Summary of the paper:

**Introduction**

Colorectal cancer (CRC) is the third leading cause of cancer accounting for 10% of cancer deaths. The increase in colorectal cancer rate is an important issue concerning people all over the world; it is a new type of cancer with 1/2 million cases and responsible for 630,000 cancer deaths (about 8% of cancer deaths per year). It is known as the fourth most common cancer type in men and the third most common cancer type in women. Asia is the most popular continent in this regard with 60% of the world's population; CRC rate is rising rapidly in this continent. Its rate has also increased in the Middle East and Iran in recent years. East Azerbaijan [located in North West of Iran with a population recently reported to be 3,724,620 including 1,882,031 males (50.9%) and 1,842,589 females (49.1%) ] has a high incidence of cancer in gastrointestinal tract. Therefore, knowing more about the biology and nature of colorectal cancer, it is possible to design effective prognostic, diagnostic, and treatment plans to help reduce the rate of this disease.

**Materials and Methods**

A total of 40 CRC samples and normal adjacent tissues were collected from 40 patients diagnosed with CRC (i.e., 18 males and 22 females) following colonoscopy and sigmoidoscopy at Imam Reza Hospital (Tabriz, Iran), the first affiliated hospital of Tabriz University of Medical Sciences. The non-tumor counterparts were obtained from a section of the resected specimens at the farthest distance from the tumor (>2 cm from tumor). All study participants were born in East Azerbaijan, Iran. The study was approved by the Research ethics Committee of Imam Reza Hospital in accordance with institutional protocol and informed consents were obtained from all patients. Resected specimens were routinely processed for histopathological assessment. A total of 48 samples were collected and 8 cases were excluded from subsequent statistical analysis for the following reasons: they had a previous malignancy and/or had undergone chemotherapy and radiation therapy.
Sample preparation and RNA isolation

Phenol based RNA extraction using TRIzol reagent (Invitrogen Carlsbad, CA) was applied according to the manufacturer's instructions. Briefly, 1 mL TRIzol LS reagent was added into homogenized tissue sample. Next, 200 μL chloroform was added, and the mixture was incubated for 2-15 minutes at room temperature. Then, a centrifugation at 12,000 rpm was followed. The aqueous phase was transferred into a new Eppendorf tube and 500 μL of 100% isopropanol was added into it. The supernant was removed, and 1 mL of 75% ethanol was added to wash the RNA pellet. The sample was then centrifuged at 7500 rpm. The supernant was eliminated and the RNA pellet was air dried. Of RNase-free water 25 μL were added into the RNA pellet. The concentration of isolated RNA was quantified by picoDrop 2000 [Bob Batty International (BBI), UK]. The extracted RNAs were stored at -80°C until cDNA synthesis. To degrade any DNA contamination in extracted RNAs, the researchers performed a 10 μL DNase I treatment reaction (Fermentas, Canada).

Reverse transcription and quantitative Real-time polymerase chain reaction

Real-time PCR was performed using SYBR® Green (purchased from Parsegen). Of diluted RT 4 μL product was added into a 10 μL PCR reaction, which also contained 10 μL SYBR Green, 1 μL primer mix (purchased from Parsegen), and 1 μL RNase-free water. MiR-383 and 5s rRNA (as a control RNA) primers were also purchased from Parsegen. All PCR reactions, including non-template controls, were run in triplicate using Rotor-Gene Q - QIAGEN Real-time PCR Detection System. Finally, the raw data were analyzed by the REST2009 Software. All samples were processed in triplicate. The threshold cycle (Ct) was defined as the cycle number at which the fluorescence passed the fixed threshold. A control without a template was included in each experiment. The final products of real-time PCR were confirmed by Polyacrylamide gel electrophoresis.

Statistical analysis

The relative expression analysis of miR-383 was performed by a randomization test using the Relative Expression Software Tool (REST) 2009. The 2-ΔΔCt method was employed to analyze the expression levels of miR-383 in CRC tissues relative to their matched non-tumor counterparts. The Ct of fluorescence for each sample was determined. ΔCt indicated the difference in expression levels with the Ct value between miR-383 and 5s rRNA (ΔCt= Ct miR-383 - Ct 5s). ΔΔCt indicated the difference in the ΔCt value between cancer tissue and the matched control (ΔΔCt=ΔCt cancer-ΔCt control). The 2-ΔΔCt value (fold value) was also calculated. It was found that when the fold value was <1, there was a low expression of miR-383 in the cancer tissues compared to their non-tumorous counterparts. The fold change less than one in expression was defined as decreased expression. All the analyses were performed with SPSS 18.0 software (Chicago, IL, USA). All cited P-values were two-sided and P<0.05 were judged as statistically significant. Receiver operating characteristic (ROC) curve was also constructed to evaluate the specificity and sensitivity of predicting CRCs and normal tissues by miR-383 expression levels. Moreover, the sensitivity/specificity at various cut off values was calculated using Sigma Plot 12.5, P<0.05 were considered to indicate a statistically significant difference.

