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ISSLS PRIZE WINNER: INHIBITION OF NF-κB ACTIVITY AMELIORATES AGE-ASSOCIATED DISC DEGENERATION IN A MOUSE MODEL OF ACCELERATED AGING

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Abstract

Study Design—NF-κB activity was pharmacologically and genetically blocked in an accelerated aging mouse model to mitigate age-related disc degenerative changes.

Objective—To study the mediatory role of NF-κB signaling pathway in age-dependent intervertebral disc degeneration.

Summary of Background Data—Aging is a major contributor to intervertebral disc degeneration (IDD), but the molecular mechanism behind this process is poorly understood. NF-κB is a family of transcription factors which play a central role in mediating cellular response to damage, stress, and inflammation. Growing evidence implicates chronic NF-κB activation as a culprit in many aging-related diseases, but its role in aging-related IDD has not been adequately explored. We studied the effects of NF-κB inhibition on IDD using a DNA repair-deficient mouse model of accelerated aging (*Ercc1*$^{-/\Delta}$ mice) previously been reported to exhibit age-related IDD.

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Methods—Systemic inhibition of NF-κB activation was achieved either genetically by deletion of one allele of the NF-κB subunit p65 (*Ercc1*$^{-/\Delta}$*p65*$^{+/-}$ mice) or pharmacologically by chronic intra-peritoneal administration of the Nemo Binding Domain (8K-NBD) peptide to block the formation of the upstream activator of NF-κB, IκB Inducible Kinase (IKK), in *Ercc1*$^{-/\Delta}$ mice. Disc cellularity, total proteoglycan content and proteoglycan synthesis of treated mice and untreated controls were assessed.

Results—Decreased disc matrix proteoglycan content, a hallmark feature of IDD, and elevated disc NF-κB activity were observed in discs of progeroid *Ercc1*$^{-/\Delta}$ mice and naturally aged wild-type compared to young WT mice. Systemic inhibition of NF-κB by the 8K-NBD peptide in *Ercc1*$^{-/\Delta}$ mice increased disc proteoglycan synthesis and ameriolated loss disc cellularity and matrix proteoglycan. These results were confirmed genetically by using the p65 haploinsufficient *Ercc1*$^{-/\Delta}$*p65*$^{+/-}$ mice.

Conclusion—These findings demonstrate that the IKK/NF-κB signaling pathway is a key mediator of age-dependent IDD and represents a therapeutic target for mitigating disc degenerative diseases associated with aging.

Keywords

NF-kB;aging; proteoglycan; disc degeneration; DNA damage repair; ERCC1-deficient mice

INTRODUCTION

Intervertebral disc degeneration (IDD) is an underlying etiology of many chronic debilitating and pathological disorders, including spinal stenosis, radiculopathy, disc herniation, and low back pain [1-3]. Although IDD is complex and multifactorial, aging is clearly the number one risk factor for many IDD-related disorders [4, 5]. Aging is closely correlated with a number of degenerative changes in the intervertebral disc (IVD), including cellular senescence, apoptosis, annular fissures, and reduced disc height [6, 7]. Loss of matrix proteoglycan (PG), a major structural component essential for disc biomechanical function, is another universal hallmark feature of disc aging [8]. Moreover, PG gene expression is decreased while expression of matrix metalloproteinases is increased in aged disc tissue [9, 10]. However, the molecular pathway(s) mediating these age-dependent disc degenerative changes are still largely unknown.

NF-κB is a family of transcription factors which play a central role in mediating cellular response to damage, stress, and inflammation [11]. Mammalian NF-κB family consists of five subunits, but the most common and abundant form is the p50/p65 heterodimer [12]. Under basal condition, NF-κB is localized primarily in the cytoplasm in an inactive state as it is being sequestered by the IκB proteins. NF-κB becomes activated in response to many different types of stress, including inflammatory, oxidative, genotoxic, mechanical and chemical stress. The canonical NF-κB activation pathway involves activation of IκB kinase (IKK), a heterotrimer consisting of two catalytic subunits, IKKα and IKKß, and a regulatory subunit termed IKKγ or NEMO (NF-κB Essential Modulator) [12]. Activated IKK then phosphorylates IκB, leading to its ubiquitination and subsequent proteosomal degradation [11]. IkB degradation allows NF-κB to translocate into the nucleus where it binds to its cognate DNA site to induce transcription of NF-κB -targeted genes [13] that regulate cell survival and growth, and production of inflammatory cytokines.

