Treating intervertebral disc degeneration in an accelerated aging model with novel therapeutics

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1. The *Ercc1*⁻/Δ mouse model, a new model for studying intervertebral disc degeneration

2. Targeting the NF-κB pathway for slowing down intervertebral disc degeneration

3. Targeting mitochondrial-generated reactive oxygen species (ROS) in intervertebral disc degeneration
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Stages of Intervertebral Disc Degeneration

Grade I: normal juvenile disc

- nucleus pulposus and anulus fibrosus can clearly be distinguished
- the nucleus pulposus has a gel-like appearance and is highly hydrated
- anulus fibrosus consist of discrete fibrous lamellae
- cartilage endplates are uniformly thick and consist of hyaline cartilage

Grade II: normal adult disc

- peripheral appearance of white, fibrous tissue in the nucleus pulposus
- mucinous material is found between the lamellae of the anulus fibrosus
- thickness of the cartilage endplate is irregular

Grade III: early stage of degeneration

- consolidated fibrous tissue in the whole nucleus pulposus
- demarcation between nucleus pulposus and anulus fibrosus is lost and extensive mucinous infiltration in the anulus fibrosus is observed
- cartilage endplates show focal defects
Grade IV: advanced stage degeneration

- clefts in the nucleus pulposus appear, usually parallel to the endplate
- focal disruptions are found in the anulus fibrosus
- hyaline cartilage of the endplate is replaced by fibrocartilage; irregularities and focal sclerosis are found in the subchondral bone

Grade V: end stage

- typical disc structure may be lost completely
- clefts extend through nucleus pulposus and anulus fibrosus
- endplates display diffuse sclerosis

Preliminary evaluation of a scheme for grading the gross morphology of the human intervertebral disc. 
*Spine* 15:411-415
A continuous process

annulus fibrosus & nucleus pulposus

Classification of age-related changes in lumbar intervertebral discs: 2002 Volvo Award in basic science.
Spine; 27(23):2631-2644
The "central hypothesis" in disc biology

**Disc PG homeostasis**
- Balanced (No net loss)
- Perturbed (net PG loss)

**Core PG, GAG... MMP expression**

**Anti-inflammatory cytokines**

**Pro-inflammatory cytokines**

Loss of functional cells

PG expression

Make

Break

Old
Aging is a major risk factor for IDD

Normal

Construction quality & maintenance
Time-dependent wears and tears (slow)
Excessive loads (sudden)
Assaults
  radiation
  wind
  current

Degenerated

GENETICS (faulty)

AGING (time-dep damage of macromolecules)

MECHANICAL (trauma)

ENVIRONMENTAL
  Smoking
  occupation
  nutrition
**The Ercc1^{-/Δ} mouse model**

**ENDONUCLEASE**¹
Repairs DNA lesions & interstrand X-links

**Progressive aging symptoms**²
- Kyphosis
- Cachexia
- DNA damage

**Genotoxic Stress**
- Loss of vision and hearing
- Reduced renal & liver function
- Neurodegeneration (dystonia, ataxia...)
- Intervertebral Disc Degeneration

~ 2-3yr wt

Intervertebral disc degeneration in Ercc1−/Δ

X-ray images

Percent Disc Height

Aggrecan fluorescence

Wt (3wks)

Ercc1−/Δ (18wks)

Wt (2yrs)

Wt (3wks)  Ercc1−/Δ (18wks)  Wt (2yrs)

Wt  Ercc1−/Δ

3-5 wks  18-20 wks  2.5 yrs

*
1. The *Ercc1*⁻/Δ mouse model, a new model for studying intervertebral disc degeneration

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NF-κB is the transcription factor most associated with mammalian aging\(^3\)

NF-κB is up-regulated in a variety of aged tissues and age-associated diseases\(^4, 5\)

- **Inflammatory diseases**
  (Rheumatoid arthritis, diabetes, etc.)

- **Non-inflammatory diseases**
  (Atherosclerosis, osteoporosis, etc.)

