Research Article

Xiangrong Zhu*, Xiongtie Wang, Xihua Chen

The diagnostic value of serum microRNA-183 and TK1 as biomarkers for colorectal cancer diagnosis

1 Introduction

Colorectal cancer is a common type of malignant tumor in the digestive system [1]. In recent years, China's aging population demographic has increased the incidence of colorectal cancer [2]. The prognosis of patients with early stages of the disease is good after undergoing radical surgery, and their 5-year survival rate is relative high [3,4]. On the contrary, patients with advanced disease often exhibit distant metastasis and a substantially reduced chance of surgical intervention. For these patients, most of them undergo conservative treatment with radiotherapy and chemotherapy. These patients also show poor prognosis and low long-term survival rates. Therefore, the early diagnosis of colorectal cancer should be improved to enhance the prognosis of patients with colorectal cancer [5,6]. Colonoscopy is an important method used for the diagnosis or screening of colorectal cancer. However, colonoscopy causes certain trauma and involves a complex operation process [7,8]. Some primary hospitals also do not have the resources to perform colonoscopy examinations. Furthermore, the large-scale application of colonoscopy to screen high-risk individuals for colorectal cancer is limited due to the relative complexity and time consumption of the procedure.

Therefore, searching for sensitive, non-invasive, and convenient methods with high specificity is the major topic on the diagnosis or screening of colorectal cancer.

Serological markers can be easily obtained and accessed without invasiveness, and they are widely used in clinical diagnosis of tumors. Studies have reported that the expression levels of miR-183 and thymidine kinase (TK) 1 (TK1) in the peripheral blood of malignant carcinoma patients were significantly increased, suggesting their potential as serological markers for cancer diagnosis [9-12]. In this study, the expression levels of serum miR-183

Keywords: colorectal cancer; diagnosis; microRNA-183; thymidine kinase 1
and TK1 in 46 colorectal cancer patients and 46 healthy controls are measured, and the feasibility and clinical value of diagnostic biomarkers for colorectal cancer are analyzed.

2 Materials and methods

2.1 Patients and controls inclusion

Colorectal cancer patients who visited our hospital for treatment from February 2015 to Jan 2017 were recruited. All patients received colonoscopy examinations and had the pathology diagnosis of malignant colorectal carcinoma. The patients did not receive any chemoradiation or target therapy before administration. Subjects who underwent routine medical examination were recruited as healthy controls. People in the healthy control group did not have any evidence or history of malignant carcinoma. Finally, 46 colorectal cancer patients were included in this study. The mean age of the colorectal cancer patients was 58.8±16.3 with 29 male and 17 female patients. The clinical characteristics of colorectal patients are shown in Table 1. For the 46 healthy controls, the mean age was 55.7±18.4 with 25 male and 21 female subjects. The age and gender distribution was not statistical different (p>0.05).

Informed consent: Informed consent has been obtained from all individuals included in this study.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the authors’ institutional review board or equivalent committee.

2.2 Serum MiR-183

Serum was collected by centrifuging blood at 400g for 10min. The supernatant was transferred to newtubes and stored at −80°C. MiR-183 was extracted from 250μl serum samples using mirVana® miRNA isolation Kit (Ambion, Austin, TX) as per the manufacturer’s instructions. TaqMan microRNA assay (Applied Biosystems, Foster City, CA) was used to relatively quantify and detect the miR-183 levels, and U6B was used as an internal control. Quantitative real-time PCR was performed by 2×SYBR Green qPCR Mix (TOYOBO, Japan) on the ABI7900 system (Applied Biosystems, Foster City, CA).

2.3 Serum TK1 detection

CIS digital chemiluminescence imaging analyzer was used to detect the serum levels of TK1. TK1 Kit was purchased from Tongkang Biotechnology Co. Ltd (Shenzhen). Enhanced chemiluminescence detection assay was applied for serum TK1 expression quantification. The specific operation was performed in strict accordance with the instructions. Serum TK1>2 pmol/L was considered as positive.

Table 1. The general characteristics of the included 46 cases

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean/n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>58.8±16.3</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>29(63.0%)</td>
</tr>
<tr>
<td>Female</td>
<td>17(37.0%)</td>
</tr>
<tr>
<td>Tumor differentiation</td>
<td></td>
</tr>
<tr>
<td>Well/moderate</td>
<td>14(30.4%)</td>
</tr>
<tr>
<td>Poor</td>
<td>32(69.6%)</td>
</tr>
<tr>
<td>Clinical stage</td>
<td></td>
</tr>
<tr>
<td>I/II</td>
<td>15(32.6%)</td>
</tr>
<tr>
<td>III/IV</td>
<td>31(77.4%)</td>
</tr>
<tr>
<td>Location</td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>25(54.3%)</td>
</tr>
<tr>
<td>Rectal</td>
<td>21(45.7%)</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>29(63.0%)</td>
</tr>
<tr>
<td>Negative</td>
<td>17(37.0%)</td>
</tr>
</tbody>
</table>
2.4 Statistical analysis

The data were analyzed by SPSS 18.0 statistical software package (IBM, Armonk, NY, USA). Measurement data is expressed by mean ± standard deviation and compared between groups with student-t test. Significance of colorectal cancer detection by serum miR-183 and TK1 was analyzed through receiver operating characteristic (ROC) curves. The association between serum miR-183 and TK1 level was evaluated by pearson’s correlation test. Two tailed p<0.05 was considered statistically significant.