An expanding body of literature link chronic activation of NF-κB to tissue aging and many age-related degenerative diseases, including the musculoskeletal disorders such as, muscular dystrophy [14], osteoarthritis [15], osteoporosis [16]. NF-κB is chronically up-regulated in various tissues of aged rodents [17-19]. Increased NF-κB activity is also observed in cells derived from

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elderly persons and patients with Hutchinson-Gilford progeria syndrome, a disease of dramatically accelerated aging. Moreover, a recent modeling study identified NF-κB as the transcription factor most associated with mammalian aging and demonstrated that expression of a subset of NF-κB effectors is increased with aging [20].

The role of NF-κB signaling in age-related IDD is far less clear, although some existing evidence suggest its involvement the process of age-associated IDD. Elevated levels of several NF-κB-targeted genes, the pro-inflammatory cytokines TNF-α, IL-1β, IL-6 and IL-8, in aged and/or painful degenerated IVDs have been reported [21-23]. Increased level of oxidative stress, a key activator of NF-κB activity, has been reported in aged and degenerative disc tissue [24]. Moreover, immunohistochemical studies also showed positive correlation between the level of disc NF-κB activity and IVD degeneration grade and patient age [24]. Intra-discal injection of 'naked' NF-κB decoy oligonucleotide proved effective in partially restoring IVD height in a rabbit annular stab disc degeneration model, indicating that activation of NF-κB is involved in disc structural changes. [25].

Based on these observations, we hypothesize that activation of NF-κB signaling pathway plays a central role in mediating age-related degenerative changes in the intervertebral disc. We tested this hypothesis by blocking NF-κB activity pharmacologically and genetically using an *in vivo* rodent model of accelerated aging due to DNA repair deficiency (*Ercc1*$^{-/\Delta}$ mice) previously reported to exhibit accelerated disc aging symptoms [5].

MATERIALS AND METHODS

Mice breeding and isolation of intervertebral discs

Experiments involving mice were approved by the University of Pittsburgh Institutional Animal Care and Use Committee and in accordance with the NIH guidelines for humane care of animals. Wild-type and *Ercc1*$^{-/\Delta}$ mice of a mixed genetic background (FVB/n:C57Bl/6J) were bred and genotyped by PCR as previously described [26]. Knockin mice of C57Bl/6J genetic background expressing eGFP under the control of an NF-κB regulatory element (NF-κB^{eGFP}) were provided by Christian Jobin, UNC Chapel Hill [27]. *p65*$^{+/-}$ C57Bl/6J mice were provided by Denis Guttridge, Ohio State University, and bred with *Ercc1*$^{+/-}$ C57Bl/6J mice to generate *Ercc1*$^{+/-}$*p65*$^{+/-}$ C57Bl/6 mice. These were then bred with *Ercc1*$^{+/\Delta}$ FVB/n mice to generate *Ercc1*$^{-/\Delta}$*p65*$^{+/-}$ mice and littermate controls (Tilstra et al. submitted) [28]. Mice were euthanized at the established time points and the spines were isolated and dissected with the aid of a 5x magnifier. Entire intervertebral discs (IVDs) were removed en bloc from the surrounding vertebral bodies through an incision along the endplates using a surgical no. 11 blade. To harvest NP tissue, an axial cut was made on the disc side of the endplate to expose the disc center, followed by gentle aspiration of the NP tissue using a sterile P-10 pipette tip under a dissecting microscope (20-40 x magnification, *Nikon SMZ645*).

8K-NBD treatment of Animals

8K-NBD (KKKKKKKKGGTALDWSWLQTE) peptide was synthesized by the peptide synthesis facility at the University of Pittsburgh, Pittsburgh, PA. *Ercc1*$^{-/\Delta}$ mice were intraperitoneally treated with 8K-NBD 10 mg/kg three times per week. Treatment began at 5 weeks of age before the animals were symptomatic and continued until 18-20 weeks of age (Tilstra et al. submitted) [28]. Disc tissues were isolated for analyses.