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NF-κB is a family of transcription factors involved in cellular response to damage. p50/p65 heterodimer is the most common factor of the NF-κB family.6

NF-κB is activated in cells by many cellular stressors; inflammatory, oxidative, genotoxic, mechanical, and chemical stress are among them.6

Two separate activation pathways have been described:

1. Canonical pathway
2. DNA damage pathway
NF-κB activation in aged human disc nucleus pulposus

Aging activates NF-κB transcription factor

Nerlich et al., ANYAS 2007;
NF-κB is activated in aging mouse discs

- NF-κB activation in aged human disc nucleus pulposus

- NF-κB activation in aged mouse discs

Activated NF-κB

NF-κB–eGFP reporter mice

Green cells = NF-κB activation

Promoter element

eGFP

Young (5 mths)

Old (28 mths)

NF-κB is activated in aging mouse discs

- NF-κB activation in aged human disc nucleus pulposus
- NF-κB activation in aged mouse discs

NF-κB gene targets include IL-1β, IL-6, iNOS, MMP-1β, MMP-3.

qRT-PCR analysis of mouse disc RNA.
NF-κB is activated in aging mouse discs

- NF-κB activation in aged human disc nucleus pulposus\(^7\)
- NF-κB activation in aged mouse discs\(^8\)
Hypothesis and Prediction

Chronic activation of NF-κB mediates age-associated disc degeneration.

Prediction

NF-κB

NF-κB
1. Pharmacological inhibition

**Ercc1−/Δ Mice**

IKK \( \xrightarrow{X} \) IκB \( \xrightarrow{\text{NBD}} \) NF-κB

**Approach:** Blocking NF-κB activity

mNBD / NBD peptide
The NBD peptide: structure

<table>
<thead>
<tr>
<th>Peptide</th>
<th>N-Transduction Domain</th>
<th>GG</th>
<th>IKKγ Blocking Peptide</th>
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</thead>
<tbody>
<tr>
<td>8K-NBD:</td>
<td>KKKKKKKKKK------------</td>
<td>GG-</td>
<td>TALDWSWLQTE</td>
</tr>
<tr>
<td>8K-mNBD:</td>
<td>KKKKKKKKKK------------</td>
<td>GG-</td>
<td>TALDASALQTE</td>
</tr>
</tbody>
</table>

Bioactivity of 8K-NBD (NEMO Binding Domain) peptide

- Inhibits IL-1β and TNFα induced NF-κB activation \textit{in vitro}
- Efficacious in animal models of
  - Inflammatory bowel disease \textit{(Dave et al., J Immunology 2007)}
  - Muscular dystrophy \textit{(Acharyya et al., JCI 2007)}
  - Arthritis \textit{(Dai et al., J Biol Chem 2004)}
The NBD peptide: functioning

**NBD peptide**

decreases NF-κB expression in Ercc1\(^{-/\delta}\) mice
**Ercc1**$^{-/\Delta}$  **Wt** (untreated)

**NBD treatment delays the onset of osteoporosis in Ercc1$^{-/\Delta}$ mice**

Vertebral Porosity ($\mu$CT)

- **mtNBD**
- **NBD**

**Porosity (% of Wt control)**

- **Wt**
- **Ercc1**$^{-/\Delta}$
- **Ercc1**$^{-/\Delta}$ mNBD
- **Ercc1**$^{-/\Delta}$ NBD

*Significant difference*
NBD treatment improves disc histology in Ercc1\(^{-/\Delta}\) mice.
NBD treatment improves disc matrix content

**GAG content (μg GAG/ng DNA)**

<table>
<thead>
<tr>
<th></th>
<th>WT NT</th>
<th>Ercc1−/− NT</th>
<th>Ercc1−/− NBD</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>600</td>
<td>#</td>
<td>#</td>
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**PG synthesis (fmoles sulfate/ng DNA)**

<table>
<thead>
<tr>
<th></th>
<th>WT NT</th>
<th>Ercc1−/− NT</th>
<th>Ercc1−/− NBD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PG</td>
<td></td>
<td></td>
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<tr>
<td>16</td>
<td>#</td>
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</table>
1. Pharmacological inhibition

IKK \rightarrow X \rightarrow \text{IκB} \rightarrow \text{NF-κB}

Non-target effects

Ercc1^{-/Δ} Mice

2. Genetic reduction

NF-κB dimer

p65^+/- Ercc1^{-/Δ} Mice

p65^+/- Ercc1^{-/Δ} Mice
NF-κB p65 haploinsufficiency delays the onset of age-related IDD

Safranin O

Ercc1^{-/Δ}

Ercc1^{-/Δ} p65^{+/−}

EP  NP  AF

Total NP GAG (μg GAG/μg DNA)

PG synthesis (μmol sulfate/μg DNA)

20 wks

WT  Ercc1^{-/Δ}  Ercc1^{-/Δ} p65^{+/−}

#  #
• Increased level of NF-κB activity in disc cells of natural aging (Wt) and progeroid (Ercc1^{-/-}) mice.

• Systemic treatment of Ercc1^{-/-} mice with the NBD peptide (NF-κB inhibitor) ameliorated age-dependent degenerative changes in discs/spine.
  - Disc cellularity
  - Disc matrix PG content
  - Disc PG synthesis
  - Vertebral bone porosity

