RESEARCH ARTICLE

MicroRNAs as Promising Biomarkers for Tumor-staging: Evaluation of MiR21 MiR155 MiR29a and MiR92a in Predicting Tumor Stage of Rectal Cancer

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Abstract

Objective: In this study, tumor-stage predictive abilities of miR21, miR155, miR29a and miR92a were evaluated in rectal cancer (RC).
Methods: Expression of miR21, miR155, miR29a and miR92a was detected and quantitated in tumor tissue and in adjacent normal tissue from 40 patients by TaqMan MicroRNA assay.
Results: Significant overexpression of miR21, miR155, miR29a and miR92a was observed in RC tissues. While high expression of miR21, miR155 and miR29a in N1-2 and C-D stages presented a potential correlation with N and Duke stages, partial correlation analysis suggested that only miR155 rather than miR21 and miR29a played a greater influencing role. Receiver operating characteristics (ROC) curve analysis showed that miR155 could discriminate N0 from N1-2 with 85.0% sensitivity and 85.0% specificity, N2 from N0-1 with 90.0% sensitivity and 96.7% specificity, and C-D stage from A-B stage with 81.0% sensitivity and 84.2% specificity.
Conclusions: Increase in expression of miR155 might represent a novel predictor for RC N and Dukes staging.

Keywords: Oncogenic microRNAs - rectal cancer - N stage - Dukes stage - miR21 - miR155 - miR29a - miR92a

Introduction

Rectal cancer (RC) is one of the most common malignant tumors. Its morbidity ranks the third place and mortality ranks the second place among all malignant tumors. Unfortunately, 50% of RC patients have already had regional or distant metastases at the time of diagnosis (Figueredo et al., 2008). So earlier diagnosing and tumor staging of RC are the key basis to choose treatment options, and directly affect patients’ prognosis and overall survival. Biochemical tumor markers such as CEA and imaging examination like CT and MRI are used for early detecting and progression monitoring. However, due to their wide variation in accuracy, they are not the “golden standard” for RC preoperative diagnosis, even less so for tumor staging (Karantanas et al., 2007; Nishiumi et al., 2012). Therefore the search for novel markers indicating RC tumor stage is needed.

MicroRNAs are noncoding small RNAs (18–25 nucleotides) that play regulating roles in cell differentiation, cell cycle progression and apoptosis (Esquela-Kerscher et al., 2006). They can be developed into microRNA-induced silencing complex (miRISC) that combines with specific 3’-untranslated-regions (3’-UTR) on mRNAs to induce mRNA degradation and inhibit protein translation (Lai et al., 2002; He et al., 2004). Therefore, microRNAs are considered as “regulators” in numerous biological events including genesis and development of carcinoma. Although not every mechanism and function of microRNAs is fully understood, certain studies suggest that abnormal expression of microRNAs is associated with a variety of tumors (Chiang et al., 2012; Iwaya et al., 2012; Hashimoto et al., 2013; Song et al., 2013). Thus it is possible that some microRNAs may have biological and clinical correlation with rectal cancer.

MiR-21, miR-155, miR-29a and miR-92a are these kinds of oncogenic microRNAs, which have been found overexpressing in several types of human malignant solid tumors (Gironella et al., 2007; Yan et al., 2008; Gebeshuber et al., 2009; Shigoka et al., 2010). But these previous studies didn’t link the microRNA expression to tumor stage quantitatively. So this study aims to further explore the quantitative relationship between expression of these microRNAs and tumor stages of RC, and evaluate their potential abilities to predict tumor stage.

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Materials and Methods

Patients and Tissue Samples
From August to October, 2012, 40 newly biopsy-proven RC patients were consecutively recruited into this study at the gastrointestinal surgery center of West China Hospital, according to the following inclusive criteria: no familial adenomatous polyposis or hereditary nonpolyposis CRC; no preoperative neoadjuvant therapy; no intestinal obstruction, perforation and any other acute abdomen conditions. From the inclusive patients, samples of tumor tissue and normal tissue (>5cm proximal to tumor site) were collected and frozen in liquid nitrogen right after surgical resection. Same well-trained laboratory technician tested CEA 3 days preoperatively, and same pathologist staged tumors according to the tumor-node-metastasis (TNM) staging system postoperatively. No treatment was administered in these 3 days. Also, same technical group detected expressions of microRNAs in normal and tumor tissue samples. The tissue samples were labeled such as to ensure that they were blind to the normal and tumor tissue identifications. The ethics committee of West China Hospital approved the study protocol (No. 2012-149 approved at June 25th 2012), and an informed consent agreement was obtained from every participant.

