Discovery and validation of new osteoarthritis biomarkers using omics approaches across the spectrum of disease

Christelle Sanchez
Bone and Cartilage Research Unit, University of Liège, 4000 Liège, Belgium

www.bcru.be
OMICs APPROACHES in OA

**Biological fluids**
- SERUM/PLASMA
- URINE
- SYNOVIAL FLUID

**Tissues**
- CARTILAGE
- BONE
- SYNOVIAL MEMBRANE
- CELL EXTRACT
- MATRISOME
- MEMBRANOME

**Cells**
- CHONDROCYTES
- OSTEOBLASTS
- SYNOVIOCYTES
- OSTEOCLASTS

+ Target of biomarker follow-up
  - VS
  - Depletion of majors proteins
  - Difficult to detect minor proteins

+ Joint specificity
  + Pathological pathways (inflammation, ...)
  - Presence in serum/urine...?

⇒ complementary approaches

D-BOARD
European partnership for Science and Discovery

www.bcru.be
OMICs APPROACHES in OA
Quantitative proteomics: The state of the art

Overview of the different workflows

Biomarker Discovery: Differential proteomics analysis pipeline

**Discovery**
- Small number of samples (3 to 10)
- Looking at ± 1000 proteins at once without any *a priori*
- Complex, expensive and time consuming analysis

**Verification / Validation**
- Average number of samples from 10 to 100
- Alternative methods
- Looking at specific proteins (10-100)
- Targeted quite complex and expensive methods

**High throughput Validation**
- Average number of samples greater than 100
- Immuno-based high throughput tests
- Looking at specific proteins (<100)
- Targeted and multiplex (low cost screening tests)

www.bcru.be
Exemple of proteomic application in new OA biomarker research:
Identification of Fibulin-3 peptides as biomarkers of OA

Fibulin 3 peptides Fib3-1 and Fib3-2 are potential biomarkers of osteoarthritis
Henrotin Y et al Arthritis Rheum 2012, 64(7):2260-2267

- Proteomic analysis of human urines by 2D-DIGE
- Identification of proteins or fragments of interest by mass spectrometry
- Production of specific antiserum directed against identified peptides
- Development of specific immunoassay of peptides
- Validation of peptide variation in OA population
Proteomic analysis: classical workflow of protein identification

Urinary proteome
Henrotin et al. Arthritis Rheum 2012

OA/N > 1.5
Increased (9)
- B-actin
- α1-microglobulin
- Fibulin-3 fragments
- Apoptosis inducing factor-2

OA/N > 0.7
Decreased (9)
- Serpins β1 et β3
- Mannan binding lectin serum proteases-2
- Kinnogen 1

**FBLN3_HUMAN**
- Mass: 54604
- Score: 130
- Queries matched: 2

<table>
<thead>
<tr>
<th>Query</th>
<th>Observed</th>
<th>Mr(expt)</th>
<th>Mr(calc)</th>
<th>Delta</th>
<th>Miss</th>
<th>Score</th>
<th>Expect</th>
<th>Rank</th>
<th>Peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>149</td>
<td>685.33</td>
<td>1368.64</td>
<td>1368.54</td>
<td>0.09</td>
<td>0</td>
<td>0.00027</td>
<td>1</td>
<td>R.CVCPVSNAMCR.E</td>
<td></td>
</tr>
<tr>
<td>444</td>
<td>965.32</td>
<td>1928.63</td>
<td>1928.73</td>
<td>-0.10</td>
<td>0</td>
<td>4.2e-06</td>
<td>1</td>
<td>R.TCQDINECETINECR.E</td>
<td></td>
</tr>
</tbody>
</table>

Cytometry
Fibulin-3: relation with cartilage

- Extracellular matrix protein highly expressed in cartilaginous tissue (Timpl, et al. 2003; Kobayashi, et al. 2007)

- Expressed in the mesenchyme giving rise to cartilage and bone, and plays a role in organizing the development of skeletal system (Ehlermann et al., 2003)

- Identified in OA cartilage by proteomic analysis (Vincourt et al., 2006)

- Intimately associated with (TIMP)-3, an inhibitor of metalloproteinases involved in the pathogenesis of OA (Klenotic et al., 2004; Kevorkian et al., 2004; Sahebjam et al., 2007)

- Negative regulator of chondrocytes differentiation (Wakabayashi, et al. 2010)

These bibliographic data suggest an implication of Fibulin-3 in the physiopathology of OA
Fibulin-3 fragments (Fib3-1 and Fib3-2): potential diagnostic biomarkers

- Immunization of rabbits
- Antiserum production
- Specific immunoassays of Fib3-1 and Fib3-2 development and validation in human serum

**Fib3-1**: TCQDINECETTNECR
**Fib3-2**: CVCPVSAMCR

<table>
<thead>
<tr>
<th></th>
<th>Sensibility</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fib3-1 (cut-off: 71.1 pM)</td>
<td>78.5%</td>
<td>68.4%</td>
</tr>
<tr>
<td>Fib3-2 (cut-off: 163.7 pM)</td>
<td>75.0%</td>
<td>86.4%</td>
</tr>
</tbody>
</table>

AUC = 0.759 (Fib3-1)  
AUC = 0.846 (Fib3-2)

ROC curves for Fib3-1 and Fib3-2 with AUC values and sensitivity versus specificity.
Immunolocalization of Fib3-1 and Fib3-2 (human)

Fib3-1

Healthy cartilage

Fib3-2

OA cartilage
Superficial layers

OA cartilage
Bone junction
Guinea pig as spontaneous model of OA
Fib3-2 kinetic in sera and correlation with histology

35-week old guinea pig, right knee, medial

Recommended OARSI histological OA score for guinea pig, parametric ANOVA /Dunnett’s post test
Proteomic paradox

Recovery of unknown proteins in chondrocytes - cartilage matrix

AND

Some known proteins are not found!

Example:
Rosenthal et al (2011) investigated protein pattern present in articular cartilage vesicles (AVCs) and didn’t find TNALP or PC1, well known enzymes located in their membrane

Due to their structure/composition/localisation, Lot of proteins are undetectable with current technics
Quantitative proteomics: a discipline in constant progress

- Sample preparation method / Specific depletions
- Protein labeling – label free methods
- Improvement in protein separation
- Protein fractionation method
- Sensibility of detection
- Evolution of databases

<table>
<thead>
<tr>
<th>Year</th>
<th>Identified proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>100</td>
</tr>
<tr>
<td>2010</td>
<td>1000</td>
</tr>
<tr>
<td>Future</td>
<td>?</td>
</tr>
</tbody>
</table>
Conclusions

- New OMICs technologies **flood** us with new potential biomarkers

- **Validation** and **qualification** of one potential biomarker is a **very long and expensive process**

- It’s essential to **start** OMICs studies with the **good samples** – representative – homogen...to gives us important informations - maybe on subgroups of the disease
Thank you!