Basic Science of Meniscus Pathology and Intervention: Molecular Analyses

Muhammad Farooq Rai, Ph.D.

Department of Orthopaedic Surgery
Musculoskeletal Research Center
St. Louis, MO, United States of America

Washington University in St. Louis
School of Medicine

ICRS, Naples, Italy: September 24, 2016
Disclosures

• No financial disclosures

• No conflict of interest
Meniscus

Meniscus is an essential component of the tibiofemoral articulation playing a role of

- shock absorption
- Weight distribution
- Lubrication
- Nutrition
- Other…
Meniscus tear

- Meniscus tears occur due to
  - Injury to the knee (trauma)
  - Contact sports
  - Abrupt stops
  - Landing on straight leg
  - Degeneration
  - Other…
Meniscus tear and OA

• Role of intact meniscus in OA is uncertain.

• Injured meniscus plays a pivotal role in OA development

• Meniscus injuries are the most common knee injuries
  • 690,000 partial menissectomies are performed each year
  • 1 M additional knee arthroscopies

Woolf et al., Best Pract Res Clin Rheumatol. 2012, 26:183-224
Post-traumatic OA

• Primary (age-onset, cause unknown)

• Secondary, post-traumatic (cause known)
  • At least 12% of OA is due to trauma
    • Average time from trauma to OA is 10 years
    • Post-traumatic OA is the model and will be the easiest to solve

• Patient population getting younger
  • Hip or knee replacement in 40’s
  • Obesity, athletics and activity level driven

Brown et al., J Orthop Trauma. 2006, 20:739-44
Stages of OA

- Initiation of Disease Process
  - 20-30 years
- ? Onset Clinical Symptoms?
  - 45 years
- Serologically Detectable
  - 60 years
- Clinically Detectable

MOLECULAR
- Biomarkers reflecting change in composition of joint tissues

PRERADIOGRAPHIC
- MRI
  - Ultrasound
  - Bone Scan
- Structural changes in bone, cartilage, and soft tissues

RADIOGRAPHIC
- Joint Failure
  - Structural changes in bone

JOINT REPLACEMENT
- Joint Death (rebirth)
- End-stage disease
Diversity in meniscus

Sanchez-Adams & Athanasiou, Cellular Mol Bioeng. 2009, 2:332
Meniscus is a complex tissue

- Meniscus displays complex geometry and anatomy
- Cellular profile ranges from fibroblast-like to chondrocyte-like
- During development, cells are similar in shape and morphology, but as it matures, cells take on distinct characteristics
  - The spindle-shaped cells of the outer meniscus: maintain a fibrous ECM rich in collagen type I.
  - The round, inner meniscus cells: produce both collagen types I and II, and glycosaminoglycan, (hyaline-like).
  - Cells intermediately located display characteristics of both cell types.
Zonal differences in cell phenotype

• Cells phenotypes vary among different parts of meniscus

Differential distribution of sGAG

Differential response to cytokines

- Zonal differences in meniscus matrix turnover and cytokine response

- Meniscal degeneration mechanisms are zonally-dependent, and may contribute to the enzymatic burden in the joint.

Matrix turnover rate is different

Fuller et al., Osteoarthritis Cartilage. 2012, 20:49-59
Medial vs. Lateral

- mRNA levels were generally higher in the medial than in the lateral meniscus.

- The medial meniscus had significantly increased levels of TGF-b, COX-2, and TIMP-1, COL-1, COL-2, COL-3, IGF-2, MMP-1.

- Levels of mRNA for iNOS and bFGF were similar in both menisci for both age groups.

Patient related factors

- Age: 40 years cut off, change in meniscus cell phenotype
- Patient’s Sex
- Obesity, important factor in OA, BMI
- Cartilage chondrosis: status of cartilage at the time of meniscus injury
- Injury pattern (± ACL tear)
- Tear type (Traumatic vs. Degenerative)
- OA vs. Normal
Age related differences: PCR

• There exist age-related gene expression differences in injured meniscus
Age related differences: Microarrays

Transcriptome Analysis of Injured Human Meniscus Reveals a Distinct Phenotype of Meniscus Degeneration With Aging

Muhammad Farooq Rai, Debabrata Patra, Linda J. Sandell, and Robert H. Brophy
Gene present in older patients