TaqMan MicroRNA Assay
Using Trizol (ABI, USA), total RNAs which contain microRNAs were isolated from tumor and normal tissue samples of the 40 patients with primary RC. MiR21, miR155, miR29a, miR92a, U6 snRNA and U47-specific probes were synthesized by ABI. The whole procedure was followed according to the TaqMan MicroRNA assay protocol (4364031 Rev.E, 01/2011) of ABI. The geometric average of two housekeeping genes, U6 snRNA and U47, was used to normalize the expression of microRNAs (Vandesompele et al., 2002). In brief, reverse transcriptase reaction was performed using Taqman MicroRNA Reverse Transcription Kit (ABI, USA). Each 25ul-reaction contained 5ng of total RNAs, 9.6ul 5xRT primer (1.6ul/gene), 0.3ul RNase Inhibitor (20U/uL), 2.5ul 10xRT buffer, 1.6ul MultiScribe Reverse Transcriptase and 0.25ul dNTPs (100mM). The reaction samples were incubated in PCR System for 30 min at 16°C, 30 min at 42°C and 5 min at 85°C, and then kept at 4°C. Taqman qPCR was performed using Taqman Universal PCR Master Mix (ABI, USA). The 3 replicates reaction (10ul/reaction) mix included 2.4ul template, 1.6ul 20×Taqman MicroRNA Assay Mix and 16ul 2×Taqman Universal PCR buffer, UNG. The reaction samples were added into 384-well optical plates. And the PCR was run on 7900 HT Sequence Detection System (ABI, USA) in the conditions of 50°C for 2min, 95 ° C for 10 min, followed by 40 cycles at 95 ° C for 15 s and 60 ° C for 1min. Relative quantification of microRNA expression was calculated by the 2−ΔΔCt method, where 2−ΔΔCt =2(2−ΔCt(target microRNA)).

Statistical Analysis
Expression levels of microRNAs were compared by paired-samples T-test or independent sample T-test according to the methods of grouping. The bivariate and partial correlations between microRNAs and tumor stage were analyzed by Spearman’s and Pearson’s correlation test. Receiver-operating characteristics (ROC) curves were established to evaluate the prognostic value of microRNA in differentiating tumor stage. P values less than 0.05 were considered statistically significant with 95% 2-sided confidence. All statistical analysis was performed with IBM SPSS 20.0 software (IBM SPSS, Inc., Chicago).
Results

Patient Characteristics

From August to October 2012, a total of 40 RC patients were recruited into this study (Table 1). There should be no significant differences about baseline information between RC tissues and normal tissues, because each pair of RC and normal tissues was obtained from a same patient.

Expression of MicroRNAs in Tumor and Normal Tissue

Between the tumor tissue and normal tissue, the statistical difference of expression was observed in miR21, miR155, miR29a and miR92a with P<0.05. In the setting of paired-samples t test, the mean (±SD) expression level of miR21 was apparently higher in tumor tissue than in corresponding normal tissue (4.122±1.973 vs. 1.825±0.661, P=0.000). So were miR155 (0.137±0.095 vs. 0.093±0.091, P=0.043), miR29a (2.220±0.834 vs. 1.863±0.730, P=0.039) and miR92a (1.437±0.581 vs. 0.761±0.241, P=0.000) (Figure 1).

Correlations between Clinicopathological Parameters and MicroRNAs Expression in Tumor Tissue

To evaluate the correlation between microRNAs expression and clinicopathological characteristics, patients were divided into different groups shown in the first column of Table 2. A statistically significant difference was observed between the group N0 and group N1-2 in miR21, miR155 and miR29a. The same phenomenon could be seen in Dukes stage (between the group A-B and group C-D). The results suggested that miR21, miR155 and miR29a might have potential association with tumor N stage (lymph node metastasis) and Dukes stage (positive metastasis).