Immunofluorescence

Wild-type NF-kB^{eGFP} mice were sacrificed at 5-6 months and 25-30 months of age. Tissues were placed in 10% formalin for 2 hours, and then transferred to 30% sucrose in phosphate

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buffered saline (PBS) overnight at 4°C. The tissues were then frozen in 2-methylbutane and embedded in optimal cutting temperature (OCT) at -20°C. Five micron sections were cut using a cryostat. Tissues were stained using HOESCT dye (Sigma) and mounted using gelvatol, as previously described [29]. Samples were allowed to incubate overnight at 4°C and were analyzed for cells expressing eGFP by fluorescence microscopy (*Nikon Eclipse* Ts100).

Histological Staining

Isolated spines were decalcified and embedded in paraffin (*Tissue Tek* processor and *Leica* embedder). Seven micrometer sections were stained with either hamatoxylin and eosin (H&E) or safranin O and fast green dyes (Fisher Scientific, Pittsburgh, PA) by standard procedure and photographed under 40-200x magnification (*Nikon Eclipse* Ts100).

1,9-Dimethylmethylene Blue (DMMB) Colorimetric Assay for Sulfated Glycosaminoglycans (GAGs)

NP isolated from six lumbar IVDs of each mouse was pooled and digested using papain at 60°C for two hours. GAG content was measured in duplicate by the DMMB procedure [30] using chondroitin-6-sulfate (*Sigma* C-8529) as a standard. The DNA concentration of each sample was measured using the PicoGreen assay (*Molecular Probes*) and used to normalize the GAG values. Average values from six reaction samples (two duplicates x three mice per group) were calculated and reported ± standard error.

Quantitation of proteoglycan synthesis

Disc organ cultures of isolated functional spine units (FSU), each consisting of vertebra, disc, vertebra, were established as previously described [31]. Four thoracic FSUs were cultured in complete growth medium (F-12/D-MEM containing 10% FCS, 1% PS, and 25 μg/ml L-ascorbic acid) for two days to equilibrate after the trauma of surgical dissection, followed by 12 hour labeling incubation with ^{35}S-sulfate (20 μCi/ml). Proteoglycan synthesis was measured by ^{35}S-sulfate incorporation as described previously [32]. The rate of proteoglycan synthesis was calculated as the fmoles of sulfate incorporated per μg DNA. Average values from six reaction samples (two duplicates x three mice per group) were calculated and reported ± standard error.

Quantitation of matrix gene expression

Total RNA was purified from whole discs using the RNeasy Plus Universal Kit (*Qiagen*). The RNA was analyzed in duplicate reactions by real-time RT-PCR (iCycler IQ4, *Bio-Rad*) to determine mRNA levels of these selected NF-κB gene targets.

Gene	Forward (5'→3')	Reverse (5'→3')
GAPDH	GAGGCCGGTGCTGAGTAT	GCGGAGATGATGACCCTTTTGG
IL-1β	GCAACTGTTCCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
IL-6	GACTTCCATCCAGTTGCCTTC	ATTTCCACGATTTCCCAGAG
MMP-1β	TCTTTATGGTCCAGGCGATGAA	CCTCTTCTATGAGGCGGGGAT
MMP-3	GGTACAGAGCTGTGGGAAGTC	GATGAGCACACAACCACACAC
iNOS	ATGACACTCTTCACCACAAGG	CAATGGCATGAGGCAGGAG

The cycle threshold (Ct) values were obtained and normalized to the housekeeping gene GAPDH. The ΔΔCt method [33] was used to calculate the relative mRNA levels of each

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target gene between *Ercc1*$^{-/\Delta}$ mice and wild-type littermates. Average values from six measurements (duplicate × 3 mice per group) are shown ± one standard error.

Statistical analysis

Values represent the average of 6 trials ± standard error (SE), with 95% confidence intervals calculated to determine statistical significance. The confidence intervals were calculated based on the t-distribution because of the small sample size.

