Levels of mirnas, mir-194, 29b markers for colorectal cancer

Circulating levels of the miRNAs, miR-194 and 29b, as clinically useful biomarkers for colorectal cancer

Colorectal cancer (CRC) has been reported as the fourth common cause of cancer mortality around the world with considerable health burden (1). Early stage diagnosis of the disease by noninvasive approaches could lead to effective treatment and better consequences (2). In this regard, microRNAs (miRNAs) in a few biological samples may deserve as early detection biomarkers for CRC (2, 3). miRNAs are endogenous small, non-protein coding RNAs that posttranscriptionally regulate expression of a broad range of biologically important genes function as either oncogenes or tumor suppressor genes depending on their physiopathological contribution and the tumour microenvironment (3, 4). Given the contribution of miRNAs in the pathogenesis of tumor cells, dysregulation of some miRNAs in tumor tissue would be conceivable. In fact, altered expression of a variety of miRNAs has been corroborated in CRC tissues (3).

Alteration in the expression of miRNAs in cancerous tissue may also be reflected in circulation (5). Therefore, altered levels of some miRNAs in plasma/serum or other body fluids of CRC patients have been extensively explored in an effort to find suitable diagnostic and prognostic biomarkers (6). In this regard, the miRNAs, miR-194 and miR-29b, may act as circulating invaluable and surrogate biomarkers for CRC. While miR-194 has been shown to be upregulated in oesophageal squamous cell carcinoma (7) and prostate cancer (8), its downregulation has been recently reported in endometrial cancer (9), primary renal cell carcinoma (10), and CRC tissues (11, 12). Notably, low expression level of miR-194 in CRC tissues was shown

to be correlated with increased tumor size (11, 12). Furthermore, the decreased level of miR-194 in stool samples was indicated to differentiate CRC patients from normal subjects (12). On the other hand, miR-29b suppresses proliferation of epithelial cells of intestinal mucosa (13), inhibits migration of colon cancer cells (14) and is significantly downregulated in osteosarcoma tissues (15). Given the involvement of miR-194 and miR-29b in some cancers including CRC and that there were no studies regarding their circulating levels in CRC, we determined the serum levels of miR-194 and miR-29b in a relatively large number of CRC patients, relative to control subjects, by real-time PCR assay to explore their diagnostic and prognostic values.

Materials and methods

Serum samples of 40 patients with colorectal adenocarcinoma and 40 control subjects were obtained at the Cancer Institute of Tehran, Iran. The obtained serum samples were further centrifugated in conical tubes for 10 min at 16000 g and 4°C in a fixed-angle rotor centrifuge and the new supernatants were immediately preserved at -80°C in multiple aliquots until further analysis. Diagnosis of patients and control subjects were based on colonoscopy and histopathological findings. None of the subjects under study had undergone any medical intervention such as radiotherapy, chemotherapy and surgery before sampling. All CRC patients underwent tumour resection and their clinical stages were determined according to the tumor-node-metastasis (TNM) staging system. Exclusion criteria included chronic or acute inflammatory conditions and any other malignancies except CRC.

The study was approved by the Ethics Committee of the Institute and all patients and control subjects provided written informed consents before enrollment in the study.

Serum RNA Extraction

For RNA isolation the frozen serum samples were thawed thoroughly on ice. Total RNA was isolated from 200 µl of serum using the miRNeasy Serum/Plasma Kit (Qiagen, CA, USA) following the manufacturer's instructions, with minor modifications. For the aqueous and organic phase separation step, 200 µL of molecular grade chloroform was added to the mixture of Qiazol solution (denaturant reagent of the kit) and serum and vortexed vigorously for 30 s, followed by incubation at room temperature for 5 min. The serum samples, after denaturation by the denaturant reagent, were spiked with 5 µL of 25 fmol synthetic cel-miR-39 (Invitrogen, CA, USA) as control. At final, total RNA was eluted from the spin column membrane by elution with 30 µL RNase-free and about 28 µL of total RNA solution was eluted. Optical density of the extracted total RNA samples were assessed at the wavelengths 260 and 280 nm on a NanoDrop spectrophotometer (NanoDrop, Wilmington, DE, USA) to determine their concentrations and purities. The isolated total RNA samples were preserved at -80°C until subsequent analysis.

Quantitative real-time PCR

The reverse transcription reaction was performed using a miScript II Reverse Transcription Kit (Qiagen, CA, USA) according to the manufacturer's protocol.

The reverse transcription reaction was carried out using a Taqman MicroRNA Reverse Transcription Kit (Applied Biosystems).

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