Gene profiling in connective tissues

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• introduction / osteoarthritis

• Dupuytren’s disease
Joint pathology - osteoarthritis

Normal joint

- Bone
- Muscle
- Capsule (ligaments)
- Synovium
- Synovial fluid
- Cartilage
- Tendon
- Tendon sheath (lined by synovium)

Osteoarthritic joint

- Bone
- Inflamed synovium
- Thickened bone with no covering cartilage
- Osteophyte
- Bone angulation (‘deformity’)
- Little remaining cartilage
- Tight, thickened capsule
Metalloproteinases and their inhibitors

Matrix metalloproteinases
23 human enzymes
(Collagenases MMP-1, -8, -13, -2, -14?)

ADAMTSs
19 human enzymes
(Aggreecanases ADAMTS-1, -4, -5, -8, -9, -15?)

TIMPs
4 human inhibitors
The balancing act of cartilage turnover…

Cartilage turnover

Cartilage degradation
The balancing act of cartilage turnover...
What are the key metalloproteinases regulated in osteoarthritic joint tissues?

Lara Kevorkian, Rose Davidson
Cartilage samples

Osteoarthritis (OA)
- femoral head from THR for osteoarthritis (n=18, age 38-81)

‘Normal’
- femoral head from THR following fracture to the neck of femur ‘NOF’ (n=15, age 52-93)

(Simon Donell, Clare Darrah, Adele Cooper - NNUH)
Polymerase chain reaction (PCR)

1. **Denature**
   - Denature

2. **Extend**
   - Extend

3. **Anneal**
   - Anneal and extend

4. **Anneal and Extend**
   - Anneal

‘Taqman’ quantitative real-time PCR
Expression profile of MMP, ADAMTS and TIMP family in normal vs. OA cartilage (mean Ct values)

**MMP**

| 1 | 2 | 3 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 19 | 20 | 21 | 23 | 24 | 25 | 26 | 27 | 28 |
|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| OA | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |

**ADAMTS**

<table>
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<th>1</th>
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<th>3</th>
<th>4</th>
<th>5</th>
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**TIMP**

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<tbody>
<tr>
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- Not detected CT=40
- Low expression CT 36-39.
- Moderate expression CT 31-35.
- High expression CT= 26-30
- Very high expression CT≤ 25
Genes that are up-regulated in OA

MMP13
Collagenase: cleaves cartilage collagen

MMP28

ADAMTS16
Unknown function

P < 0.001

# Metalloproteinase expression profiling in synovium

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<th>$P$ value</th>
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<tr>
<td></td>
<td>↑ in OA</td>
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<tr>
<td>&lt;0.05</td>
<td>MMP9</td>
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<td>TIMP2</td>
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Davidson et al (2006) ART 8:R124
Conclusions

• First expression profiles to assay all MMPs, ADAMTSs and TIMPs in cartilage and synovium

• MMP28 and ADAMTS16 expression is significantly increased in end-stage OA

Hypothesis
MMP-28 and ADAMTS-16 play key roles in the metabolism of joint tissues and the destruction of cartilage in osteoarthritis
Dupuytren’s disease

Phillip Johnston
Background

• Dupuytren’s Disease
  – fibroproliferative disorder of palmar fascia
  – altered extracellular matrix turnover
Aetiology

- Alcohol
- Smoking
- Manual work
  - Vibration tools
- Injury
Aetiology

• Diabetes
• Epilepsy
• Hyperlipidaemia
• Rheumatoid disease
• Lederhosen’s
Prevalence

- Mainly men of north-European descent
  - 2 - 42% depending on study population

- Approx. 2 million people in the UK are thought to have Dupuytren’s disease
Metalloproteinases and DD

• Extracellular matrix deposition and remodelling

• Broad spectrum MMP inhibitors in cancer clinical trials showed toxicity described as ‘musculoskeletal syndrome’
  • Dupuytren's disease and frozen shoulder
Samples

- 20 patients with Dupuytren’s disease
  - Samples divided cord / nodule

- 20 controls (palmar fascia from carpal tunnel decompression)

(Adrian Chojnowski - NNUH)
Results

Conclusions

• first expression profiles to assay all MMPs, ADAMTSs and TIMPs in DD
  • a number of key genes are regulated

Hypothesis

• increased collagen biosynthesis mediated by increased ADAMTS-14
  • increased TIMP-1 blocking MMP-1 and MMP-13-mediated collagenolysis
  • contraction enabled by MMP-14 mediated pericellular collagenolysis which may escape inhibition by TIMP-1
Correlation with clinical outcomes

- 22 patients; primary fasciectomy for DD
- Clinical scores
  - Pre op
  - 3 months post op (interim)
  - 14 months (11 – 16) post op (final)
- Range of movement
- Grip strength
- DASH (disabilities of the arm, shoulder and hand)
- MHQ (Michigan Hand Outcome Questionnaire)
- Vancouver Scar Scale
Correlations

• Reduction in fixed flexion deformity
  – (overall)
    • MMP-13 (p = 0.062, R = 0.463)
    • ADAMTS-5 (p = 0.058, R = 0.467)
    • ADAMTS-14 (p = 0.034, R = 0.517)
    • ADAMTS-16 (p = 0.032, R = 0.521)

• Reduction in fixed flexion deformity
  – (interim to final follow up)
    • MMP-13 (p = 0.005, R = 0.727)
    • MMP-14 (p = 0.006, R = 0.714)
    • ADAMTS-14 (p = 0.003, R = 0.752)
Conclusions

• potential to predict disease progression
Summary

• Focused expression profiling in human connective tissues can help build hypotheses on disease mechanisms

ABI microfluidic card

Affymetrix GeneChip
Acknowledgements

Clark lab
Kirsty Culley
Rose Davidson
Ursula Rodgers
Alison Surridge
Tracey Swingler
Jasmine Waters
(David Young)

Edwards lab
Dylan Edwards
Caroline Pennington
Clara Sampieri

Simon Donell
Clare Darrah
Adele Cooper
Adrian Chojnowski
Phil Johnston
Debbie Larson
Sarah Lewis

Institute of Orthopaedics
UEA Norwich
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