RESULTS

NF-κB is activated in intervertebral discs of natural and accelerated aging mice

Previous analysis of human discs reported a positive correlation between NF-κB activation in disc tissue and aging [24]. To verify this finding in mice, we used knockin mice expressing eGFP under the control of an NF-κB regulatory element (NF-κB^{eGFP}) (Clauson et al, in preparation). In these mice, green fluorescence from eGFP expression indicates activation of the NF-κB pathway. Through fluorescent microscopy, eGFP was detected in the nucleus pulposus of old (>2 yrs), but not young (5-6 months) wild-type (WT) NF-κB^{eGFP} mice (Fig. 1A), indicating increased NF-κB activity disc tissues of aged mice. To further confirm this result, we analyzed disc expression of selected genes known to be induced by activated NF-κB, including the interleukins (IL-1β, IL-6), the matrix metalloproteinases (MMP-1β, MMP-3), and inducible nitric oxide synthase (iNOS). The level of mRNA expression of these NF-κB gene targets was higher in discs of old WT mice compared to young WT mice (Fig. 1B). Similarly, expression of these NF-κB responsive genes also was generally greater in discs of accelerated aging progeroid *Ercc1*$^{-/\Delta}$ mice (5-6 mths) compared to those in their WT littermates (Fig. 1B). Together, these data provide evidence of increased NF-κB activity in discs with aging.

Genetic reduction of NF-κB mitigates loss of disc matrix proteoglycan in progeroid Ercc1$^{-/\Delta}$ mice

Accelerated aging *Ercc1*$^{-/\Delta}$ mice exhibit distinct age-related histopathologic changes in their discs, including reduced safranin O staining for disc proteoglycans (PG) [5]. To test whether these degenerative changes are mediated by NF-κB, we measured disc PG content using the *Ercc1*$^{-/\Delta}$ mouse strain containing a genetic deletion of one allele of the NF-κB subunit p65 (*Ercc1*$^{-/\Delta}$*p65*$^{+/-}$ mice). Compared to *Ercc1*$^{-/\Delta}$ mice, *Ercc1*$^{-/\Delta}$*p65*$^{+/-}$ mice exhibited higher levels of disc PG content as assessed qualitatively by histological safranin O staining (Fig. 2A) and quantitatively by DMMB assay for total disc GAG content (Fig. 2B). In addition, net loss of disc PGs in *Ercc1*$^{-/\Delta}$ mice could be caused by decreased PG synthesis. We determined PG synthesis by measuring the level of ^{35}S incorporated into the discs of mice *ex vivo*. Disc PG synthesis from *Ercc1*$^{-/\Delta}$*p65*$^{+/-}$ mice (9.1 ± 1.7 fmoles sulfate/ng DNA) was 30% higher than that from *Ercc1*$^{-/\Delta}$ mice (6.1 ± 1.7 fmoles sulfate/ng DNA), but was still about 30% lower than that from WT mice (12.4 ± 1.1 fmoles sulfate/ng DNA) (Fig. 2B). Thus, genetic reduction of NF-κB mitigated PG loss in progeroid *Ercc1*$^{-/\Delta}$ mice in part through enhancing PG matrix synthesis.

8K-NBD inhibition of IKK/NF-κB attenuates disc histopathological changes and PG loss associated with aging in progeroid Ercc1$^{-/\Delta}$ mice

To independently confirm the beneficial effects of reducing NF-κB activity by genetic depletion, we treated *Ercc1*$^{-/\Delta}$ mice using the 8K-NBD peptide that reduces NF-κB activation by inhibiting the formation of the IKK protein complex (Fig. 3). The 8K-NBD peptide, consisting of an 8 lysine protein transduction domain [34] fused to the 11 amino acid 8K-NBD peptide, was shown previously to have therapeutic efficacies in animal models of

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inflammatory bowel disease, muscular dystrophy, and arthritis [14, 35, 36]. *Ercc1*$^{-/\Delta}$ mice at 5 weeks of age, were either untreated or treated with 10 mg/kg of 8K-NBD three times per week intraperitoneally until the age of 18-20 weeks (Fig. 4A). This dose and treatment regiment was selected because it was previously shown to be therapeutic in mouse models of muscular dystrophy[14] and inflammatory bowel disease [36]. Compared to untreated control, 8K-NBD-treated mice showed noticeable improvement in nucleus pulposus (NP) matrix proteoglycan content by safranin O histological staining (Fig. 4B). 8K-NBD treatment also resulted in increased cellularity in the endplate and denser matrix network within the NP as assessed by H&E staining (Fig. 4B).

