Original Article
Differential microRNA expression in the serum of patients with nephrotic syndrome and clinical correlation analysis

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Abstract: Many different microRNAs existed in nephrotic syndrome patients, and they may be involved in nephrotic syndrome occurrence. In order to further clarify miRNAs expression changes in nephrotic syndrome patients and their correlation with clinical features, this study investigated differential microRNA expression in the peripheral serum of patients with nephrotic syndrome and analyzed the correlation between miRNA with largest overexpression level and clinical features. miRNAs microarray was applied to screen different expressed miRNAs in nephrotic syndrome patients. Real-time PCR was performed to verify miRNA expression level. SPSS software was used to analyze correlation between miRNA expression and clinical features. Compared with healthy subjects, 35 miRNAs overexpressed and 24 miRNAs down-regulated in patients. After real-time PCR verification, 6 miRNAs up-regulated in nephrotic syndrome patients, including hsa-miR-181a, hsa-miR-210, hsa-miR-30a, hsa-miR-942, hsa-miR-192 and hsa-miR-586. miRNA-30a significantly overexpressed in nephrotic syndrome patients and with no difference between genders. miRNA-30a expression level in drug resistant nephrotic syndrome patients was obviously higher than the drug sensitive patients. miRNA-30a up-regulated most significantly in mesangial proliferative glomerulonephritis among different pathology types, while it decreased most obviously in glomerular lesions, miRNA differently expressed in the serum of nephrotic syndrome patients. miRNA-30a could be treated as the molecular marker in predict drug resistance and pathological type of nephrotic syndrome.

Keywords: Microarray, miRNA-30a, nephrotic syndrome, clinical correlation

Introduction

Nephrotic Syndrome (NS) is a comprehensive disease characterized as glomerulus basement membrane permeability increase by multiple factors (such as oxidative stress, immune, etc.). Clinical characteristics mainly include proteinuria, hypoalbuminemia, edema, and hyperlipidemia. At present, the diagnosis of nephrotic syndrome pathology mainly relies on the renal biopsy. However, renal biopsy is an invasive examination but lack of sensitivity and specificity. Therefore, searching for specific and sensitive method to identify the pathological types of nephrotic syndrome is of great significance.

MicroRNAs is a kind of non-coding RNA about 19-24 nucleotides that exist in eukaryotes. It could degrade mRNA or inhibit mRNA transcription through the 3'-UTR region of the target mRNAs. Because of its conservative feature in evolution and important role in the physiological function, it could be treated as predictors for disease classification and clinical process based on specific expression. Many studies have shown that microRNAs could regulate kidney cells in a variety of different pathological types of nephrotic syndrome. For example, miRNA-192 is the only miRNA that has been found expressed in glomerular mesangial cells, and it may affect pathological occurrence by participating in extracellular matrix proteins accumulation. In addition, the miRNA-29c expression changes causes transforming growth factor β1 (TGF-β1) abnormal expression, leading to renal interstitial fibrosis occurrence [1-3]. A variety of miRNAs play an impor-
Differential expressed miRNA in nephrotic syndrome

This study investigated miRNAs expression in the serum of nephrotic syndrome patients and discussed the correlation between overexpressed miRNA and clinical features, providing auxiliary examination indexes for nephrotic syndrome.

Materials and methods

**Taqman low density miRNA microarray**

800 μl blood was extracted from 25 cases of nephrotic syndrome patients and 20 cases of health controls. RNA was extracted and stored at -80°C. MiRNA expression level was detected by ABI Company. U6 was selected as control. The study protocol was approved by the Research Ethics Committee of our hospital, and all patients gave their informed consent before study commencement.

**Serum RNA extraction**

RNA was extracted and stored at -80°C for RT-PCR.

**Real-time PCR**

MiRNA was reverse transcribed to DNA using PCR kit (Takara, Japan). Each real-time RT-PCR reaction (in 20 μL) contained 2.5×SYBR Green Mixture, 5 μM primers and 2 μL template cDNA. The cycling conditions consisted of an initial, single cycle of 30 s at 95°C, followed by 40 cycles of 30 s at 95°C, 30 s at 55°C, and 60 s at 72°C. PCR amplifications (ABI 7500 PCR amplifier, Applied Biosystem) were performed in three duplicates for each sample.

**Statistical analysis**

Numerical data were presented as means and standard deviation (± SD). All statistical analyses were performed using SPSS13.0 software (SPSS Inc., USA). Differences between multiple groups were analyzed by t-test or one-way ANOVA. *P* < 0.05 was considered as significant difference.

