Expression of microRNAs in peripheral blood mononuclear cells associated with the progression of knee osteoarthritis

INTRODUCTION: Osteoarthritis (OA) is the most widespread acquired connective tissue disorder that affects the synovial joints. Many studies which focused on the articular cartilage of OA such as chondrocytes, extracellular matrix, and chemokines were conducted. However, the lesion of OA is not limited articular cartilage as well as bone and synovial tissues. Especially, the inflammation in synovial tissues has been well documented, suggesting that synovial tissues might play a crucial role in the pathogenesis and progression of OA. Several studies demonstrated that mononuclear cells such as T cells aggregated in OA synovial tissues, which participated in the cartilage degradation. Many evidence clarified that OA might be the systemic diseases rather than a joint disease.

Recently, it has become evident that genetic alterations in non-coding genes can also contribute to the pathogenesis of human disease. A new class of small non-coding RNAs, named microRNAs (miRNAs), regulates gene expression. Many miRNAs are evolutionarily conserved across phyla, and several miRNAs exhibit a tissue-specific or developmental stage-specific expression pattern and have been reported to be associated with human diseases such as cancer, leukemia, and viral infection. miRNAs might play a role in joint disorder, such as rheumatoid arthritis (RA) and OA.

The objective of this study was to investigate the expression pattern of microRNAs in peripheral blood mononuclear cells (PBMC) from patients with OA, as a new biomarker for OA diagnosis.

METHODS: This study was approved by the Institutional Review Board of Hiroshima University and was conducted in accordance with the Helsinki Declaration. Thirty six patients (seven males, twenty eight females; mean age 68.0 ± 1.0 years, range 32-83; mean Body Mass Index(BMI) 25 ± 4.1, range 19.6-41.4) with OA, and ten healthy subjects (nine males, one female; mean age 68.0 ± 1.0 years, range 32-82; mean Body Mass) were included in this study. OA was diagnosed according to the American College of Rheumatology criteria. The volunteers were healthy with no gross obesity, inferior limb malalignment, and history of knee injury or knee disorders.

We chose 4 miRNAs, miR-146a, miR-155, miR-181a and miR-223, which express in immune cells and regulate immune function and inflammation, and analyzed their expression in OA PBMC compared with healthy subjects, and examine their altered expression in difference OA stage, and correlation the expression of these miRNAs with BMI, age, FTA and serum keratan sulfate level.

Human peripheral blood was collected from healthy subjects and OA patients and the expression of miR-146a, miR-155, miR-181a and miR-223 was analyzed using real-time PCR. To examine the expression level of miR-146a, miR-155, miR-181a and miR-223 in OA PBMCs, these samples were devided into three grades (grade II, III and IV) according to Kellgren-Lawrence classification.

Statistical analysis was performed performed using Stat View version 5.0 statistical package. Significance was set at P<0.05.

RESULTS: The average relative expression levels of miR-146, miR-155 and miR-223 were 5.8, 7.6, and 12.6-fold, respectively, higher for OA patients than for healthy subjects (P<0.01 for miR-146a, miR-223 and P=0.05 for miR-155, as determined by Mann-Whitney U test). The average relative expression levels of miR-181a was not significantly different between OA patients and healthy subjects (Figure 1).

As for the relative expression levels of miR-146a, miR-155 and miR-223 according to Kellgren-Lawrence classification, that of miR-146, 181a and miR-223 were intensly expressed in OA PBMCs with a low grade of the Kellgren-Lawrence classification. That of miR-155 was not significantly different between each grades (Figure 2).

Clinical characteristics of the patients allowed us to study potential correlations between microRNAs expression and clinico-pathological parameters. We selected age, BMI, femorotibial angle (FTA) and the concentration of the keratin sulfate (KS) as clinico-pathological parameters (Table 1).

microRNA expression correlates with age miR-181a is correlated with age (p=0.034, r=0.4157).

microRNA expression correlates with BMI

These four microRNAs are not correlated with BMI.

microRNA expression correlates with FTA

miR-146 is correlated with femorotibial angle (p=0.030, r=0.4219)

miR-223 is correlated with the concentration of keratan sulfate (p=0.037, r=0.406). MiR-146, MiR-155 and miR-181a are not correlated with age.

DISCUSSION: miRNAs which express in immune cells and regulate immune function and inflammation are expressed intensely in OA patients, compared with healthy subjects. This results suggests that these miRNAs which express specifically in OA peripheral blood play a role in OA pathogenesis, and especially the expression of miRNAs is concerned with progressive OA. This study leads to become the new biological blood marker to diagnose early OA and predict its prognosis, and new therapeutic target for treatment of OA.

And by combining miRNA expression level and clinico-pathological marker, the pathogenesis of OA may be elucidated.

Table 1. Association of clinico-pathological parameters with the relative expression of miRNAs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>miR-146a</th>
<th>miR-155</th>
<th>miR-181a</th>
<th>miR-223</th>
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<tr>
<td>Age</td>
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<td>0.4157</td>
<td>0.037</td>
<td>0.406</td>
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<tr>
<td>BMI</td>
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<td>0.4219</td>
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<td>0.406</td>
</tr>
<tr>
<td>FTA</td>
<td>0.030</td>
<td>0.4219</td>
<td>0.037</td>
<td>0.406</td>
</tr>
<tr>
<td>KS</td>
<td>0.037</td>
<td>0.406</td>
<td>0.037</td>
<td>0.406</td>
</tr>
</tbody>
</table>

Figure 1. Expression of miRNAs in PBMC of OA patient and healthy volunteer

Figure 2. Expression of miRNAs in PBMC of OA patients and healthy subjects

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