To confirm the therapeutic effects of 8K-NBD, we also measured disc matrix PG content using the quantitative DMMB assay for total GAG. Total GAG of NP tissue of 8K-NBD treated *Ercc1*$^{-/\Delta}$ mice (555 ± 21 μg GAG/ng DNA) was greater than that of untreated *Ercc1*$^{-/\Delta}$ controls (295 ± 31 μg GAG/ng DNA) but was still lower than that of WT mice (662 ± 29 μg GAG/ng DNA) (Fig. 4C, left graph). Disc PG synthesis from 8K-NBD treated *Ercc1*$^{-/\Delta}$ mice (11 ± 2 fmoles sulfate/ng DNA) also showed a substantial increase over that of the untreated *Ercc1*$^{-/\Delta}$ mice (6 ± 2 fmoles sulfate/ng DNA) (Fig. 4C, right graph). Thus consistent with the effects observed using the NF-κB genetic depletion approach, 8K-NBD inhibition of NF-κB also appeared to mitigate PG loss in progeroid *Ercc1*$^{-/\Delta}$ mice in part through enhancing PG matrix synthesis.

DISCUSSION

Increasing evidence implicates chronic activation of NF-κB as a major mediator of many age-associated degenerative diseases. In this report, we used a mouse strain harboring a NF-κB^{eGFP} reporter to demonstrate that there is a significant increase in the percent of cells in the disc with activated NF-κB in aged mice. Moreover, in addition to demonstrating that NF-κB is upregulated in the disc with aging, we demonstrate that activation of NF-κB plays an important role in age-associated disc degeneration. Systemic inhibition of NF-κB activation by chronic administration of the 8K-NBD peptide in *Ercc1*$^{-/\Delta}$ mice mitigated age-related IDD, including loss of disc matrix proteoglycan and cellularity. This result was confirmed genetically by crossing *Ercc1*$^{-/\Delta}$ mice into a p65 haploinsufficient background to reduce NF-κB, thus demonstrating that the therapeutic effect of 8K-NBD on disc health is a result of direct inhibition of NF-κB pathway and not other nonspecific targets. These results extend our previous study demonstrating that genetic depletion of one allele of the p65 subunit of NF-κB or pharmacologic inhibition of NF-κB attenuated numerous age-related pathologies in *Ercc1*$^{-/\Delta}$ mice including impaired gait, epidermal atrophy, sarcopenia, anemia, liver and kidney dysfunction, incontinence and trembling [28].

Previous studies reported evidence of increased NF-κB activation in aged and degenerated human disc [24]. Elevated levels of NF-κB target genes, including the pro-inflammatory cytokines TNF-α, IL-1β, IL-6 a n d I L-8, have been documented in human degenerative discs [21-23, 37, 38]. Immunomorphological analysis revealed a higher level of carboxymethyl-lysine (CML), a biomarker of oxidized protein, in degenerated intervertebral discs from aged patients compared to young normal discs [24]. Age-associated oxidative damage in disc is also evident by the accumulation of advanced glycation end products (AGEs), produced by nonenzymatic glucosylation and oxidation of proteins and lipids [39, 40]. These findings suggest that cells in aged discs are exposed to oxidative stress, a known activator of the NF-κB pathway.

Our study also indicates enhanced disc NF-κB activity in mice with age. This is consistent with the idea that NF-κB is activated in response to cellular stress and damage, including DNA damage, which stochastically accumulate with age [11, 41]. In the case of *Ercc1*$^{-/\Delta}$ mice,

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which age rapidly due to a DNA repair deficiency, accumulation of DNA damage is likely the source that triggers NF-κB activation in disc tissue. Indeed, in a separate study we have demonstrated through immuno and biochemical analyses that NF-κB is activated in a variety of tissue types in the ERCC1-deficient mouse model of accelerated aging, including liver, kidney and muscle [28], as well as in tissues of naturally aged mice including liver, muscle, gastrointestinal tract, and bone marrow (Clauson et al, in preparation). Together, these observations strongly suggest that during the aging process the NF-κB pathway plays a vital role in mediating the effects of various stressors on the changes in cellular transcription program in disc tissue leading to the development of age-related IDD.