3 Results

3.1 Serum miR-183 level and its diagnostic value for colorectal cancer

The median serum relative expression of miR-183 was 1.33 (0.34-5.65) and 0.88 (0.26-4.67) in colorectal cancer patients and healthy controls respectively with significant statistical difference (p<0.05). Figure 1a. Using serum miR-183 as the diagnostic reference, the colorectal cancer diagnosis sensitivity, specificity and AUC was 65.22%, 63.04% and 0.69 respectively, (Table 2, Figure 1b).

Table 2. The colorectal cancer diagnosis value for serum miR-183 and TK1

<table>
<thead>
<tr>
<th>Markers</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Cut-off</th>
<th>Likelihood ratio</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>MiR-183</td>
<td>65.22%</td>
<td>63.04%</td>
<td>1.10</td>
<td>1.77</td>
<td>0.69</td>
</tr>
<tr>
<td>TK1</td>
<td>84.78%</td>
<td>78.26%</td>
<td>2.26</td>
<td>3.9</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Figure 1. Serum miR-183 expression and its diagnostic value for colorectal cancer (a: scatter plot for miR-183 relative expression in colorectal patients and healthy controls; b: ROC curve of serum miRNA-183 as a biomarker for colorectal cancer diagnosis)

Figure 2. Serum TK1 expression and its diagnostic value for colorectal cancer (a: scatter plot for TK1 relative expression in colorectal patients and healthy controls; b: ROC curve of serum TK1 as a biomarker for colorectal cancer diagnosis)
3.2 Serum TK1 level and its diagnostic value for colorectal cancer

The median serum levels of TK1 were 3.33 (0.78-5.78) pmol/L and 0.99 (0.34-4.46) pmol/L in colorectal cancer patients and healthy controls respectively with significant statistical difference (p<0.05). Figure 2a. The diagnostic sensitivity, specificity and AUC was 84.78%, 78.26% and 0.88 respectively for serum TK1 used as a reference for colorectal diagnosis (Table 2, Figure 2b).

3.3 Correlation between serum miR-183 and TK1 in colorectal patients

The Pearson correlation test was used to evaluate the correlation between serum miR-183 and TK1 in colorectal cancer patients. However, no significant correlation between serum miR-183 and TK1 was found in colorectal patients (p>0.05), Figure 3.

![Figure 3. Correlation between serum miR-183 and TK1 in colorectal patients](image)

4 Discussion

MicroRNA (miRNA) is a non-coding, single-stranded RNA molecule (approximately 22 nucleotides in length) encoded by endogenous genes. These molecules are involved in the regulation of post-transcriptional genes in animals and plants. To date, approximately 30,000 miRNA molecules have been found in animals, plants, and viruses [13]. Most miRNA genes are present in the genome in the form of single-copy genes, multiple-copy genes, or gene clusters. MiRNAs are tissue-specific and play a regulatory role by binding with the 3' untranslated region of the target gene to degrade or inhibit target gene expression. Many miRNAs have tumor-specific expression and differential expression in the peripheral blood of cancer patients compared to normal population. A certain relationship also exists between miRNA and pathology type, differentiation, clinical stage, and the treatment response of tumors. The MiR-183 family contains miRNAs of miR-183, miR-182, and miR-96, which are transcribed from chromosome 7 (7q31–34), are evolutionally conserved, and are expressed abnormally in many human tumors [14,15]. MiR-183 is expressed abnormally in various malignant solid tumors and is associated with clinicopathological features and prognosis. Zhou et al. [16] used real-time PCR assay examine the expression of miR-183 in tumor and adjacent tissues of 94 patients with colorectal cancer. The results showed that the increased expression of miR-183 is closely related to advanced clinical stage, lymph node and distant metastases, and poor prognosis of colorectal cancer patients, indicating that miR-183 may serve as a predictive biomarker for the prognosis or the aggressiveness of colorectal cancer. Zaporozhchenko et al. [9] found that the serum level of miR-183 in patients with lung cancer is significantly higher. Given its high sensitivity and specificity, miR-183 can be used as a serological marker for the diagnosis of lung cancer. In the current study, the serum level of miR-183 in colorectal cancer patients is significantly higher than those in healthy controls. This result suggests that miR-183 can be used as a tumor serum marker for distinguishing patients with colorectal cancer from patients without the disease. A diagnostic test was conducted, and the results show that the sensitivity and specificity for diagnosing colorectal cancer based on serum miR-183 are 65.22% and 63.04%, respectively. The area under the ROC curve is 0.69, showing a dissatisfaction diagnostic efficacy. This may be related to the small number of patients involved in the study and a certain degree of heterogeneity in the clinical characteristics of the patients.

TK is the rate-limiting enzyme of the DNA recovery synthesis pathway. TK1 is closely associated with the cell cycle, with TK1 levels being low in the G1 phase. TK1 begins to increase in the S phase and peaks in the G2 phase [17]. In general, the TK1 level in healthy human serum is low because of rapid cell proliferation; however, TK1 significantly increases in patients with malignant tumors [18]. Alegre et al. [19] analyzed the relationship between the serum level of TK1 and the clinical characteristics of 40 patients with lung cancer. The results showed that TK1 was significantly increased in the serum of patients with early stage lung cancer and it could be a serological marker for early diagnosis of lung cancer. In the present study, the sensitivity and specificity for diagnosing colorectal cancer
based on serum TK1 is 84.78% and 78.26, respectively. The area under the ROC curve is 0.88, which presents a good clinical application value.

Therefore, TK1 and miR-183 serum levels can be preemptively examined for individuals at high-risk of colorectal cancer to determine the necessity of colonoscopy examination.

Conflict of interest: Authors state no conflict of interest

References