Metabolic Profiles of Synovial Fibroblasts: Implications for Disease Processes in Early Inflammatory Arthritis

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NO DISCLOSURES
Fibroblasts in RA synovium

- Synovial fibroblasts play a key role in the persistence of inflammation and joint destruction in RA.
  - Numbers are expanded
  - They directly invade cartilage
  - They contribute to bone destruction though activation of osteoclasts
  - They drive leuckoyte recruitment and retention through chemokine and cytokine production
Fibroblasts in RA synovium

• In the joint, fibroblasts have limited access to nutrients and oxygen within the poorly vascularised hypoxic synovium and yet expansion of fibroblast numbers still occurs.

• This suggests fibroblasts may adapt their metabolism to this environment and this process may be involved in driving persistence of chronic inflammation.
Metabolomic analysis

• Metabolomics can be used to assess global metabolic pathways in cells and tissue.

• We have used NMR-based metabolomic fingerprinting to assess:
  1. differences in metabolite fingerprints in fibroblasts from patients with established RA, early arthritis and healthy controls
  2. if inflammatory cytokine production by fibroblasts relates to their metabolic profile
NMR metabolomics

NMR spectrum

Spectrum segmented into “bins”

Data bins from groups of spectra assessed by PCA

Identify metabolites

PCA analysis identifies regions of the spectrum which allow segregation
Healthy controls

Early arthritis that eventually resolves

Early arthritis that evolves into RA

Established RA

Metabolism instantly halted using methanol at -50°C

polar metabolites

protein layer

non-polar metabolites

NMR
<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (n=6)</th>
<th>Early resolving arthritis patients (n=6)</th>
<th>Early RA patients (n=6)</th>
<th>Established RA (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years); median (IQR)</strong></td>
<td>43 (34-52)</td>
<td>40 (33-54)</td>
<td>50 (48-65)</td>
<td>55 (46-66)</td>
</tr>
<tr>
<td><strong>Female; number (%)</strong></td>
<td>2 (33)</td>
<td>4 (67)</td>
<td>3 (50)</td>
<td>3 (50)</td>
</tr>
<tr>
<td><strong>Symptom duration (weeks); median (IQR)</strong></td>
<td>-</td>
<td>4 (2-7)</td>
<td>5 (4-10)</td>
<td>38 (26-152)</td>
</tr>
<tr>
<td><strong>CRP (mg/ml); median (IQR)</strong></td>
<td>-</td>
<td>7.5 (0-20.5)</td>
<td>22 (3.75-33.5)</td>
<td>48 (3.5-66.5)</td>
</tr>
<tr>
<td><strong>RF positive; number (%)</strong></td>
<td>-</td>
<td>0 (0)</td>
<td>5 (83)</td>
<td>5 (83)</td>
</tr>
<tr>
<td><strong>Anti CCP antibody positive; number (%)</strong></td>
<td>-</td>
<td>0 (0)</td>
<td>6 (100)</td>
<td>6 (100)</td>
</tr>
<tr>
<td><strong>Joint biopsied</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ankle; n (%)</td>
<td>0 (0)</td>
<td>2 (33)</td>
<td>2 (33)</td>
<td>2 (33)</td>
</tr>
<tr>
<td>Knee; n (%)</td>
<td>6 (100)</td>
<td>4 (67)</td>
<td>2 (33)</td>
<td>4 (67)</td>
</tr>
<tr>
<td>MCP; n (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (33)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>
PLSDA of synovial fibroblast metabolite profile data
Fibroblasts from inflamed joints produce more IL6 in culture
Metabolic profiles in cultured synovial fibroblasts from patients correlate with fibroblast IL6 production
Metabolic profiles in cultured synovial fibroblasts correlate with patients' serum CRP level at the time of biopsy
<table>
<thead>
<tr>
<th>Ranked importance</th>
<th>Metabolites correlated with secreted CRP (ppm)</th>
<th>Metabolites correlated with patient IL-6 (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cholesterol (0.9-1.1)</td>
<td>Citrate (2.54, 2.66, 2.62)</td>
</tr>
<tr>
<td>2</td>
<td>Fatty acids (0.8-0.84, 2.22-2.24)</td>
<td>Carnosine (2.69)</td>
</tr>
<tr>
<td>3</td>
<td>Leucine (0.94-0.96)</td>
<td>Pyroglutamate (2.52)</td>
</tr>
<tr>
<td>4</td>
<td>Citrate (2.54, 2.66, 2.62)</td>
<td>Alanine (1.48)</td>
</tr>
<tr>
<td>5</td>
<td>Pyroglutamate (2.52)</td>
<td>Lactate (1.31, 1.33)</td>
</tr>
<tr>
<td>6</td>
<td>Carnosine (2.69)</td>
<td>Glycerol (3.57)</td>
</tr>
<tr>
<td>7</td>
<td>Alanine (1.48)</td>
<td>Leucine (0.94-0.96)</td>
</tr>
<tr>
<td>8</td>
<td>Lactate (1.31, 1.33)</td>
<td>Acetylglycine (3.76, 3.77)</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>Glucose (3.38, 3.5, 3.68)</td>
</tr>
</tbody>
</table>
Conclusions

• Metabolomics provides a novel “systems” approach to disease mechanisms in RA.

• We have found that the metabolic profile of an individual early arthritis patient’s cultured synovial fibroblasts predicts the subsequent course of the patient’s disease.

• There was a significant association between CRP levels in the patients’ serum and the metabolic profile of their synovial fibroblasts. This implies that fibroblasts from inflammatory arthritis patients retain their metabolic fingerprint during culture ex vivo.

• There was also a significant association between the metabolomic fingerprint of synovial fibroblasts and their IL6 production, raising the possibility that IL6 production drives or is driven by significant changes in metabolism.

• This may provide insights into the abnormal function of fibroblasts in RA (1,2). It may also partially explain our observation that serum metabolites differ in patients with different types of inflammatory arthritis(1).

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- Steve Young
- Karim Raza
- Andrew Filer
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- Kath Howlett
- Anne Garfield
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- Ulrich Günther
- Chris Buckley
How does NMR work?

Nuclei are charged and many have spin which makes them magnetic.

Higher energy state: magnetic field opposes applied field.

Lower energy state: magnetic field aligned with applied field.

Applied Magnetic Field

Energy gap in field corresponds to radio frequency.

NO FIELD
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<th>Metabolites correlated with fibroblast secreted IL-6</th>
</tr>
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</table>
| 1                 | Cholesterol                                              | Citrate  
Warburg effect – cells using glycolysis during growth |
| 2                 | Fatty acids                                               | Carnosine  
a dipeptide His-Ala. Antioxidant, anti-ageing, possible growth factor for fibroblasts. Carnosine synthase expressed by mesenchymal stem cells |
| 3                 | Leucine                                                   | Pyroglutamate  
cyclised form of glutamic acid. Found in skin. Increased levels seen in problems with glutathione metabolism. Increased in fibroblasts in which ATP synthesis depressed which lead to lower GSH and higher PG |
| 4                 | Citrate                                                   | Alanine cell growth |
| 5                 | Pyroglutamate                                             | Lactate  
Warburg effect – cells using glycolysis during growth |
| 6                 | Carnosine                                                 | Glycerol  
lipolysis as energy source |
| 7                 | Alanine                                                   | Leucine |
| 8                 | Lactate                                                   | Acetylglucose |
| 9                 | Glucose                                                   | Glucose  
Warburg effect – cells using glycolysis during growth |