Value of combined detection of serum miR-21, miR-195 and miR-222 in the diagnosis of early breast cancer

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ABSTRACT

Objective: To study level of miRNA in the serum of early breast carcinoma patients and to evaluate the clinical diagnostic value of combined detection of early breast carcinoma.

Methods: A total of 54 cases of early breast carcinoma, 58 cases of benign breast diseases and 70 cases of healthy physical examination women were selected as the research subjects, to analysis serum miR-21, miR-195 and miR-222 levels by fluorescence quantitative PCR method and to analyze the diagnostic value of single and combined detection in early breast cancer by receiver operating characteristic curve.

Results: The relative expression levels of miR-21, miR-222 and miR-195 in early breast carcinoma patients were significantly higher than those in benign breast disease and healthy controls; there was no significant difference in the relative expression of miR-21, miR-222 and miR-195 in the benign breast disease group and healthy control group; receiver operating characteristic curve analysis showed that AUC of miR-21, miR-195 and miR-222 in the diagnosis of early breast carcinoma were 0.805, 0.86 and 0.848 respectively, the sensitivity were 63.3%, 70.0% and 70.0%, and the specificity were 86.7%, 93.3% and 90.0%; AUC, sensitivity and specificity of the combined detection were 0.974, 93.3% and 96.7% respectively.

Conclusion: miR-21, miR-195 and miR-222 levels in serum of patients with early breast carcinoma rise, the combined detection of the 3 indicators have a high diagnostic value for early breast carcinoma, and contribute to early breast carcinoma screening and diagnosis.

1. Introduction

Breast cancer is one of the most common malignancies of women, and the trend of incidence and mortality was significantly upward recently. The key of improving survival rates is the diagnosis of early breast cancer and the timely treatment. The nationwide-used serum protein processing is low at specificity and sensitivity is not high to diagnosis the early breast cancer. MicroRNAs (miRNA) is an endogenous non-coding small RNA, transcription of genes regulated function. In recent years, several studies have shown aberrant miRNA expression is closely related with the occurrence and development of breast cancer, it has high specificity and sensitivity on early diagnosis of breast cancer. The author detected a variety of serum miRNA jointly, investigate the clinical value in the early diagnosis of breast cancer, as follows.

2. Case information and treatment methods

2.1. General materials

Early breast cancer group were 54 early breast cancer patients treated in our hospital from June, 2014 to October, 2015, diagnosed TNM I breast cancer, aged 27 to 64 years, the average age (45.89 ± 8.94) years; patients with benign breast disease benign breast disease group for the same period in our hospital, 58 patients, aged 25 to 65 years, mean age (46.24 ± 8.58) years; Female 70 cases of healthy control group for the same period in our hospital healthy, aged 24 to 60 years, mean age (43.89 ± 8.46) years. Patients in
each group on gender, age difference was not statistically significant ($P>0.05$), comparable.

2.2. Research method

2.2.1. Sample collection

Each group were collected at admission or examination of peripheral blood 5 mL, 4 °C, 3 000 r / min centrifugation for 5 min in serum and stored at -80 °C ultra-low temperature freezer for miRNA detection.

2.2.2. miRNA detection

Blood RNA extraction kit according to the steps of extracting total miRNA, after the reverse transcription reaction, using fluorescence quantitative PCR tiny RNA-21 (miR-21), tiny RNA-195 (miR-195) and micro RNA-222 (miR-222). Amplification conditions were 95 °C 10 min; 95 °C 15 s, 60 °C 60 s; 40 cycles, each sample was repeated three times to take the mean. In U6 SnRNA as an internal reference, using SDS 1.4 software to calculate the Ct value, relative to the U6 SnRNA of 2△△Ct represents the relative expression of miRNAs.

$$\triangle Ct = Ct \text{ value of the target miRNA - internal reference U6 SnRNA}$$

△△Ct values: △△Ct = group of early breast cancer or benign breast disease group of target miRNA △△Ct- healthy control group of target miRNA △△Ct; △△Ct substituting 2 - △△Ct calculation, and the healthy control group target gene is 100, calculated the relative expression of miRNAs in the observation group.

2.3 Statistical methods

Using SPSS 13.0 statistical analysis software. Measurement data were expressed as mean ± standard deviation (Mean ± sd), compared using t test; count data expressed as a percentage, compared using the $\chi^2$ test. $P<0.05$ indicates significant difference.

3. Results

3.1. Compare the amount of relative expression levels of serum miRNAs

Each miRNA relative expression of early breast cancer group were significantly higher, the difference was statistically significant ($P<0.05$); each miRNA benign breast disease group relative expression group and the control group compared the difference was not significant sex ($P>0.05$); the relative expression of each miRNA early breast cancer group were significantly higher than those with benign breast disease group, the differences were statistically significant ($P<0.05$). As shown in Table 1.

3.2. Diagnostic value analysis

Using receiver operating characteristic curve (receiver operating characteristic curve, ROC) compare the ability to identify individual miRNA diagnosis of early breast cancer and benign breast disease group, and each miRNA adopt joint diagnosis, analysis of its diagnostic value. ROC curve showed that serum miR-21, miR-195 area under the curve and miR-222 early diagnosis of breast cancer (area under the curve, AUC) were 0.805, 0.867 and 0.848 ($P<0.05$), specificity were 86.7%, 93.3% and 90.0%, sensitivity was 63.3%, 70.0% and 70.0%; said miRNA joint diagnosis, AUC, were as high specificity and sensitivity 0.974, 96.7% and 93.3%. As shown in Table 2 below.

