Clinics and Research in Hepatology and Gastroenterology (2015) 39, 508—515

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ORIGINAL ARTICLE

Evaluation of microRNAs-29a, 92a and 145 in colorectal carcinoma as candidate diagnostic markers: An Egyptian pilot study

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Available online 23 February 2015

Summary
Background: Colorectal cancer (CRC) is one of the most common malignant neoplasms in Egypt, and interestingly in young age. Adenomatous polyps and inflammatory bowel diseases (IBD) are considered the commonest pre-malignant lesions for CRC. A possible diagnostic role for different microRNAs on CRC has been suggested by numerous studies.
Aim of work: To assess the serum expression of 3 microRNA markers (miR-29a, miR-92a and miR-145) in pre-malignant and malignant colorectal lesions.
Patients and methods: The 60 patients studied were divided into 4groups: CRC group (25patients), IBD group (11patients), adenomatous polyps group (14 patients) and control group (10 patients). The serum expression of the 3 markers (miR-29a, miR-92a and miR-145) has been assessed by RT-PCR.
Results: All CRCs were sporadic cases. Significant downregulation of miR-145 in CRC group was reported at all levels, i.e. when compared to normal, among the 3 studied groups, and when compared between CRC and non-CRC groups. Significant upregulation of miR-29a in CRC was reported when compared to normal, but no significant difference existed either among the 3 studied groups or between CRC and the other 2 groups. All 3 miRNAs studied were positively inter-correlated.
Conclusions: miR-145 may be considered a promising non-invasive reliable diagnostic marker in CRC. Extended studies are needed to ascertain the diagnostic role of miRNAs in CRC.

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http://dx.doi.org/10.1016/j.clinre.2014.12.008
2210-7401/

Introduction

Colorectal cancer (CRC) constitutes the second in females, and the third in males regarding most commonly diagnosed cancers, with $> 6 \times 10^5$ deaths in 2008 [1]. In Egypt, it was diagnosed in 29—31% of patients aged ≤ 40 years, and was detected in 11—15% of colonoscopies [2]. MicroRNAs (miRNAs) are small, non-coding RNAs. They are expressed endogenously, and regulate gene expression post-transcriptionally [3]. Their mis-expressions or mutations are involved in many human cancers, indicating their function as oncogenes or tumour suppressors. Therefore, they might be applied in the diagnosis and treatment of cancer as well [4,5]. Also, they play role in oncogenesis via either pro- or anti-apoptosis, plus their coordination with/without synergism with many other deregulated miRs [6]. Multiple miRNAs are dysregulated in CRC, and have been correlated with biogenesis of tumor growth and progression [7]. They are considered potential biomarkers for CRC diagnosis, prognosis and drug-response prediction [8]. miR-29a promoted CRC and its metastasis by regulating matrix metalloproteinase and E-cadherin, through its direct targeting KLF4 [9], the identified tumour suppressor gene involved in cell proliferation, migration and invasion [10]. Similarly, miR-92a, which was upregulated in CRC, stimulated epithelial to mesenchymal transition, with negative influence on phosphatase and tensin homologue lucoferase activity [11]. On the contrary, miRNA-145 was downregulated in most primary CRC, particularly the aggressive forms, via targeting fascin-1 [12], elementary in neoangiogenesis [13]. On the other hand, miR-145 is stimulated by the main tumour suppressor gene, *p53*, in response to DNA damage, involved in its post-transcriptional pathway [14]. Accordingly, they may reflect the therapeutic outcome of CRC including its recurrence, and metastasis [15].

Patients and methods

The current study was a cross-sectional study applied on adult patients who attended the Gastrointestinal Endoscopy Unit in Kasr AL-Ainy Hospital, Cairo University, referred to colonoscopy for:

- variable colonic symptoms, including alarming symptoms of CRC;
- screening for CRC.

Exclusion criteria include:

- patients previously received chemotherapy or hormonal therapy for CRC;
- recent diagnosis of IBD (< 8 years);
- patients having cancer at any other site;
- hyperplastic and inflammatory polyps.

Patients were divided into 4 groups according to the diagnosed disease:

- group I: CRC;
- group II: adenomatous polyps;
- group III: IBD;
- group IV: others, with negative colonoscopic examination, which served as a control group.

All patients were recruited after a written informed consent and the study protocol was approved by the Ethics Review Committee of Kasr Al-Ainy, Cairo University Hospital.

All enrolled patients were subjected to:

- full history taking and thorough clinical examination;
- laboratory investigations:
  - stool analysis, and fecal occult blood testing (FOBT) for those who had no overt blood in stools,
  - complete blood count (CBC), and erythrocyte sedimentation rate,
  - liver biochemical profile,
  - tumor marker: carcinoembryonic antigen (CEA);
- colonoscopy by Pentax®, model number (EC 3840 L) $\pm$ biopsies;
- imaging: Abdominal ultrasound, and computed tomography, to assess the staging of CRC by applying American Joint Committee on Cancer (AJCC) [16];
- special test of the study:
  - RNA extraction and amplification: 8 mL of blood are collected in EDTA tube for centrifugation once sera are separated, then followed immediately by RNA extraction (the concentration was measured by nanodrop). We used miRneasy mini kit for miRNA extraction, miScript RT II for miRNA reverse transcription, and miSCript Primer Assay and miSCript SYBR Green PCR Kit for PCR amplification,
  - specific MicroRNA assay: the sequences of the selected miRNAs were done by using ready-made primers and pre-designed by miSCript system, Qiagen® Gmbh and they have their patent for all primer sequences. The housekeeping primer (*HK* gene) was SNORD68, with all rights reserved by the company, such that hsa-miR-29a, hsa-miR-92a, and and hsa-miR-145 are ID of miRNAs-29a, 92a and 145, respectively.

Delta CT analysis ($2^{-\Delta\Delta Ct}$) method was used, so the results were expressed as fold change compared to the control sample, and not expressed as absolute value. Control value, considered the normal value, was assumed equaled 1.

Statistical analysis

Quantitative variables were expressed by mean and standard deviation (SD) or expressed by median and inter quartile range (IQR) whenever values are not evenly expressed, for non-parametric data. They were compared by *t*-student or ANOVA test when appropriate. Qualitative variables were compared by Chi^2 or Fischer's exact test when appropriate. Receiver operator characteristic (ROC) curves were constructed to assess the value of MiRNA in diagnosis of CRC and to assess area under the curve (AUC). AUC < 0.60 is considered unreliable discriminator in diagnosing CRC, AUC 0.7—0.89 is considered a potential discriminator, while AUC $\geq$ 0.9 is a significant discriminator.

Spearmen and Pearson correlations were done for correlating quantitative variables. Correlation was considered