Expression level of miRNA in the peripheral blood of patients with multiple myeloma and its clinical significance

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Abstract: Objective: To investigate the expression level of serum miRNA-192-5p and its clinical value in the diagnosis and care of patients with multiple myeloma (MM). Methods: Eighty-eight patients with MM admitted to our hospital from June 2017 to April 2020 were selected as the observation group. In addition, 70 patients who received osteoporosis testing in our hospital in the corresponding period but were excluded from having MM and haematological malignancy were selected as the control group. The relative expression level of serum miRNA-192-5p was detected. The expression level of serum miRNA and its correlation with patient-related clinical parameters were compared and analyzed. The ROC curve was used to analyze its diagnostic efficacy for MM. Results: The relative expression level of serum miRNA-192-5p in MM patients was remarkably lower than that in the control group (P < 0.05); the AUC area of serum miRNA-192-5p in patients with a diagnosis of MM was 0.853, with a cutoff value of 0.72, the sensitivity of 86.30%, and the specificity of 81.20%, P = 0.030. The relative expression level of miRNA-192-5p in the serum of patients with high β2-MG and creatinine levels was markedly reduced compared to that in patients with low β2-MG levels (P < 0.05); the relative expression level of miRNA-192-5p in the serum of patients with low hemoglobin and albumin levels was markedly reduced compared to that in patients with normal hemoglobin and albumin (P < 0.05); and there was significantly negative correlation between the relative expression level of miRNA-192-5p in the serum of MM patients and IgG and IgA levels, respectively (P < 0.05). Conclusion: miRNA-192-5p may serve as an auxiliary diagnostic tool in the diagnosis of MM. Furthermore, because there is certain correlation between serum miRNA-192-5p and MM progression and prognosis, it may be regarded as a novel marker for MM monitoring.

Keywords: Multiple myeloma, miRNA-192-5p, diagnosis

Introduction

Multiple myeloma (MM) is a common malignant proliferative disease of plasma cells characterized by unlimited proliferation and accumulation of plasma cells in the bone marrow. It is classified as a B-cell lymphoma by the World Health Organization [1]. Its incidence accounts for approximately 13% of hematologic malignancies [2], ranking only second to leukemia [3]. The clinical manifestations of MM patients are nonspecific in the early stage, but symptoms such as bone pain, pathological fractures, and liver, spleen, or kidney lesions may occur as the disease progresses, with bone pain as the major symptom. These patients may delay treatment due to misdiagnosis, and the 5-year survival rate of patients’ remains low [4]. Consequently, the medical community is always working to search for effective biomarkers that can accurately diagnose MM, assess disease progression and determine the prognosis of patients. MM pathogenesis remains unclear, but it is thought to be related to viral infection, genetics, genetic mutation and the bone marrow microenvironment. MicroRNA (miRNA) is a non-coding small RNA with a length of about 22 nucleotides. It functions in regulating the expression of tumor genes through the inhibition or degradation of mRNA, and exerts an essential effect in the occurrence, development and drug resistance of tumors. Currently, good results have been found by using several miRNAs as new biomarkers in the assessment of
miRNA in the peripheral blood of multiple myeloma patients

Table 1. Main reagents and instruments

<table>
<thead>
<tr>
<th>Name</th>
<th>Manufacturer</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA extraction kit</td>
<td>Thermo Fisher Scientific (USA)</td>
<td>TAKARA (Dalian, China)</td>
</tr>
<tr>
<td>Reverse transcription reagent</td>
<td>Epicentre</td>
<td>Guangzhou RiboBio Co., LTD</td>
</tr>
<tr>
<td>Fluorescence quantitative PCR kit</td>
<td>TAKARA (Dalian, China)</td>
<td>Applied Biosystems (USA)</td>
</tr>
<tr>
<td>MiRNA-192-5p PCR primer</td>
<td>Guangzhou RiboBio Co., LTD</td>
<td></td>
</tr>
<tr>
<td>Real-time quantitative PCR amplification instrument</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol, chloroform, etc.</td>
<td>Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China)</td>
<td></td>
</tr>
</tbody>
</table>

diagnosis, treatment and prognosis for many diseases [5]. For the past few years, some scholars have found that abnormally expressed miRNA can inhibit or promote the expression of oncogenes to regulate the proliferation and migration of MM [6, 7]. It has been confirmed by current studies that miRNA-192-5p exerts a major impact on influenza viral infections and it has been used to develop a live attenuated influenza vaccine [8]. However, the association between miRNA-192-5p and the development and progression of MM is not yet clearly known. Therefore, this study focused on the diagnostic value of the expression level of serum miRNA-192-5p for MM, and further analyzed its relationship with patient-related clinical data so as to offer a reference standard for clinical diagnosis and therapy.

