MicroRNA Expression Profiles Associated with Response to Adalimumab in Patients with Early Rheumatoid Arthritis

Results from the OPERA Study

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Disclosures

• None

• The clinical study was supported by unrestricted grants from Abbott Laboratories, Denmark and Meda Pharmaceuticals, Denmark

• The companies were not involved in study set-up, data collection, analysis or publishing of data

Evidence-based medicine

- Aiboudiotis A et al. J Rheumatol 2011;38:645-51
- Skov K et al. J Rheumatol 2011;38:2066-71
- Li et al. J Rheumatol 2011;38:2382-91
- Tottori et al. J Rheumatol 2011;38:1281-8

Background

TNF blocking agents

- Improve the outcome in patients with severe RA
- Not effective in all patients

Factors associated with response to TNF blocking agents

Concomitant treatment with DMARDs

Low HAQ at baseline

Negative Anti-CCP

Anti drug antibodies

Smoking

Genetic variations

Their ability to predict response in clinical practice is poor

Epigenetics

The study of heritable information that is carried by the genetic material but is not encoded in the DNA sequence*

Epigenetics such as microRNAs may represent an important link between clinical, serological, environmental and genetic factors influencing response to TNF blocking agents

microRNA (miRNA)

~22 nt single stranded non-coding RNAs

Regulators of gene expression

Play essential role in basic biological functions

Development and fine-tuning of the immune system

2042 human miRNAs (miRBase.org)

>100 miRNAs in immune cells


O’Connell RM et al. Nat Rev Immunol 2010

10/27/2013
miRNA and RA

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Peripheral blood mononuclear cells</th>
<th>Synovial fluid</th>
<th>Fibroblast-like synoviocytes</th>
<th>Synovium</th>
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</thead>
<tbody>
<tr>
<td>miR-16</td>
<td>↑</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-21</td>
<td>↓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-34a</td>
<td>↓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-124a</td>
<td>↓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-146a</td>
<td>↑, ↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>miR-155</td>
<td>↑</td>
<td></td>
<td></td>
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<tr>
<td>miR-203</td>
<td>↑</td>
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<tr>
<td>miR-223</td>
<td>↑</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>miR-323-3p</td>
<td>↑</td>
<td>↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-346</td>
<td>↑</td>
<td></td>
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</tr>
</tbody>
</table>

Aim
To investigate the association between miRNAs and treatment response in pre-treatment whole blood from 180 patients with early RA enrolled in the OPERA Study.

The OPERA Study
- OPERA* (Optimized treatment algorithm for patients with Early RA)
- Danish investigator initiated, prospective, double blinded study
- 180 patients with early treatment-naive rheumatoid arthritis (ACR 1987)
- Disease duration <6 months
- DAS28 >3.2
- 1 year follow-up

Participating hospitals

Outcome measures in the miRNA sub-study
- Primary outcome
  - EULAR response
    - good versus moderate/no response
- Secondary outcomes
  - DAS28 remission
  - ACR/EULAR Boolean remission

Methods
- Pre-treatment whole blood collected in Paxgene RNA tubes
- miRNA expression analysis
  - TaqMan® Human MicroRNA assay LDA A Card v2.0 (Applied Biosystem)
  - 377 miRNAs
    - Cycle threshold (Ct)
      - The number of cycles in PCR
      - Low Ct values = high expression of the miRNA
- Normalization of raw Ct values
  - 120 most expressed
  - Rank
  - Quantile

* Hørslev-Petersen K et al. Ann Rheum Dis Online First 7 Mar 2013
Identification of miRNA associated with response

1. Interaction between miRNAs and treatment for each outcome
   p < 0.05 in all 3 normalization methods → potential miRNAs

2. Kolmogorov-Smirnov tests for each outcome measure
   Outcome measures with p <0.05
   Potential miRNAs from step 1

3. Multi-variate model
   Backward elimination

1. Potential miRNAs
   - EULAR response
     - 9 miRNAs
   - DAS28 remission
     - 5 miRNAs
   - ACR/EULAR Boolean remission
     - 8 miRNAs