- Pathway: Inflammation and proliferation

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Gene Name</th>
<th>Microarray Fold-change</th>
<th>Microarray P</th>
<th>QGP assay Fold-change</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCER1A</td>
<td>Fc fragment of IgE, high affinity I, receptor for; alpha polypeptide</td>
<td>4.15</td>
<td>0.000</td>
<td>3.00</td>
</tr>
<tr>
<td>IFI27</td>
<td>Interferon, alpha-inducible protein 27</td>
<td>3.21</td>
<td>0.000</td>
<td>1.75</td>
</tr>
<tr>
<td>CD36</td>
<td>CD36 molecule (thrombospondin receptor)</td>
<td>3.21</td>
<td>0.003</td>
<td>4.33</td>
</tr>
<tr>
<td>DHRS9</td>
<td>Dehydrogenase/reductase (SDR family) member 9</td>
<td>3.16</td>
<td>0.017</td>
<td>2.98</td>
</tr>
<tr>
<td>DARC</td>
<td>Duffy blood group, chemokine receptor</td>
<td>3.07</td>
<td>0.022</td>
<td>1.11</td>
</tr>
<tr>
<td>SCARA5</td>
<td>Scavenger receptor class A, member 5 (putative)</td>
<td>2.95</td>
<td>0.031</td>
<td>1.96</td>
</tr>
<tr>
<td>LYN</td>
<td>Latexin</td>
<td>2.87</td>
<td>0.002</td>
<td>2.17</td>
</tr>
<tr>
<td>IL7R</td>
<td>Interleukin 7 receptor</td>
<td>2.83</td>
<td>0.000</td>
<td>1.31</td>
</tr>
<tr>
<td>OAS2</td>
<td>2'-5'-oligoadenylate synthetase 2, 69/71kDa</td>
<td>2.80</td>
<td>0.000</td>
<td>1.99</td>
</tr>
<tr>
<td>WISP2</td>
<td>WNT1 inducible signaling pathway protein 2</td>
<td>2.59</td>
<td>0.039</td>
<td>1.63</td>
</tr>
<tr>
<td>CX3CR1</td>
<td>Chemokine (C-X3-C motif) receptor 1</td>
<td>2.28</td>
<td>0.022</td>
<td>2.86</td>
</tr>
<tr>
<td>CDC25C</td>
<td>Cell division cycle 25 homolog C</td>
<td>1.61</td>
<td>0.024</td>
<td>1.40</td>
</tr>
<tr>
<td>GDNF</td>
<td>Glial cell derived neurotrophic factor</td>
<td>1.57</td>
<td>0.011</td>
<td>1.27</td>
</tr>
<tr>
<td>CCL8</td>
<td>Chemokine (C-C motif) ligand 8</td>
<td>1.56</td>
<td>0.031</td>
<td>1.24</td>
</tr>
<tr>
<td>CCNF</td>
<td>Cyclin F</td>
<td>1.55</td>
<td>0.021</td>
<td>1.32</td>
</tr>
</tbody>
</table>
Genes absent in older patients

- Pathway: Cartilage homeostasis

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Gene Name</th>
<th>Microarray Fold-change</th>
<th>Microarray P</th>
<th>QGP assay Fold-change</th>
</tr>
</thead>
<tbody>
<tr>
<td>COL2A1</td>
<td>Collagen, type II, alpha 1</td>
<td>-10.38</td>
<td>0.018</td>
<td>-14.66</td>
</tr>
<tr>
<td>CHAD</td>
<td>Chondroadherin</td>
<td>-6.73</td>
<td>0.014</td>
<td>-6.57</td>
</tr>
<tr>
<td>COL11A2</td>
<td>Collagen, type XI, alpha 2</td>
<td>-6.14</td>
<td>0.006</td>
<td>-10.59</td>
</tr>
<tr>
<td>COL9A2</td>
<td>Collagen, type IX, alpha 2</td>
<td>-5.99</td>
<td>0.003</td>
<td>-5.98</td>
</tr>
<tr>
<td>SCIN</td>
<td>Scinderin</td>
<td>-4.88</td>
<td>0.005</td>
<td>-7.41</td>
</tr>
<tr>
<td>FGFR3</td>
<td>Fibroblast growth factor receptor 3</td>
<td>-4.65</td>
<td>0.007</td>
<td>-4.36</td>
</tr>
<tr>
<td>SHC4</td>
<td>SHC (Src homology 2 domain containing) family, member 4</td>
<td>-4.55</td>
<td>0.016</td>
<td>-7.94</td>
</tr>
<tr>
<td>CILP2</td>
<td>Cartilage intermediate layer protein 2</td>
<td>-4.32</td>
<td>0.030</td>
<td>-3.04</td>
</tr>
<tr>
<td>SOX8</td>
<td>SRY (sex determining region Y)-box 8</td>
<td>-4.23</td>
<td>0.015</td>
<td>-1.12</td>
</tr>
<tr>
<td>SBSPCN</td>
<td>RPE-Spondin</td>
<td>-4.14</td>
<td>0.038</td>
<td>-3.37</td>
</tr>
<tr>
<td>S100A1</td>
<td>S100 calcium binding protein A1</td>
<td>-4.08</td>
<td>0.012</td>
<td>-12.47</td>
</tr>
<tr>
<td>GLDN</td>
<td>Glomiadin</td>
<td>-4.06</td>
<td>0.031</td>
<td>-4.94</td>
</tr>
<tr>
<td>MT1E</td>
<td>Metallothionein 1E</td>
<td>-3.86</td>
<td>0.003</td>
<td>-1.33</td>
</tr>
<tr>
<td>S100A13</td>
<td>S100 calcium binding protein A13</td>
<td>-3.82</td>
<td>0.014</td>
<td>-1.04</td>
</tr>
<tr>
<td>HAPLN1</td>
<td>Hyaluronan and proteoglycan link protein 1</td>
<td>-3.70</td>
<td>0.008</td>
<td>-4.69</td>
</tr>
<tr>
<td>LEPREL1</td>
<td>Leprecan-like 1</td>
<td>-3.36</td>
<td>0.016</td>
<td>-4.14</td>
</tr>
<tr>
<td>SOX9</td>
<td>SRY (sex determining region Y)-box 9</td>
<td>-2.98</td>
<td>0.042</td>
<td>-2.53</td>
</tr>
<tr>
<td>VEGFA</td>
<td>Vascular endothelial growth factor A</td>
<td>-2.86</td>
<td>0.029</td>
<td>-1.71</td>
</tr>
<tr>
<td>ACAN</td>
<td>Aggrecan</td>
<td>-2.52</td>
<td>0.032</td>
<td>-3.37</td>
</tr>
<tr>
<td>SPRY4</td>
<td>Sprouty homolog 4</td>
<td>-2.09</td>
<td>0.038</td>
<td>-1.51</td>
</tr>
<tr>
<td>SULF2</td>
<td>Sulfatase 2</td>
<td>-2.07</td>
<td>0.031</td>
<td>-2.16</td>
</tr>
<tr>
<td>FGF18</td>
<td>Fibroblast growth factor 18</td>
<td>-1.88</td>
<td>0.033</td>
<td>-1.58</td>
</tr>
<tr>
<td>WNT16</td>
<td>Wingless-type MMTV integration site family, member 16</td>
<td>-1.67</td>
<td>0.022</td>
<td>-3.45</td>
</tr>
</tbody>
</table>
Differences due to sex