Materials and methods

General data

Eighty-eight patients with MM admitted to our hospital from June 2017 to April 2020 were selected as the observation group. There were 55 males and 33 females aged 51-78 years (mean: 61.80 ± 5.40 years). All the patients in the observation group were eligible for the relevant diagnostic criteria as specified in the Guidelines for the Diagnosis and Management of Multiple Myeloma in China (2017 revision) [9]; Inclusion criteria: (1) Plasma cells in bone marrow increased by > 30%; (2) Biopsy confirmed plasmacytoma; (3) Serum monoclonal immunoglobulin (M protein) IgG greater than 35 g/L; IgA greater than 20 g/L; IgM greater than 15 g/L; IgD greater than 2 g/L; IgE greater than 2 g/L; Urine monoclonal immunoglobulin greater than 1 g/24 h. (4) Extensive osteoporosis and/or osteolytic lesions. Exclusion criteria: those patients who were complicated with other malignant tumors, severe heart, liver and kidney and other organ diseases, and other hematologic disorders. By referring to the general data of patients in the observation group, 70 patients who underwent osteoporosis testing in our hospital in the corresponding period but were excluded from having MM and haematological malignancy were selected as the control group. There were 38 males and 32 females aged 52-80 years (mean: 63.18 ± 6.80 years). No statistically significant difference in the general data between the two groups was noted (P > 0.05). This study was approved by our hospital ethics committee, and all patents provided an informed consent.

Assay of the expression level of serum miRNA-192-5p

Primary reagents and instruments: RNA extraction kit was used to extract RNA from tissues. After measuring the RNA concentration, the amount of substrate RNA needed for reverse transcription was saved. The remaining RNA was stored in a freezer at -80 degrees C. The reverse transcription kit was used to reverse transcribe RNA into cDNA, and MiRNA-192-5 PCR primers and cDNA were added to a reaction tube to perform fluorescent quantitative PCR. The reagents required were from a fluorescent quantitative PCR kit, and a real-time quantitative PCR amplifier was used for amplification. The tissue and supernatant were centrifuged using a high-speed centrifuge, and ethanol and chloroform were used for RNA purification. The primary reagents and instruments used in this study are displayed in Table 1.

Assay: Extraction of the RNA in serum: 400 μL of serum samples were prepared in enzyme-
free EP tubes, and then 1000 μL of Trizol LS lysis reagent was added, mixed well, and let rest at room temperature for 5 min; the resulting solution was centrifuged at 13500 r/min for 5 min at 4°C, and the supernatant was collected with another EP tube; 200 μL of trichloromethane solution (Trizol LS/trichloromethane: 5:1) was added, mixed well, and let sit at room temperature for 5 min; the resulting solution was centrifuged at 13500 r/min for 5 min at 4°C, and the supernatant was taken with another EP tube; 500 μL of isopropanol was added, and inverted at 4°C for 2 h; the resulting solution was centrifuged at 13500 r/min at 4°C for 10 min, and the supernatant was discarded; 1 ml of 75% ethanol was added, the centrifuge tube was gently inverted, and then centrifuged at 13500 r/min for 5 min at 4°C, and then the supernatant was discarded and dried; 10 μL of DEPC was added to dissolve, the concentration of total RNA was measured and stored in a freezer at -80°C.

Reverse transcription of miRNA-192-5p: add 11 μL of RNase-free water to 4 μL of total RNA samples and 2 μL of primer working solution, mix, centrifuge, allow to stand at 70°C for 10 min, incubated with ice for 2 min, and then RT reaction was performed (Table 2); after the end of the RT reaction, the extracted product of the reverse transcribed cDNA was placed on ice for cooling or stored in a freezer at -80°C.

The relative expression level of miRNA-192-5p was measured by the real-time fluorescence quantitative PCR: primer sequences of miRNA-192-5p and U6 as internal references are shown in Table 3. We prepared the real-time quantitative PCR reaction system, mixed, and centrifuged at 3000 r/min; added 20 μL of prepared mixture into the PCR plate, and set three parallel controls; then we performed the PCR plate sealing, centrifuged, and put it on ice or in a refrigerator at 4°C, and waited for the PCR reaction. The reaction procedure was as described below: 95°C for 3 min; 95°C for 15 s, 60°C for 30 s, and 72°C for 25 s, 40 cycles in total; 95°C for 15 s; and 60°C for 15 s. Three wells were averaged for each sample. The relative expression level of miRNA-192-5p was calculated by the following formula: 2-ΔΔCt.