3. miRNAs associated with EULAR response

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Treatment</th>
<th>Normalisation</th>
<th>OR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-22</td>
<td>Adalimumab + methotrexate</td>
<td>120 most expressed</td>
<td>15.32</td>
<td>3.43-137.06</td>
<td>0.001</td>
</tr>
<tr>
<td>miR-22</td>
<td>Adalimumab + methotrexate</td>
<td>Rank</td>
<td>10.26</td>
<td>2.29-95.66</td>
<td>0.01</td>
</tr>
<tr>
<td>miR-22</td>
<td>Adalimumab + methotrexate</td>
<td>Quantile</td>
<td>12.18</td>
<td>2.30-140.45</td>
<td>0.01</td>
</tr>
<tr>
<td>miR-22</td>
<td>Placebo + methotrexate</td>
<td>120 most expressed</td>
<td>0.54</td>
<td>0.22-1.28</td>
<td>0.17</td>
</tr>
<tr>
<td>miR-22</td>
<td>Placebo + methotrexate</td>
<td>Rank</td>
<td>0.60</td>
<td>0.26-1.40</td>
<td>0.22</td>
</tr>
<tr>
<td>miR-22</td>
<td>Placebo + methotrexate</td>
<td>Quantile</td>
<td>0.71</td>
<td>0.39-1.40</td>
<td>0.46</td>
</tr>
<tr>
<td>miR-886</td>
<td>Adalimumab + methotrexate</td>
<td>Rank</td>
<td>2.06</td>
<td>0.79-6.19</td>
<td>0.16</td>
</tr>
<tr>
<td>miR-886</td>
<td>Adalimumab + methotrexate</td>
<td>Quantile</td>
<td>2.01</td>
<td>0.82-5.88</td>
<td>0.15</td>
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<tr>
<td>miR-886</td>
<td>Placebo + methotrexate</td>
<td>Rank</td>
<td>0.33</td>
<td>0.11-0.78</td>
<td>0.02</td>
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<tr>
<td>miR-886</td>
<td>Placebo + methotrexate</td>
<td>Quantile</td>
<td>0.40</td>
<td>0.14-1.57</td>
<td>0.06</td>
</tr>
</tbody>
</table>

The effects are reported for a CT inter-quartile range increase

Baseline characteristics and response

<table>
<thead>
<tr>
<th></th>
<th>Adalimumab + MTX (N=89)</th>
<th>Placebo-adalimumab + MTX (N=91)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
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<tr>
<td>Age (years)</td>
<td>56</td>
<td>54.2</td>
<td>0.71</td>
</tr>
<tr>
<td>Female sex (%)</td>
<td>63</td>
<td>69</td>
<td>0.46</td>
</tr>
<tr>
<td>Disease duration (days)</td>
<td>88</td>
<td>83</td>
<td>0.74</td>
</tr>
<tr>
<td>Rheumatoid factor (%)</td>
<td>70</td>
<td>74</td>
<td>0.67</td>
</tr>
<tr>
<td>Anti-CCP (%)</td>
<td>70</td>
<td>60</td>
<td>0.17</td>
</tr>
<tr>
<td>DAS28</td>
<td>5.5</td>
<td>5.6</td>
<td>0.53</td>
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</tbody>
</table>

1 year follow-up

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Adalimumab + MTX (N=89)</th>
<th>Placebo-adalimumab + MTX (N=91)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EULAR response (%)</td>
<td>82</td>
<td>74</td>
<td>0.28</td>
</tr>
<tr>
<td>DAS28 remission (%)</td>
<td>74</td>
<td>49</td>
<td>0.001</td>
</tr>
<tr>
<td>ACR/EULAR Boolean remission (%)</td>
<td>48</td>
<td>30</td>
<td>0.014</td>
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</table>

Step 2. Kolmogorov-Smirnov tests

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Normalisation</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EULAR response</td>
<td>120 most expressed</td>
<td>0.008</td>
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<tr>
<td>DAS28 remission</td>
<td>Rank</td>
<td>0.011</td>
</tr>
<tr>
<td>ACR/EULAR Boolean remission</td>
<td>0.035</td>
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</table>