• Little differences in gene expression between males and females

• CCL3L1 was highly expressed in meniscus from female patients
Obesity related differences

- More age-related differences in gene expression than BMI-related differences
- More changes in gene expression between overweight and obese patients than between lean and overweight patients

Rai et al., Arthritis Rheumatol. 2014, 66:2152-64
Changes in relation to chondrosis

- Some differences in gene expression in meniscus in relation to cartilage changes i.e. chondrosis

Changes related to injury pattern

- Combined ACL and meniscus injury resulted in increased expression of inflammatory cytokines and decreased expression of matrix synthesizing genes.

Differences due to tear type

A. Degenerative (complex, horizontal, or flap tear pattern)

B. Traumatic (longitudinal tear pattern)

Traumatic vs. Degenerative Tears

- Increased in traumatic tears:
  - MMP1
  - MMP3
  - IL-8
  - CXCL6

Changes by disease (OA) status

Normal Menisci

OA Menisci

Sun et al., BMC Musculoskelet Disord. 2010, 28:11-19
## Processes effected (normal vs. OA)

<table>
<thead>
<tr>
<th>Biological process</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune response</td>
<td>Increased in OA</td>
</tr>
<tr>
<td>Inflammatory response</td>
<td>Increased in OA</td>
</tr>
<tr>
<td>Cytokine production</td>
<td>Increased in OA</td>
</tr>
<tr>
<td>DNA repair</td>
<td>Decreased in OA</td>
</tr>
<tr>
<td>Cellular biosynthetic process</td>
<td>Decreased in OA</td>
</tr>
</tbody>
</table>
Other considerations

- Biomechanics and Mechanobiology
- Tissue engineering strategies
- Joint homeostasis, inflammation
- Interaction with other tissues
- Diversity in cell population
Our approach

- Mencius injury as predictor for OA: info from cartilage changes

- PCR test at the time of arthroscopy: gene expression will dictate treatment options

- Biomarkers surrogates in serum and/or synovial fluid

- Investigating the diverse cell population to a better understanding of the pathophysiology and regenerative processes of the meniscus.
Road to the precision medicine!

- Complex nature of the tissue
- Complex tear patterns: traumatic, degenerative
- Multiple confounders (patients’ related factors)
- Joint as an organ: role of other tissues
- Restoration of joint homeostasis is challenging
- Road to precision medicine...
“Effective methods for diagnosis, screening, treatment monitoring, and prognostication for meniscal disorders are desperately needed, and those that include assessment of meniscal biology appear to hold the most promise for providing sensitive, specific and accurate strategies for clinical implementation. By pursuing this translational approach to the meniscus, research can be effective in optimizing diagnosis, prevention and treatment for the millions of patients who suffer from meniscal disease.”

Credit: Dr. James Cook
Acknowledgements

K99 Pathway to Independence (Rai)
R00… (Rai)

R01 (Sandell PI, Rai Co-I)
Supplement to R01 (Sandell, Rai)

P30 Musculoskeletal Research Center (Sandell)
P30 Pilot and Feasibility Award (Rai)

T32 Metabolic Skeletal Disorders  (Civitelli)
T32 Postdoctoral Award (Rai)

AOSSM/Sanofi Biosurgery (Rai, Brophy)