Statistical methodology

In current study, the data obtained were addressed by the SPSS 20.0 statistical software. Quantitative data were presented as (x ± sd) and analyzed by t-test; qualitative data were presented as frequency or percentage and analyzed by χ² test; the diagnostic efficacy of serum miRNA-192-5p for MM was analyzed based on ROC curves; and the correlation of relative expression level of serum miRNA-192-5p with relevant clinical parameters in MM patients was analyzed by the Pearson coefficient test. P < 0.05 suggested a statistically significant difference.

Results

Comparison of the relative expression levels of serum miRNA-192-5p between the two groups

The relative expression level of serum miRNA-192-5p was 0.66 ± 0.20 in MM patients and 1.14 ± 0.28 in the control group. Moreover, the relative expression level of serum miRNA-192-5p was notably lower in the observation group than in the control group (t = 12.560, P < 0.001), as shown in Figure 1.

ROC curve analysis of serum miRNA-192-5p in the diagnosis of multiple myeloma

The results of ROC curve analysis showed that the AUC area of miRNA-192-5p for MM diagnosis was 0.853, with a cutoff value of 0.72, the sensitivity of 86.30%, and the specificity of 81.20% (P = 0.030) (Figure 2).

Comparison of the relative expression level of serum miRNA-192-5p in patients with different clinical parameters of multiple myeloma

The relative expression level of serum miRNA-192-5p in MM patients with high β2-MG level was markedly lower than that in MM patients with normal β2-MG level (P < 0.05), the relative expression level of serum miRNA-192-5p in
miRNA in the peripheral blood of multiple myeloma patients

The content of miRNA-192-5p in the serum of MM patients was significantly negatively correlated with that of IgG and IgA, respectively (P < 0.05) (Table 5).

Discussion

The specific pathogenesis of multiple myeloma is fiendishly complex and still unclear. Although targeted drug therapy has led to certain improvements in the prognosis of patients at this stage, it has still failed to radically cure this disease. Therefore, it is of great significance to find tumor markers that predict the risk of early MM and monitor the prognosis for timely treatment, enhancement of efficacy and improvement of prognosis. Studies have revealed that cytokines, bone marrow microenvironment, and activation of proto-oncogenes exert an important influence on the pathogenesis of MM, and these factors will eventually function in regulating the abnormal proliferation and apoptosis of MM cells through several signaling pathways such as NF-κB and Ras/Raf/Mek/Erk [10, 11]. In recent years, miRNA has become a hot topic in virology, oncology, embryonic development etc., with high conservation and tissue specificity, it acts as a regulator of several metabolic processes in vivo. Small miRNA can regulate the capability of most proteins to code and translate biologically. It is thought to be closely related to the proliferation and apoptosis of cancer cells, and miRNA has been confirmed to function in anti-oncogenes or pro-oncogenes in the development and progression of various tumor diseases such as leukemia, lymphoma, and breast cancer [12-14]. In pathological studies of MM, miRNA is considered to be an important gene regulatory element. For example, miR-186 inhibits the proliferation of MM cells by inhibiting Jagged1 [15, 16].

Table 3. Primer sequences of miRNA-192-5p and U6 as internal references

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Primer sequences</th>
<th>Annealing temperature</th>
<th>Product length</th>
</tr>
</thead>
<tbody>
<tr>
<td>U6</td>
<td>F: 5’GCTTCGCAGCACATATAAAT3’</td>
<td>60</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>R: 5’CGCTTCAGCAATTGGCTGAT3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>miRNA-192-5p</td>
<td>F: 5’GGGGCTGACCTATGAATTG3’</td>
<td>60</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>R: 5’CAGTGCGTCTGCAGGAGT3’</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Comparison of the relative expression levels of serum miRNA-192-5p between the two groups.

Figure 2. ROC curve analysis of serum miRNA-192-5p in the diagnosis of multiple myeloma.