**Results**

**Microarray result analysis**

Taqman low density microarray was applied to detect serum miRNA expression changes in

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**Table 1.** Up-regulated miRNAs in the serum of nephrotic syndrome patients

<table>
<thead>
<tr>
<th>miRNA ID</th>
<th>NS patient signal strength</th>
<th>Healthy subjects signal strength</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsa-miR-923</td>
<td>17130</td>
<td>10324</td>
<td>1.66</td>
</tr>
<tr>
<td>hsa-miR-942</td>
<td>10286</td>
<td>6584</td>
<td>1.56</td>
</tr>
<tr>
<td>hsa-miR-181a</td>
<td>14987</td>
<td>10643</td>
<td>1.41</td>
</tr>
<tr>
<td>hsa-miR-1308</td>
<td>1467</td>
<td>364</td>
<td>4.03</td>
</tr>
<tr>
<td>hsa-miR-151</td>
<td>1044</td>
<td>548</td>
<td>1.90</td>
</tr>
<tr>
<td>hsa-miR-222</td>
<td>887</td>
<td>432</td>
<td>2.05</td>
</tr>
<tr>
<td>hsa-miR-191</td>
<td>765</td>
<td>345</td>
<td>2.22</td>
</tr>
<tr>
<td>hsa-miR-210</td>
<td>11243</td>
<td>10189</td>
<td>1.10</td>
</tr>
<tr>
<td>hsa-miR-30a</td>
<td>2340</td>
<td>870</td>
<td>2.69</td>
</tr>
<tr>
<td>hsa-miR-231</td>
<td>1743</td>
<td>786</td>
<td>2.22</td>
</tr>
</tbody>
</table>

---

**Table 2.** Down-regulated miRNAs in the serum of nephrotic syndrome patients

<table>
<thead>
<tr>
<th>miRNA ID</th>
<th>NS patient signal strength</th>
<th>Healthy subjects signal strength</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsa-miR-16</td>
<td>2443</td>
<td>2614</td>
<td>0.93</td>
</tr>
<tr>
<td>hsa-miR-510</td>
<td>843</td>
<td>2170</td>
<td>0.39</td>
</tr>
<tr>
<td>hsa-miR-172</td>
<td>2659</td>
<td>3430</td>
<td>0.78</td>
</tr>
<tr>
<td>hsa-miR-1245</td>
<td>2848</td>
<td>2934</td>
<td>0.97</td>
</tr>
<tr>
<td>hsa-miR-633</td>
<td>3445</td>
<td>5676</td>
<td>0.61</td>
</tr>
<tr>
<td>hsa-miR-320</td>
<td>545</td>
<td>789</td>
<td>0.69</td>
</tr>
<tr>
<td>hsa-miR-1324</td>
<td>2911</td>
<td>3455</td>
<td>0.84</td>
</tr>
<tr>
<td>hsa-miR-626</td>
<td>3004</td>
<td>3435</td>
<td>0.87</td>
</tr>
<tr>
<td>hsa-miR-383</td>
<td>31426</td>
<td>53435</td>
<td>0.87</td>
</tr>
<tr>
<td>hsa-miR-422a</td>
<td>2736</td>
<td>3812</td>
<td>0.72</td>
</tr>
</tbody>
</table>
Differential expressed miRNA in nephrotic syndrome

Figure 1. qRT-PCR verification. **P < 0.05 compared with healthy subjects.

Table 3. Correlation analysis between miR-30a expression and NS patients’ clinical features

<table>
<thead>
<tr>
<th>Index</th>
<th>NS</th>
<th>Healthy subjects</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>7.8 ± 4.5</td>
<td>2.3 ± 1.3</td>
<td>0.023</td>
</tr>
<tr>
<td>Female</td>
<td>7.2 ± 3.6</td>
<td>1.5 ± 0.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Drug resistance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hormone sensitive</td>
<td>6.3 ± 3.6</td>
<td>2.0 ± 1.3</td>
<td>0.003</td>
</tr>
<tr>
<td>Hormone resistance</td>
<td>8.6 ± 4.5 **</td>
<td>2.0 ± 1.3</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Pathological type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPGN</td>
<td>9.8 ± 4.5</td>
<td>2.0 ± 1.3</td>
<td>0.023</td>
</tr>
<tr>
<td>PCL</td>
<td>7.2 ± 3.6 **</td>
<td>2.0 ± 1.3</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>IGN</td>
<td>2.4 ± 4.5 **</td>
<td>2.0 ± 1.3</td>
<td>0.431</td>
</tr>
<tr>
<td>GMC</td>
<td>2.2 ± 3.6 **</td>
<td>2.0 ± 1.3</td>
<td>0.329</td>
</tr>
</tbody>
</table>

**P < 0.05, compared with healthy; **P < 0.05, compared with MPGN; ***P < 0.05, compared with PCL.

The screening standard was fold change ≥ 1.5 and P < 0.05. 6 miRNAs up-regulated in nephrotic syndrome patients, including hsa-miR-181a, hsa-miR-210, hsa-miR-30a, hsa-miR-942, hsa-miR-192 and hsa-miR-586 (Figure 1). miR-30a exhibited the largest overexpression level.

Correlation analysis between miR-30a expression and NS patients’ clinical features

A total of four kinds of different pathological types including mesangial proliferative glomerulonephritis (MPGN), podocyte lesion (PCL), and glomerular interstitial nephritis (IGN), and glomerular lesions (GMC) according to the kidney biopsy. MiR-30a expression level in different pathological types was listed in Table 3. MiRNA-30a significantly overexpressed in nephrotic syndrome patients and with no difference between genders. MiRNA-30a expression level in drug resistant nephrotic syndrome patients was obviously higher than the drug sensitive patients. miRNA-30a up-regulated most significantly in mesangial proliferative glomerulonephritis among different pathology types, while it decreased most obviously in glomerular lesions.

