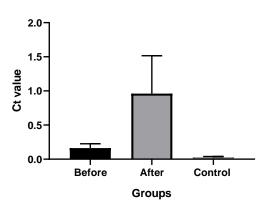
MATERIALS AND METHODS

A total of 39 whole blood samples were collected from CRC patients before and after surgical intervention at Qaem hospital in Mashhad, Iran. Age- and gender-matched healthy individuals (39 controls) with no history of cancer were also enrolled in the study. The study was performed between March 2018 and September 2019 in the Department of Biotechnology at Golestan University of Medical Sciences, Gorgan, Iran. Ethical approval was obtained from the Ethics Committee of Golestan University of Medical Sciences. In addition, an informed written consent form was obtained from all participants. First, sera were separated from the blood samples by centrifugation at 12,000 g for 10 min at 4 ◦C. Then, miRNA extraction was carried out using RNx-PLUS kit (Sinagene Company) according to the manufacturer's protocol. The purity and quality of the extracted RNAs was checked and samples with absorbance of 1.8-2 at 260/280 nm were used. Moreover, the concentration of the extracted RNAs was assessed using a bio-photometer (Eppendorf, Germany), which produced an average concentration of 110 ng/µl. Finally, real-time PCR was performed using specific primers for the miR-21. Internal control (*let-7d* and *let-7g*) was used due to the relativity of the method. For the real-time PCR reaction, 10 µl SYBER Green master mix, 5 µl of the synthetized cDNA and 4 µl specific primers were mixed in a total volume of 20 µl. The reaction was carried out using the ABI 7300 model (Applied Biosystems) at optimal temperatures. Next, miR-21 expression level was calculated based on the ΔΔCT method. Data were analyzed using SPSS statistical software (version 22) and Graph Pad.

RESULTS

Based on the results, miR-21 was overexpressed in in postoperative serum samples from CRC patients who underwent curative surgery (P=0.022) (Table 1). The obtained Ct values from the real-time expression analysis indicated that miR-21 level increased almost 4.5-fold in sera of patients after tumor removal (Figure 1)

Table 1- Expression analysis of miR-21 after tumor removal

Gene	Type	Reaction efficiency	Expression	Std.error	95% c.i.	P(h1)	Result
miR-21	**Target**	**1.0**	**4.683**	**0.566 - 41.907**	**0.096 - 378.272**	**0.022**	**up**
let7-D	**Reference**	**1.0**	**0.772**				
let7-G	**Reference**	**1.0**	**1.296**				

Figure 1- Ct values of real-time PCR experiment for hereditary CRC patients and healthy controls

DISCUSSION

In this study, we aimed to explore the association between miRNA expression patterns and the diagnostic outcome of hereditary CRC. Since the beginning of the new century, the link between miRNAs and human diseases, particularly cancer, has been established. Although most miRNAs are intracellular, a large number of miRNAs have been detected outside cells in various body fluids. They are stable in cell culture supernatants and in many body fluids such as blood, urine, saliva and milk (12).

Results from recent studies indicate that circulating miRNAs could serve as potential diagnostic and prognostic markers in different types of cancers and other diseases. Additionally, early and timely diagnosis of cancer is one of the main objectives of today's cancer research projects worldwide. Thus, there has been a need for non-invasive biomarkers that would improve the early detection of different types of cancer including CRC (13, 14).

Furthermore, the expression pattern of miRNAs in the circulation could be relevant to the risk of disease progression in CRC.

However, inclusion criteria for evaluating miRNA expression from patients with hereditary cancer have long been considered to use patients without diabetes, metabolic diseases and hereditary cancer. Moreover, cases with history of medications, radiation therapy or even smoking have been excluded from the miRNA expression analysis studies (15).

CONCLUSION

Our study verified previous results concerning the exclusion of samples from patients with hereditary cancer when investigating diagnostic potential of miRNAs expression in cancer studies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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