Liquid biopsy for chronic rejection after kidney transplantation - a novel non-invasive biomarker involved in Inflammation Amplifier -

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Materials and Methods

1) CAAMR: Chronic active antibody-mediated rejection

In kidney transplantation (KTx), chronic active antibody-mediated rejection (CAAMR) is particularly a problem because of 25% account for graft loss even after 2000. However, it remains unclear what progresses the stage of CAAMR. For this reason, CAAMR has no diagnostic biomarkers. Detection of the process mechanism will permit early intervention and preservation of allograft function.

2) Inflammation Amplifier (IA)

We previously found an excessive NF-kB-activating mechanism in non-immune cells is induced by the co-activation of NFkB and STATs, which is essential for the development of various disease models and associated with several human diseases.

3) Liquid biopsy using urine samples

In recent years, liquid biopsy technology enables minimally invasive diagnosis. In particular, it has been actively conducted to analysis of exosomes, which contain disease-specific information.

Purpose

This study aims to research a novel biomarker for CAAMR based on IA using liquid biopsy technique.

Results

1) RNA sequence analysis

- 6646 genes were stimulated by IL-6 + IL-17 or TNFα using HRGEC, RPTEC, and MVEC.
- 25 genes were high reactivity after 6 and 24 hours.
- No relationship with kidney diseases
- 5 genes were possibility of new biomarkers

2) Immunohistochemistry (IHC)

To confirm the expression of KIAM protein in kidney tissue, we performed IHC using frozen sections of transplanted kidney biopsies.

Ex.1 RNA sequence analysis

Ex.2 Immunohistochemistry (IHC)

Table 1: Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>NED (n=20)</th>
<th>IF/TA (n=19)</th>
<th>CNI-T (n=17)</th>
<th>CAAMR (n=22)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, Male</td>
<td>60%</td>
<td>47%</td>
<td>76%</td>
<td>68%</td>
<td>NS</td>
</tr>
<tr>
<td>Age at transplant</td>
<td>43±22</td>
<td>29±20</td>
<td>44±16</td>
<td>26±19</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Observe period (year)</td>
<td>3.1±4.1</td>
<td>3.9±6.3</td>
<td>6.9±4.7</td>
<td>8.9±4.2</td>
<td>&lt;0.01</td>
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<tr>
<td>ABO incomp. yes</td>
<td>49%</td>
<td>16%</td>
<td>29%</td>
<td>5%</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Living donor</td>
<td>85%</td>
<td>74%</td>
<td>76%</td>
<td>77%</td>
<td>NS</td>
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<tr>
<td>Kidney age (year)</td>
<td>56±11</td>
<td>56±15</td>
<td>58±12</td>
<td>56±11</td>
<td>NS</td>
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<tr>
<td>Dialysis period (year)</td>
<td>3.4±5.8</td>
<td>4.1±6.8</td>
<td>6.0±6.8</td>
<td>4.7±8.7</td>
<td>NS</td>
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<td>HLA mismatch</td>
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<td>2.6±1.5</td>
<td>2.8±1.8</td>
<td>2.8±0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Kidney age†</td>
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<td>13±2</td>
<td>14±2</td>
<td>12±2</td>
<td>NS</td>
</tr>
</tbody>
</table>

Ex.1: RNA sequence analysis

Ex.2: Immunohistochemistry (IHC)

Conclusion

Urinary exosomal KIAM has a potential for diagnostic biomarker of CAAMR.