Differential gene expression of ECM-related genes after in vivo CXL in rabbits

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Keratoconus

Extracellular matrix degeneration:
- loss of collagen fibril orientation
- corneal thinning
- biomechanical weakening

Aim of corneal cross-linking:
- To increase the biomechanical resistance of the cornea.
- To stop ECM degeneration.
Previous studies:
Mechanical, morphological and structural changes

- Elastic modulus
- Demarcation line
- Resistance to enzymatic digestion
- Collagen fiber diameter
Proteomic level: Cross-linking of collagens and/or proteoglycans?

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<th>MW</th>
<th>Coll I</th>
<th>Coll I+RFUVA</th>
<th>Coll III+RFUVA</th>
<th>Coll IV+RFUVA</th>
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- cross-links: • among collagens
  • among proteoglycans
  • between collagens and proteoglycans

REF. Zhang Y. J Biol Chem 2011
How long is cross-linking effective?

- Stability for at least 10 years.  
  Raiskup F. et al, 2015, JCRS

- Progression after several years in some cases. However, re-stabilization after repeated CXL treatment.  
  Hafezi F. et al, 2014, JRS
Keratoconus also has a genetic component.

• How can CXL stop the progression of the disease on the long term?

• Does CXL only have an immediate effect, or does it permanently affect gene expression?
Possible mechanisms on the molecular level:

- Triggering of mechanisms activated by oxidative stress
- Stimulation of mechanosensitive pathways
Experimental set-up:

- 15 New-Zealand white rabbits
- Different CXL protocols to study different stiffening effects:
  - 3 mW/cm², 30 min
  - 9 mW/cm², 10 min
  - 18 mW/cm², 5 min
  + riboflavin-only control
  + virgin controls
Analysis:

- 1 week post treatment
- RNA extraction
- **RNAseq** (HighSeq 2500, Illumina)
- **Statistical analysis** considering
  - the overall effect of CXL
  - the graded stiffening effect
Results:

- total of 9335 transcripts
- **297** significantly differentially expressed between CXL conditions and controls.
- **51%** of the differentially expressed genes were stiffening dependent.
Results:

Absolutely highest differentially expressed genes:

- **Down-regulation of enzyme activity:** Glycolysis (enolase 1 alpha, tranke tole), protease inhibition (cystatin, alpha-2-macroglobulin-like 1)

- **Up-regulation of enzymatic cross-linking:** (transgluatimnase 2)

- **Down-regulation of extracellular matrix (ECM) synthesis:** (collagen type-I alpha-1 / alpha-2, collagen type-VI alpha-2, keratocan)
Results:

Relatively highest differentially expressed genes:

• **Altered membrane transport** (*cytohesin 1 interacting protein, solute carrier organic anion transporter family, solute carrier family 13 member 5*) and receptor binding (*EPH receptor B1, Kazal-type serine peptidase inhibitor domain 1, integrin, beta-like 1*)

• **Down-regulation of ECM relevant components**: (*cysteine-rich angiogenic inducer 61, keratocan, olfactomedin-like 1, thrombospondin 4, fibromodulin*)

• **Up-regulation of cross-linking enzyme** *transglutaminase 2*
Reduced ECM degradation after CXL:

- **down-regulation of ENO1 after CXL**
  - Enolase 1 (ENO1) overexpression has been reported in context with increased ECM degradation and cancer invasion.

- **TKT down-regulation after CXL**
  - Transketolase (TKT) inhibition: to suppress tumor growth and lactate-based ECM degradation

- **Down-regulation of collagen-I, collagen-VI and keratocan** confirms the reduced ECM degradation after CXL.
Gene expression is to 51% stiffening-dependent

Potential mechanosensitive pathways:

- **Thrombospondin 4:** mechano-sensing molecule in the cardiac contractile response to mechanical stress
  - up-regulation in response to hypertension

- **Keratoconus:** constantly increasing corneal strain (progressive stromal thinning)
  - comparable to cardiac hypertension

- **after CXL:** thrombospondin 4 down-regulation
  - decreased corneal strain
Conclusions:

- CXL treatment affects gene expression.
- Gene candidates have been identified suggesting that CXL treatment decreases the ECM degradation on the molecular level.
- Differential gene expression is to 51% stiffening-dependent suggesting that mechano-sensitive pathways are involved.
Thank you for your attention