Aging causes many degenerative changes to intervertebral discs, particularly loss of disc proteoglycan matrix which is highly detrimental as this is typically accompanied by a concomitant decrease in disc hydration and reduced ability of the tissue to resist compression [42, 43]. Our study shows that by decreasing NF-κB activity in *Ercc1*$^{-/\Delta}$ mice, PG synthesis was improved and PG loss was alleviated. *Ercc1*$^{-/\Delta}$ mice harbor a high level of senescent cells in the disc [5]. Senescent chondrocytes also have been reported to have compromised capacity to synthesize new matrix [44]. Thus the decline in disc PG synthesis in *Ercc1*$^{-/\Delta}$ mice could be due to increased cellular senescence. Since cell senescence is a well-demonstrated consequence of cellular response to DNA damage [41, 45], our results suggest that the accumulation of DNA damage in the DNA repair-deficient *Ercc1*$^{-/\Delta}$ mice promotes cellular senescence in their intervertebral discs through the mediatory action of NF-κB.

It should be noted that neither our pharmacologic (8K-NBD treatment) nor genetic (*p65*$^{+/-}$) intervention completely revert the age-related disc degenerative changes in *Ercc1*$^{-/\Delta}$ mice to the normal disc phenotype observed in their control littermates. This is not unexpected because disc aging process is complex and likely involves other molecular pathways besides the NF-κB signaling pathways. Moreover, it is not possible to attribute how much of the observed therapeutic effects in our study are the direct effects of NF-κB inhibition in disc tissue. Alternatively, it is also possible that the therapeutic effects on discs are also due to the systemic response to NF-κB inhibition which, as we have shown in a separate study, delays the onset of many other age-related pathologies in ERCC1-deficient mice and improves the overall health of these animals [28].

In summary, our findings suggest that DNA damage promotes age-dependent intervertebral disc degeneration and aging and that NF-κB activation mediates both natural and accelerated disc aging and age-related disc diseases. This study also suggests that the IKK/NF-κB signaling pathway is a potential therapeutic target for treating age-related disc degenerative disorders.

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KEY POINTS

■ NF-κB activity is elevated in aged and degenerated disc tissue of naturally aging mice and accelerated aging *Ercc1*$^{-/\Delta}$ mice.

■ Reduction of NF-κB activity in accelerated aging *Ercc1*$^{-/\Delta}$ mice either by genetic depletion of an allele of the NF-κB subunit p65 or by pharmacologic inhibition using the Nemo Binding Domain (8K-NBD) peptide to block the IκB Inducible Kinase mitigates loss of disc matrix proteoglycan.

■ NF-κB-mediated signal transduction pathway plays an important role in age-related IDD and represents as a potential therapeutic target for treating age-dependent disc pathologies.

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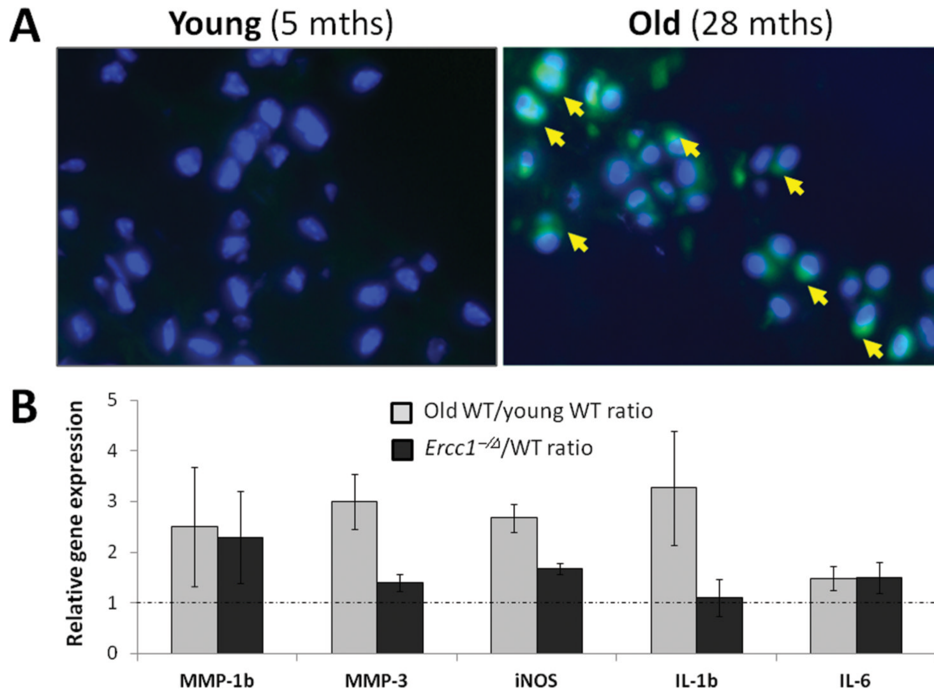