Quantile normalization

High Cycle threshold (CT) value = low miRNA expression
Conclusions

- miR-22 and miR-886.3p in pre-treatment whole blood were associated with EULAR good response to adalimumab
- Validation studies are needed to investigate the utility of these miRNAs as predictive biomarkers of response to TNF blocking agents

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- Aarhus Hospital, Denmark
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- King Christian 10th Hospital for Rheumatic Diseases, University of Southern Denmark, Grundriss, Denmark
- Department of Medicine and Oncology, Copenhagen University Hospital, Herlev, Denmark

Backup slides
**Interaction between miRNAs and treatment**

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Treatment</th>
<th>Normalization</th>
<th>CR</th>
<th>95% CI</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>miR-22</td>
<td>Placebo</td>
<td>Rank</td>
<td>0.54</td>
<td>0.22</td>
<td>0.28</td>
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<tr>
<td>miR-22</td>
<td>Adalimumab</td>
<td>Rank</td>
<td>0.60</td>
<td>0.26</td>
<td>0.40</td>
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<tr>
<td>miR-866p</td>
<td>Placebo</td>
<td>Rank</td>
<td>0.51</td>
<td>0.14</td>
<td>0.78</td>
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<tr>
<td>miR-22</td>
<td>Adalimumab + methotrexate</td>
<td>Rank</td>
<td>10.26</td>
<td>2.39</td>
<td>6.66</td>
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<tr>
<td>miR-866p</td>
<td>Adalimumab + methotrexate</td>
<td>Rank</td>
<td>2.00</td>
<td>0.79</td>
<td>3.30</td>
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<td>miR-22</td>
<td>Placebo</td>
<td>Quantile</td>
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<td>1.80</td>
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<tr>
<td>miR-866p</td>
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<td>Quantile</td>
<td>0.80</td>
<td>0.14</td>
<td>0.97</td>
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<tr>
<td>miR-22</td>
<td>Adalimumab + methotrexate</td>
<td>Quantile</td>
<td>12.18</td>
<td>3.30</td>
<td>43.45</td>
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<tr>
<td>miR-866p</td>
<td>Adalimumab + methotrexate</td>
<td>Quantile</td>
<td>3.01</td>
<td>0.00</td>
<td>5.88</td>
</tr>
</tbody>
</table>

**miRNAs associated with DAS28 remission independent of treatment group**

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Fold-change</th>
<th>CR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-22</td>
<td>0.83</td>
<td>0.54</td>
<td>0.14</td>
<td>0.20</td>
</tr>
<tr>
<td>miR-24</td>
<td>2.83</td>
<td>1.54</td>
<td>0.19</td>
<td>0.02</td>
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<tr>
<td>miR-330</td>
<td>0.36</td>
<td>0.24</td>
<td>0.18</td>
<td>0.10</td>
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<tr>
<td>miR-148a</td>
<td>2.36</td>
<td>1.31</td>
<td>1.03</td>
<td>0.03</td>
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<tr>
<td>miR-152</td>
<td>1.85</td>
<td>1.07</td>
<td>2.43</td>
<td>0.03</td>
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<tr>
<td>miR-211</td>
<td>0.60</td>
<td>0.36</td>
<td>0.95</td>
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<td>miR-375</td>
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<td>0.32</td>
<td>0.89</td>
<td>0.01</td>
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<td>0.37</td>
<td>0.89</td>
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<td>miR-512</td>
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<td>0.22</td>
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<td>miR-636</td>
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<td>0.27</td>
<td>0.62</td>
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<tr>
<td>miR-652</td>
<td>0.64</td>
<td>0.44</td>
<td>0.95</td>
<td>0.00</td>
</tr>
</tbody>
</table>

- Cyr61 mediates joint inflammation and damage in RA
- miR-22 targets Cyr61 and thereby inhibits Cyr61 expression
- Wild type p53 activates miR-22 transcription
- Mutant forms of p53 (common in stromal tissue from RA patients) have lost the ability to activate miR-22
- Down-regulation of miR-22 contributes to over-expression of Cyr61 in RA FLS