MM patients with high creatinine level was notably lower than that in MM patients with normal creatinine level (P < 0.05), and the relative expression level of serum miRNA-192-5p in MM patients with low hemoglobin and albumin levels was remarkably lower than that in MM patients with normal hemoglobin and albumin level (P < 0.05) (Table 4).
miRNA in the peripheral blood of multiple myeloma patients

11q13, and evolutionarily conserved. It has the potential to promote the expression of p53, activate downstream related signaling pathways and inhibit tumor development; miRNA-192-5p, one of the two mature chains produced by miRNA-192, is a multifunctional regulator that provides positive feedback on p53 and becomes an indispensable part in the physiopathology of different diseases [17-19]. Studies have proven that miRNA-192-5p is involved in the pathogenesis of liver cancer by inhibiting the expression of ZEB2 mRNA [20]. Highly expressed miRNA-192-5p has been shown to significantly inhibit the proliferation of MM cells, with the inhibition becoming more pronounced over time, and also to enhance the apoptotic effect of the mainstream chemotherapy drug bortezomib on MM cells [20]. The results of this study showed that the relative expression level of serum miRNA-192-5p in MM patients (0.66 ± 0.20) was remarkably lower than that in the control group (1.14 ± 0.28) (P < 0.05), suggesting that miRNA-192-5p showed low expression levels in MM, that it may be involved in the occurrence and development of MM, and that it may function as an oncogenic suppressor gene in MM, which was similar to the above study conclusions.

At present, bone marrow biopsy is still the gold standard for the MM diagnosis. It is traumatic and prone to cause infection from multiple rounds of testing [21]. Recent studies have shown that certain specific miRNA molecules can be detected in the peripheral blood serum of patients with a variety of tumor diseases, and these circulating miRNA molecules exhibit good tumor correlation, tissue specificity and stability. Additionally, the detection of serum miRNA is minimally invasive, simple and convenient. It has been proven by the results of domestic and foreign studies that the serum detection levels of multiple miRNAs have high sensitivity, specificity and accuracy in disease diagnosis and prognosis evaluation [22, 23]. Therefore, miRNA is expected to be a novel biomarker for the diagnosis of tumors. The results of ROC curve analysis showed that the AUC area of serum miRNA-192-5p for MM diagnosis was 0.853, with a cutoff value of 0.72, sensitivity of 86.30%, and specificity of 81.20%, P = 0.030. To further investigate the clinical value of serum miRNA-192-5p on MM, the analysis of the relative expression level of serum miRNA-192-5p and clinically relevant parameters of patients, this study found that the relative expression level of serum miRNA-192-5p in the serum of patients with high β2-MG and creatinine levels was notably lower than that in patients with low β2-MG levels (P < 0.05), the relative expression level of serum miRNA-192-5p in the serum of patients with low hemoglobin and albumin levels was significantly lower than that in patients with normal hemoglobin and albumin levels (P < 0.05), and the relative expression level of serum miRNA-192-5p in MM patients was significantly negatively correlated with IgG and IgA levels, respectively (P < 0.05); which all indicated that there was a certain correlation between serum miRNA-192-5p and the severity of MM, and the lower expression level was associated with the worse prognosis of patients.

In summary, since miRNA-192-5p shows abnormally low expression in the serum of MM patients, it may serve as an auxiliary diagnostic tool in the diagnosis of MM. The limitation of

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**Table 4.** Comparison of the relative expression level of serum miRNA-192-5p in patients with different clinical parameters of multiple myeloma (X ± sd)

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>n</th>
<th>miRNA-192-5p</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>β2-MG</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>≤ 3.5 mg/L</td>
<td>54</td>
<td>0.72±0.20</td>
<td>3.795</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>&gt; 3.5 mg/L</td>
<td>34</td>
<td>0.56±0.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 150 μmol/L</td>
<td>50</td>
<td>0.71±0.19</td>
<td>3.318</td>
<td>0.002</td>
</tr>
<tr>
<td>&gt; 150 μmol/L</td>
<td>38</td>
<td>0.59±0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>43</td>
<td>0.72±0.22</td>
<td>2.679</td>
<td>0.008</td>
</tr>
<tr>
<td>Low</td>
<td>45</td>
<td>0.60±0.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>36</td>
<td>0.73±0.22</td>
<td>2.884</td>
<td>0.005</td>
</tr>
<tr>
<td>Low</td>
<td>52</td>
<td>0.61±0.17</td>
<td></td>
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</tbody>
</table>

**Table 5.** Analysis of correlation between serum miRNA-192-5p and immunoglobulin in patients with multiple myeloma

<table>
<thead>
<tr>
<th>Indicator</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>-0.608</td>
<td>0.005</td>
</tr>
<tr>
<td>IgA</td>
<td>-0.660</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
this study is that the number of patients included is small, and long-term follow-up is not performed. The influence of serum miRNA-192-5p on the prognosis of MM patients has not been discussed. In the future, longer-term follow-up trials will be needed to obtain more comprehensive clinical data. Furthermore, because there is certain correlation between serum miRNA-192-5p and MM progression and prognosis, it may be regarded as a novel marker for MM monitoring.

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Disclosure of conflict of interest

None.

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