For these 3 candidate microRNAs: miR21, miR155 and miR29a, bivariate and partial correlation analysis was used to further determine which microRNA had greater influence on N and Dukes stage. As Table 3 presented, a bivariate correlation analysis was used to hypothesis-test association and causality between tumor stage (N and Dukes stage) and microRNAs (miR21, miR155 and miR29a). From the results, these 3 microRNAs were all found to have relationship with N and Dukes stage in bivariate correlation. But when partial correlation analysis was used to remove the effects of any two microRNAs, spurious relationship was uncovered: only miR155 stood out and presented a strong correlation with
In this study, we found that the expression levels of miR21, miR155, miR29a and miR92a in RC tissues were significantly higher than those in non-RC normal tissues. Expression levels of miR21, miR155 and miR29a in RC tissues seemed to have underlying relation with tumor stage. Although the increase of expression is much larger in miR21 (the largest) and miR29a than miR155 (Figure 1), further statistical analysis revealed a surprising result. After controlling any two factors, only miR155 still maintained the positive correlation with N and Dukes stages (Table 3).

For molecular mechanism, miR21, miR155, miR29a and miR92a are closely related to tumor development. MiR21 was demystified that it could down-regulate the protein expression of programmed cell death 4 (PDCD4), which plays a role as suppressors of transformation, tumor genesis, progression, invasion and metalloproteinase activation, and as an inducer of apoptosis (Asangani et al., 2008). Knockdown of PDCD4 decreased the expressions of epithelial-specific proteins, and increased the expressions of mesenchymal-specific proteins in vitro and in vivo, and the rate of wound closure and migration capacity in wound-healing assays and Boyden chamber migration assays, suggesting that knockdown of Pdc4 results in epithelial to mesenchymal transition (EMT) and promotes cell migration (Wang et al., 2013). It was reported that miR155 could down-regulate TP53INP1, which is a pro-apoptotic stress-induced p53 target gene (Tomasini et al., 2002). It can interact with p53 and the homeodomain-interacting protein kinase-2 within the p53-promyelocytic leukemia nuclear bodies, modulating p53 transcriptional activity (Tomasini et al., 2003).

The Predictive Value of MiR155 for N and Dukes Staging in Rectal Cancer

To further assess the ability of miR155 to distinguish tumor N and Dukes stages, receiver operating characteristics (ROC) curve analysis was used. As shown in Figure 2-4, N0 stage could be differentiated from N1-2 stage by miR155 with an AUC of 0.855 (95% CI: 0.730-0.980), so could N2 stage be differentiated from N0-1 stage with an AUC of 0.975 (95% CI: 0.930-1.000). Also, C-D stage could be apart from A-B stage with an AUC of 0.835 (95% CI: 0.706-0.963). For miR155, at the cutoff value of 0.125, the sensitivity, specificity and accuracy were 85.0%, 96.7% and 95.0%, the +LR and -LR were 31.3, and 9.6 in discriminating N0 from N1-2. At the cutoff value of 0.165, the sensitivity, specificity and accuracy were 85.0%, 85.0% and 85.0%, the +LR (positive likelihood ratio) and –LR (the positive likelihood ratio) were 5.677 and 0.176 correspondingly in discriminating N0 from N1-2. In discriminating C-D stage from A-B stage at the cutoff value of 0.125, the sensitivity, specificity and accuracy were 81.0%, 84.2% and 82.5%, the +LR and –LR were 5.127 and 0.266.

Discussion

In previous studies, it was found high expression of miR21 in CRC tissue, which was associated with lymph node metastasis, distant metastasis and tumor staging (Slaby et al., 2008). A following joint research involving Chinese and American CRC patients uncovered the relation between low survival rate and high expression of miR21. The overexpression in C stage correlated with low chemotherapy sensitiveness and early recurrence. The expression of miR21 was significantly lower in RC tissue than non-RC normal tissue. Consequently, the result of this study may provide clinical application in evaluating the adjuvant therapy for RC patients. It was suggested that miR21 serves as a potential marker for identifying the metastasis and dissemination in CRC treatment.
Among other studies, miR155 was found to be overexpressed in colorectal cancer and has been associated with poor prognosis. The authors conclude that these results suggest that miR155 could represent a potential novel predictor for colorectal cancer, and future studies are needed to confirm these findings in larger cohorts.

References


Gebescher CA, Zlatoulkal K, Martinez J (2009). MiR29a suppresses tristetraprolin, which is a regulator of epithelial polarity and metastasis. EMBO Rep, 10, 400-5.