4. Discussion

The incidence of breast cancer accounts for nearly one-third of women, seriously affecting the lives and safety of the patient, the importance and difficulty are in a high incidence of breast cancer, early diagnosis rate, death rate in clinical diagnosis and treatment. For more than 1.0 cm diameter breast lesions generally super effectively resolved by conventional mammography and B, but the sensitivity for early lesions smaller than 5 mm in diameter was low, and routine serum protein deposit with early breast cancer detection sensitivity and lower specificity problem, so look for high specificity, high sensitivity of early breast cancer biomarkers is becoming increasingly urgent.

Clinical studies, miR-21 in breast cancer patients has increased significantly, with the role of oncoproteins, can inhibit tumor cell apoptosis and promote tumor cell proliferation and invasion metastasis, the study also confirmed that miR-21 in the early breast the relative expression level of the cancer is far higher than the control group and benign breast disease group, with significant differences ($P<0.05$); closely related to the degree of malignancy of miR-195 and breast cancer, studies have shown that inhibit tumor cell proliferation and invasion of serum concentrations of miR-195 in patients was significantly higher than healthy controls, this study of early breast cancer group relative expression of miR-195 was significantly higher and benign breast disease group ($P<0.05$); studies have shown that miR-222 levels of gene expression by the change, promote invasion and metastasis of breast cancer cells, compared with the healthy control group, miR-222 expression in plasma are significantly increased, miR-early breast cancer in this study group 222 relative expression levels significantly higher than the control group and benign breast disease ($P<0.05$). Numerous studies show that, miRNA tumor occurrence and development plays a role in tumor suppressor genes or oncoproteins, whose aberrant expression is closely related to the occurrence of certain tumors, in recent years a number of studies have shown that when the development of breast cancer, blood plasma miRNA abnormal expression, miRNA has the potential to become an early diagnosis of breast cancer biomarkers.

The diagnostic value of this study using ROC curve to evaluate miRNA in early breast cancer, AUC greater, indicating that the ability to identify the higher the index, the greater the clinical diagnostic value, and therefore can be used to indicate the size of the AUC accuracy of diagnosis. Generally considered: AUC<0.5 showed no diagnostic value; 0.5<AUC<0.7 indicating a lower clinical diagnostic accuracy;
Table 1
Serum levels of miRNA in each group comparisons.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>miR-21</th>
<th>miR-195</th>
<th>miR-222</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control</td>
<td>70</td>
<td>100.0±14.6</td>
<td>100.0±14.8</td>
<td>100.0±18.9</td>
</tr>
<tr>
<td>Early breast cancer</td>
<td>54</td>
<td>143.3±19.4#</td>
<td>154.1±22.6#</td>
<td>146.3±18.6#</td>
</tr>
<tr>
<td>Benign breast disease</td>
<td>58</td>
<td>111.2±20.3</td>
<td>107.1±20.1</td>
<td>108.6±21.3</td>
</tr>
</tbody>
</table>

Notes: Compared with healthy control group *representation p<0.05; Compared with benign breast disease group # representation p<0.05

Table 2
ROC curve analysis.

<table>
<thead>
<tr>
<th>Factor</th>
<th>AUC</th>
<th>95% confidence interval</th>
<th>P</th>
<th>Specificity (%)</th>
<th>Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-21</td>
<td>0.805</td>
<td>0.695-0.915</td>
<td>0.000</td>
<td>86.7</td>
<td>63.3</td>
</tr>
<tr>
<td>miR-195</td>
<td>0.867</td>
<td>0.778-0.957</td>
<td>0.000</td>
<td>93.3</td>
<td>70.0</td>
</tr>
<tr>
<td>miR-222</td>
<td>0.848</td>
<td>0.750-0.945</td>
<td>0.000</td>
<td>90.0</td>
<td>70.0</td>
</tr>
<tr>
<td>union</td>
<td>0.974</td>
<td>0.941-1.008</td>
<td>0.000</td>
<td>96.7</td>
<td>93.3</td>
</tr>
</tbody>
</table>

0.7<AUC<0.9 indicates a certain clinical diagnostic accuracy; high AUC>0.9 clinical diagnostic accuracy. In this study, benign breast disease group compared with early breast cancer group in plasma miR-21, miR-195 and miR-222 in the AUC were 0.805, 0.867 and 0.848 (P<0.05), the results showed that all indicators early diagnosis of breast cancer has a certain accuracy. Plasma miR-21, miR-195 and miR-222 in the United diagnosis, AUC up to 0.974 (P<0.05), AUC higher than individual indicators detection, specificity and sensitivity, respectively, 96.7% and 93.3%, indicating that the joint diagnosis can improve the accuracy of diagnosis of early breast cancer.

To sum up, early stage breast cancer patients plasma miR-21, miR-195 and higher miR-222 levels, an important reference value for the diagnosis of early breast cancer detection through the index, and the joint detecting the miRNA may increase diagnosis of early breast cancer is expected to become early screening and diagnosis of breast cancer clinic.

References


