Bioinformatic Prediction of Neuropathic Pain Signaling Pathways in Rheumatoid Arthritis after high throughput miRNA analysis

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Abstract
microRNAs (miRNAs) are a special subclass of RNAs which tend to prevent the expression of genes at the post-transcriptional level by “silencing” them. That is, gene expression is prevented. This ultimately affects the pathways that they are a part of and the products produced by these pathways. Due to the ability of miRNAs to bind to any base pair complementary to its own base, inhibiting expression of the sequence it is found is known to be often involved in multiple cellular pathways. The regulation between a specific outcome and a specific pathway that a miRNA is involved is hard to establish because of the sheer number of potential pathways one miRNA could be involved in. Bioinformatic prediction is useful because it allows you to identify the most probable pathways. That is, through the careful analysis of miRNA targets and the prediction of the targets, interactions in biological mechanisms are uncovered. In this project, we predicted the pathways related to deregulation of miR-223-3p and miR-16-5p. Both miRNAs were correlated with neuropathy-related clinical measures (neuropathic pain (ID Pain)) and the Toronto Clinical Neuropathy Score (TCNS) respectively. Pearson correlation coefficients were calculated between scores of 60+ and 90+ to carry out a gene ontology analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG). Conducted through DAVID Software from the National Institute of Biomedical Imaging and Health Sciences, KEGG identified 72 pathways as the most likely affected by deregulation of miR-223-3p and miR-16-5p. Several pathways on this list, including the Fos family, autophagy and sphingolipid signaling pathways are involved in RA and neuropathic outcomes. Future studies will have to determine whether both miRNAs affect these pathways directly or in relation to RA.

Introduction
• Rheumatoid Arthritis is a chronic disease that causes inflammation and pain of the joints. It affects the joints of 1.5 million adults in the United States annually.¹
• Neuropathic pain, small-fiber neuropathy, and peripheral neuropathy present more frequently in patients with RA in comparison with subjects without the disease.²
• The mechanism underlying this phenomenon is poorly understood.

Methods

Stage 1

High Throughput Analysis of 800 miRNAs Nanotagging nCounter

Top 20 Hits Selected for Analysis

Clinical Variable Correlations
SPSS statistical analysis of clinical variables and miRNA level

miRNA Target Identification
miRDB, TargetScan, miRWalk online database

Pathway Identification and Ranking
DAVID software generated KEGG pathway and GO term list

Stage 2

Visual Map of Pathway
DAVID also provided pathway maps for each identified pathway

Results

Table 1: Demographic and Clinical Characteristics of RA Patients in ERA Study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>RA patients</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>57.5 ± 2.1</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>357/308</td>
</tr>
<tr>
<td>Disease Duration (years)</td>
<td>18.9 ± 5.5</td>
</tr>
<tr>
<td>DMARD Use</td>
<td>80.0%</td>
</tr>
<tr>
<td>SLEDAI</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>60</td>
</tr>
</tbody>
</table>

Figure 1: MicroRNA biogenesis and mechanism of gene regulation. Modified from Lin Y et al., Cancer Prevention Research, 2013.

Figure 2: miR-223-3p and miR-16-5p correlate with measures of neuropathy and pain. (A) miR-16-5p positively correlates with the Toronto Clinical Neuropathy Score (TCNS) (2017). (B) miR-223-3p positively correlates with ID Pain, a measure of neuropathic pain.

Figure 3: Bar graph indicating a group of significant KEGG pathways affected by the deregulation of miR-223-3p and miR-16-5p. Values represent p-values of each pathway analysis. The pathway with the most significant p-value is indicated by a solid dot. (A) miR-223-3p; (B) miR-16-5p.

Figure 4: Unsupervised hierarchical cluster analysis of miRNAs identified in association to clinical RA characteristics. Twenty RA patients were included in this analysis.

Table 2: Ranking of target miRNAs

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Target</th>
<th>p-value</th>
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<tbody>
<tr>
<td>miR-16-5p</td>
<td>MAP2K1</td>
<td>0.014</td>
</tr>
<tr>
<td>miR-223-3p</td>
<td>MAP2K1</td>
<td>0.556</td>
</tr>
</tbody>
</table>

Figure 5: The Neurotrophin Signaling Pathway. Modified from david-d.ncifcrf.gov.

Figure 6: Macrophage biogenesis and mechanism of gene regulation. Modified from Lin Y et al., Cancer Prevention Research, 2013.

Figure 7: miRNAs are endogenous, single-stranded, non-coding RNAs that are 18–25 nucleotides long.³

Figure 8: They regulate gene expression in cells throughout the body, including those of the nervous system.³

Figure 9: Recent studies have shown that miRNAs play a key role in the pathogenesis of RA and other autoimmune diseases.⁴

Figure 10: Similarly, studies have shown that miRNAs play a role in neuropathy and neuropathic pain.⁵ ⁶

Figure 11: miR-143a-3p and miR-145-3p have been related to neuropathy in human and mice.⁷ In addition, miR-126 and miR-135 have been shown to be associated with neuropathic pain.⁸ ⁹

Figure 12: Only one study has described the association of miR-8-3p with neuropathic pain in RA.⁹

References