Discussion

Several researches suggested that miRNAs participate in a variety of disease by inhibiting mRNA expression and can be used as molecular diagnostic markers [11-13]. MiRNA-192 expression level in kidney was significantly higher than that of the bone marrow, while it plays an important role in the renal epithelial sodium ion transport. It has been found that kidney disease can lead to specific circulating miRNA expression change. Studies have suggested that miR-16 and miR-320 expression level significantly elevated in patients with acute kidney disease [14]. At the same time, serum miRNA showed stronger stability than the cells. Gui J et al. found that serum miR885-5p might be a potential biomarker for liver pathology [15]. MiR-126 differentially expressed in multiple tumors including renal cell...
Differential expressed miRNA in nephrotic syndrome
carcinoma, and can be used as the marker to
differentiate transparent cell carcinoma and papillary carcinoma [16]. Thus, quantitative
detection of miRNAs in the blood can be treat-
ed as a new method to detect and monitor kid-
ney disease.

In this study, TaqMan low density microarray
was applied to detect serum miRNAs expres-
sion in patients with nephrotic syndrome. Since
the microarray may have false positive result,
real-time PCR was used for validation. 35 miR-
NAs up-regulated in nephrotic syndrome
patients such as hsa-miR-30a, hsa-miR-221,
and hsa-miR-181a, of them miRNA-181a has
been reported. Sui W et al. found that miR-
181a, miR-483-5p, and miR-557 differentially
expressed in nephrotic syndrome, and might be
used as peripheral blood biomarkers for diag-
nosis [17]. Zhu et al. showed that after trans-
fection with Anti-miRNA-181a, tubular epitheli-
al cells apoptosis degree reduced treated by
DDP, indicating miR-181a may down-regulate
BAX expression and impact kidney disease
[18]. Our study also detected 24 down-regulat-
ed miRNAs, such as hsa-miR-320 and hsa-
miR-510. Johan M Lorenzen suggested that
plasma miR-320 and miR-16 expression level
decreased, while miR-210 increased (P <
0.0001) in patients with acute renal failure
[14]. They could prompt patient’s survival rate
and act as new biomarkers. In the study about
kidney transparent cell carcinoma, miR-210
overexpressed significantly in the cancer tissue
and adjacent normal tissue, and obviously cor-
related with shorter overall survival and dis-
ease recurrence [19].

Real-time PCR was used for miRNA verification.
The results showed that serum hsa-miR-181a,
hsa-miR-210 and hsa-miR-30 still up-regulated
in patients compared with the healthy control.
The pathogenesis of primary nephrotic syn-
drome is still unclear. Mathieson PW study
revealed that nephrotic syndrome occurrence
may be associated with glomerular layer sertoli
cell defects [20]. When the Dicer enzyme was
knockout in sertoli cells, adult mice appeared
significant proteinuria in four or five weeks after
birth, following with kidney failure. Some patho-
logical changes such as sertoli cell damage,
capillary expansion were due to the lack of miR-
NAs [21, 22], suggesting miRNAs play an impor-
tant role in maintaining normal renal function.
At the same time, it was found that miR-30a
plays an important effect on sertoli cell struc-
ture and function. Thus, it is reasonable to
speculate that miR-30a plays an important role
in nephrotic syndrome occurrence. Current
studies also found that serum miR-30a-5p,
miR-151-3p, miR-150, miR-191, and miR-19b
increased in children with nephrotic syndrome,
while urine miR-30a-5p expression also up-reg-
ulated [23]. Also, some researchers found that
[24], miR-30a may affect kidney development
and regulate kidney maturity by targeting trans-
scription factor Xlim1/Lhx1.

Correlation analysis between miRNA-30a and
kidney disease clinicopathological characteristics
showed that miR-30a failed to present dif-
ference between different genders, while it was
different between drug resistant or sensitive
cases. MiRNA-30a overexpressed more signifi-
cantly in drug resistant cases. In patients with
different pathological types, miR-30a showed
obvious statistical difference in mesangial pro-
iferative glomerulonephritis and sertoli cell
lesions with glomerular interstitial nephritis
and glomerular lesions. It may be because of
the miR-30a influence on sertoli cell apoptosis
and proliferation.

Based on previous studies results, miRNA-30a
overexpressed in kidney development [25].
Together with our results, it suggested that
miR-30a may help diagnose drug resistance
and pathological type. It still needs further
investigation about the mechanism of miR-30a
influence on nephrotic syndrome occurrence
and different pathological types. At the same
time, since the population study might be
affected by many factors, further in vitro study
is needed.

Multiple miRNAs abnormal expressed in the
serum of nephrotic syndrome patients, sug-
gesting that miRNAs could be treated as the
serum biomarkers to differentiate pathological
classification and drug resistance. This study
only studied miR-30a, whether other miRNAs
changes exist in the serum of patients with
nephrotic syndrome and related in different
pathological types still need further research.

Disclosure of conflict of interest
None.

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References


