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# MicroRNA expression patterns of tumors in early-onset colorectal cancer patients

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ARTICLE INFO

*Article history:*
Received 29 November 2013
Received in revised form
27 February 2014
Accepted 18 March 2014
Available online 24 March 2014

*Keywords:*
miR-106a
miR-125b
miR-143
Colorectal cancer
Early onset

ABSTRACT

*Background:* The expression of microRNAs (miRNAs) may differ in tumors from patients with different ethnic origins and ages. The aims of the present study were to clarify the appropriate alterations of miRNA expression associated with the early stages of carcinogenesis in early-onset Turkish colorectal cancer (CRC) patients and to define specific biomarkers that could be used as new diagnostic and prognostic markers for this population.
*Materials and methods:* The expression profiles of 38 different miRNAs associated with CRC were evaluated using miRNA polymerase chain reaction arrays in tumors and surgical margin tissue samples from 40 sporadic early-onset Turkish CRC patients. The relationships between the miRNA expression profiles and the characteristics of the tumors and patients were evaluated.
*Results:* The expression of miR-106a was found to be upregulated, and miR-143 and miR-125b levels were found to be downregulated in tumor tissues compared with the normal tissues. The high expression level of miR-106a (2.93-fold; $P = 0.031$) and low expression level of miR-125b (2.42-fold; $P = 0.063$) were observed in tumors with lymph node metastases compared with the normal colorectal mucosa samples. However, the deregulation of these miRNAs was not significantly associated with survival (log-rank $P > 0.05$).
*Conclusions:* The present results implied that miR-106a and the miR-125b were associated with the formation and invasion of colorectal tumors. Thus, these miRNAs might be used as significant prognostic factors and indicators of early-stage CRC. Further studies and validations are required; these miRNAs may provide novel molecular targets for CRC treatment.



## 1. Introduction

Colorectal cancer (CRC) is the third most common cancer in the world. Despite progress in its diagnosis and treatment, the overall 5-y survival rate is 40%, and approximately, half of the patients die due to the development of distant metastases [1]. To predict the outcome of CRC cases, the most common technique is to evaluate tumor node metastasis (TNM) status. Up to now, there have been significant advances in our understanding of the natural history, etiology, and molecular

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http://dx.doi.org/10.1016/j.jss.2014.03.057

mechanisms underlying CRC development [2]. Accordingly, many tumor markers have been identified [3,4]. However, none of these markers has been shown to have a significantly improved prognostic value for cancer recurrence compared with the tumor stage. Therefore, there is a need to develop better classifiers to differentiate these cases to decide who would benefit from therapy [5].

Most research on CRC has focused on the genetic and epigenetic changes in the protein-coding genes to determine their role in CRC initiation and progression [5]. Recently, microRNAs (miRNAs), which are involved in the regulation of cancer-modulating genes in many cancer types, have also come into focus to better understand the molecular biology of CRC [6]. To date, nearly 200 miRNAs have been found to be deregulated in CRC [6,7]. Therefore, aberrant expression of miRNAs might be potential diagnostic and prognostic biomarkers for cancers [6]. The expression patterns of miRNAs may differ in tumors from patients with different ethnic origins [8]. Although there a few studies that focused on miRNA alterations in CRC in different population [9,10], no study has examined the miRNA expression patterns and their potential use as early markers for CRC in the Turkish population yet. Furthermore, colorectal tumors in younger patients are characterized by more advanced tumor stages and worse prognoses than those in older patients [11]. These tumors are possessed by different molecular markers [12] and varied miRNA expression profiles [9]. We believe that it had better to evaluate miRNA expression pattern of tumors separately in early- and advanced-onset patients to ensure group homogeneity, and so more reliable data could be obtained. However, there are few studies clarifying the role of miRNA regulation in specifically early-onset CRC development [13].

Evidence supports a role for miRNA at every stage of CRC initiation, progression, and development [14]. To improve our knowledge of miRNA regulation in CRC, recent studies have examined the possibility of using miRNA expression patterns as early detection biomarkers for this disease [5]. Therefore, the aim of this study was to clarify the relevant alterations in miRNA expression that are associated with recurrence and metastases in the early-stage of CRC in early-onset Turkish patients.

## 2. Materials and methods

### 2.1. Patient selection and outcomes

The CRC archive database at the Uludag University, Medical Faculty, Department of General Surgery, was used to collect the clinical information and follow-up data of these CRC patients. Basic demographic, clinical, and tumor characteristics were analyzed. The assessment of the tumor grade was performed according to the World Health Organization criteria by an academic surgical pathologist with subspecialty expertise in gastrointestinal pathology. The parameters obtained from the medical records of the 40 patients included age, tumor location, lymph node metastases (LNM) (evaluated by optical microscopy), pathologic stage (assessed by the TNMs classification), tumor histologic grade, and the presence of vascular invasion in the tumors. Tumor samples from all stages were evaluated to identify biomarkers related with invasion and metastasis. Only CRC patients who were <50 y old were included to obtain a homogenous group. All patients were considered sporadic cases. Ten patients with rectal cancer were defined as tumors located between 12 and 16 cm from the anal verge. These patients were considered upper rectum cancer. For stage II (T2N0M0) CRC, surgery is usually the only therapy that is necessary, although chemotherapy is not standard treatment for stage II and upper rectum cancer. Therefore, selected patients did not receive preoperative chemotherapy and/or radiation. Thus, the untreated study cohort let avoiding the confounding influence of the treatment on the tumor composition and clinical outcomes. Forty patients with primary CRC were enrolled in this study. Informed consent was obtained from all the alive patients. The study was approved by the local Ethics Committee (2011-2/7) and conformed to the ethical standards of the Helsinki Declaration. The study endpoint was disease recurrence. Only patients with recurrent disease or those without recurrence and at least 2 y of follow-up were included. Time to recurrence or disease-free interval was defined as the time from the date of surgery to the date of confirmed tumor relapse for the recurrent patients and from the date of surgery to the date of the last follow-up for the disease-free patients.

### 2.2. RNA extraction

Small RNA-enriched total RNA was extracted from 40 tumor and eight nontumor formalin-fixed paraffin-embedded tissues from CRC patients using the miRNeasy Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's protocol. The nucleic acid concentration and purity were determined using a Nanodrop 2000 UV spectrophotometer (Thermo Scientific, USA); an A260:A280 ratio >2.0 and an A260:A230 ratio >1.8 were used as cutoffs.

### 2.3. Real-time polymerase chain reaction–based miRNA expression profiling

Complementary DNA was synthesized from 5 ng of small RNA-enriched total RNA using the $RT^2$ miRNA First Strand Kit (QIAGEN, Germantown, MD). The samples were analyzed for the presence and differential expression of 38 miRNAs that have been previously related to CRC formation using custom RT2 miRNA polymerase chain reaction (PCR) arrays (RT2 Profiler; SABiosciences, Frederick, MD) according to the manufacturer's instructions using a LightCycler 480II (Roche Diagnostics, Wilmington, DE). The accession numbers of the primers are shown in Table 1. The RNA input was normalized to the endogenous control, which included SNORD 44, SNORD 47, and SNORD 48 for the miRNAs and TATA-binding protein for the protein-encoding genes. The initial copy number in the samples and the threshold cycle (Ct) for miRNA expression were determined using the Light Cycler 480II software (Roche Diagnostics). The average Ct value of up to three housekeeping genes from this assay was used as a baseline to normalize the PCR array data. The miRNA reverse transcription control assay was used to test the efficiency of the miScript II Reverse Transcription Kit reaction by detecting the synthesis of the kit's built-in miRNA external RNA control.