Expression levels of miR-383 in colorectal cancer and normal tissues

To further compare the overall level of miR-383 expression in colorectal cancer linked to normal tissues, the researchers entered the Ct values of all the samples into the REST 2009 software. The results of the randomization test showed that miR-383 expression in tumor samples decreased 8.9 times more than normal tissues (Figure 1).

The association between the expression of miR-383 and clinicopathological characteristics

With regard to clinicopathological characteristics, no significant relationship was observed between the miR-383 expression and gender (P=0.655), age (P=0.408), histological grade (P=0.103), and tumor location (P=0.894) (Table 1).

Capability of miR-383 to function as a colorectal cancer tumor marker

ROC curve was constructed and the area under the curve (AROC) was calculated to evaluate the specificity and sensitivity of predicting CRCs and normal tissues by miR-383 expression levels. Based on the analysis of ROC curves, miR-383 showed an AROC of 70%. The expression of miR-383 with a value of 0.70, compared with 1.0, revealed that this miRNA has a high sensitivity and specificity; thus it can detect tumor samples compared to non-tumor ones in colorectal cancer, and consequently it can be considered as a tumor marker (Figure 2).

![Figure 1](image1.png)

Figure 1. Differential Expression of miR-383. miR-383 relative expression in tumor samples showed a significant decrease (8.9 times) compared to normal samples with confidence interval of 95%, P=0.0.

![Figure 2](image2.png)

Figure 2. Receiver operating characteristic (ROC) for biomarker in detection of colorectal cancer. The ROC curve was automatically generated from 40 points of cutoff values set by the software SigmaPlot. The area under the ROC curve is 0.70 out of 1, with 68 and 75% sensitivity and specificity respectively.
Discussion

Recently, a correlation between miRNAs function and colorectal cancer pathogenesis is supported by a number of molecular studies on the expression of miRNAs in clinical samples. Thereafter, investigation of miRNAs would be useful to expand our insight to better understand colorectal carcinogenesis as a potential tumor oncogene by analyzing associated mRNA targets and miRNA-mediated pathways.\(^{21,22}\) There are many reports on the effects of miRNAs on oncogenes and tumor suppressor genes.\(^{23}\)

One study showed that down regulation of miR-383 was correlated with enhanced proliferative activity of germ cells in maturation arrest patients.\(^{24}\) MiR-383 prevented tumor suppressor, interferon regulatory factor-1 (IRF1), and reduced target levels of IRF1, cyclin D1, CDK2 and p21.\(^{24}\) Moreover, it is reported that expression of miR-383 was downregulated in patients with non-obstructive azoospermia.\(^{24,26}\) In another research, it was found that miR-383 strictly targeted insulin-like growth factor 1 receptor (IGF1R). Downregulation of miR-383 promotes glioma cell invasion by targeting IGF1R; it also results in the activation of AKT signaling following upregulation of matrix metalloproteinase-2.\(^{27}\)

As a likely target of miR-383, growth arrest and DNA-damage-inducible protein GADD45 gamma can induce apoptosis and inhibit cell growth in response to stress shock.\(^{28}\) A recent study, also, showed that miR-383 expression was decreased in hepatocellular carcinoma (HCC). It was also reported that this miR is associated with tumor progression and prognosis of HCC patients. Furthermore, miR-383 inhibits hepatocellular carcinoma cell proliferation via targeting APRIL (13th member of the tumor necrosis factor). It has also been revealed by the recent studies that low levels of miR-383 were correlated with high levels of APRIL expression in clinical HCC samples.\(^{16}\)

Overall, the conclusions obtained from the recent studies show that miR-383 has a critical role in cell cycle regulation, proliferation, and apoptosis in cancer. However, its functional and regulatory roles in all human cancers have not been clearly elucidated yet.

Conclusions

In summary, as far as the authors are concerned, this study is the first report on the expression patterns of miR-383 in CRC tissues. The results of this study indicated that miR-383 has significantly downregulated in CRC samples compared to normal counterparts. The capability of miR-383 expression level to function as a tumor marker to distinguish CRCs from normal counterparts was also assessed in the present study suggesting that this miR has a high sensitivity and specificity; therefore, it can be regarded as a tumor marker in diagnosing CRC. However, further studies should be conducted with numerous sample sizes to find the relationship between this miRNA and clinicopathological features. The researchers of the present study believe that it is just the beginning of a long and still unknown path of highly promising investigation of miR-383.

Table 1. Relationships between miR-383 expression levels in cancer tissue samples from patients with colorectal cancer and clinicopathological characteristics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>N.</th>
<th>miR-383 relevant expression (2-ΔΔCt)</th>
<th>P</th>
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<tbody>
<tr>
<td>Gender</td>
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<tr>
<td>Male</td>
<td>18</td>
<td>21.2283±4.28057</td>
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<tr>
<td>Female</td>
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<td>22.901±5.76414</td>
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<td>Age</td>
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<td>&lt;6</td>
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<td>22.1188±6.05476</td>
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<td>≥60</td>
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<td>21.9874±4.53612</td>
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<td>Histological grade</td>
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<td>Tumor location</td>
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<td>Rectum</td>
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<td>22.0059±4.9004</td>
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Data presented as mean±standard deviation; P values obtained using ANOVA test. No significant relationship was found between miR-383 expression levels and clinicopathological features.

References