• Similar protective effects were seen with p65 genetic haploinsufficiency.
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3. Targeting mitochondrial-generated reactive oxygen species (ROS) in intervertebral disc degeneration
Reactive oxygen species (ROS) are chemically reactive molecules (e.g. $O_2^-$, $H_2O_2$, $OH^-$). ROS are a natural byproduct of normal oxidative metabolism. Mitochondria are the main source of ROS within most cells.
Production of ROS is an **inevitable** biochemical consequence of oxygen metabolism

Antioxidant systems maintain a controlled balance against oxidative stress within the cell
Numerous studies have shown that oxidative damage increases with age in many tissues and various organisms.\textsuperscript{9, 10, 11, 12}

**Neurodegenerative diseases** (Patel VP, Int J Clin Exp Pathol 2011)
- Lou Gehring’s disease (ALS)
- Parkinson’s disease
- Alzheimer’s disease
- Huntington’s disease

**Cardiovascular diseases** (Wang JC, Circ Res 2012)
- Reperfusion injury following hypoxia

**Chronic fatigue syndrome** (Nijs J, Man Ther 2006)

**Gastric cancer** (Handa O, Redox Rep 2011)
...but intervertebral disc is hypoxic!

Nutrition of the intervertebral disc.
Spine; 29 (23):2700-2709
Vascular ingrowth in degenerated intervertebral discs

Adams MA, Clin Biomech 2010

Classification of age-related changes in lumbar intervertebral discs: 2002 Volvo Award in basic science.
Spine; 27(23):2631-2644
Disc cells from 20 wk old Ercc1<sup>−/Δ</sup> mice show greater mitochondrial death (arrows) compared to wild-type littermates by transmission electron microscopy.

**Ercc1<sup>−/Δ</sup> mice**  
(DNA repair defect)

**Accelerated aging**
Growing human NP (nucleus pulposus) cells

*in vitro* increases ROS production
The XJB-5-131 radical scavenger

XJB-5-131 is a mitochondria-targeted ROS scavenger

XJB-5-131 has been previously shown to be therapeutic in rodent models of hemorrhagic shock and sepsis.\(^\text{13}\)

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Professor of Pharmaceutical Sciences
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Assessing the effects of oxidative stress *in vitro* on human disc cell cultures

Assessing the effects of oxidative stress *in vivo* on *Ercc1*-Δ mice testing the XJB-5-131 radical scavenger
High oxygen levels have catabolic effects on human disc cells \textit{in vitro}.

Reduced matrix synthesis.
**XJB-5-131 rescues in vitro effects of high O₂**

- **Gene Expression**
  - XJB-5-131 rescues in vitro effects of high O₂ on gene expression for Agc, ADAMTS4, ADAMTS5, MMP1, and MMP3.
  - XJB-5-131 increases relative gene expression (%).

- **PG Synthesis**
  - XJB-5-131 rescues in vitro effects of high O₂ on PG synthesis (%).

- **Graphs**
  - Comparison of relative gene expression and PG synthesis under 5% and 20% O₂ conditions with and without XJB-5-131.
Systemic treatment with XJB in Ercc1\(^{-}/\Delta\) mice

*Ercc1\(^{-}/\Delta\) mice*  
(DNA repair defect)

Accelerated aging

9 wks \[\rightarrow\] 25 wks

Systemic anti-oxidant therapy in *Ercc1\(^{-}/\Delta\) mice* to slow down IDD

Treatment:
- XJB-5-131 by IP injection  
  2mg/Kg, 3x/week  
  Treatment start: 4 weeks  
  Treatment end: 12 weeks

Age (weeks)

0 4 8 10 12 14 16 20
XJB treatment improves disc histology in Ercc1^{-/Δ} mice

XJB-5-131

Neg. control
XJB treatment improves disc matrix content

Total GAG content (µgGAG/ng DNA)

- NT
- Oil
- XJB

PG Synthesis (fmoles sulfate/ng DNA)

- NT
- Oil
- XJB

* indicates p < 0.07
Conclusions

• Oxidative stress has been well established as a key driver of aging.

• We showed that ROS were produced in disc cells at high oxygen tension and this correlates with:
  ➢ Reduced PG (new matrix) synthesis
  ➢ Enhanced expression of catabolic factors

• Depleting ROS in disc cells using XJB-5-131 rescued matrix homeostatic imbalance

• Mitochondria-targeted free radical scavengers could be therapeutic in delaying the onset of age-related IDD
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