Figure 1. Increased NF-κB activation in disc tissue of natural and accelerated aging mice

A, Disc sections from NF-κB^{eGFP} young (5 months, left image) and old (28 months, right image) WT mice were imaged using fluorescent microscopy to detect eGFP expression (green) and nuclei were counter-stained with Hoechst (blue). Representative images of the nucleus pulposus region are shown. Arrows, eGFP-positive cells. **B**, disc mRNA levels of selected NF-κB target genes as determined by qRT-PCR. Gray, the ratios of disc mRNA level of old (23-28 months) to young (5-6 months) wild-type mice. Black, the ratios of disc mRNA level of progeroid *Ercc1*$^{-/\Delta}$ mice (5-6 months) to their WT littermates.

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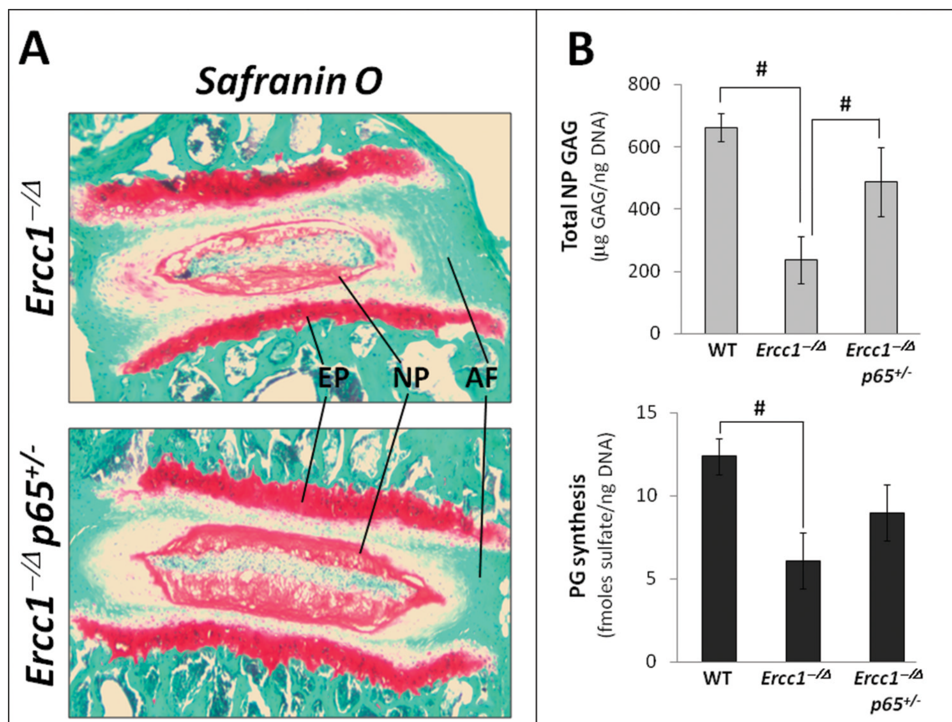

Figure 2. Genetic depletion of the p65 NF-κB subunit mitigates age-associated disc proteoglycan loss and histopathologic changes

A, Safranin O/fast green histologic staining of disc sections. Endplate (EP), nucleus pulposus (NP), and annulus fibrosus (AF) are indicated. Red, safranin O staining of proteoglycan. **B**, DMMB assay for total GAG of NP tissue (top graph) and proteoglycan synthesis as measured by ^{35}S incorporation by disc organotypic culture (bottom graph). # denotes $p < 0.05$.

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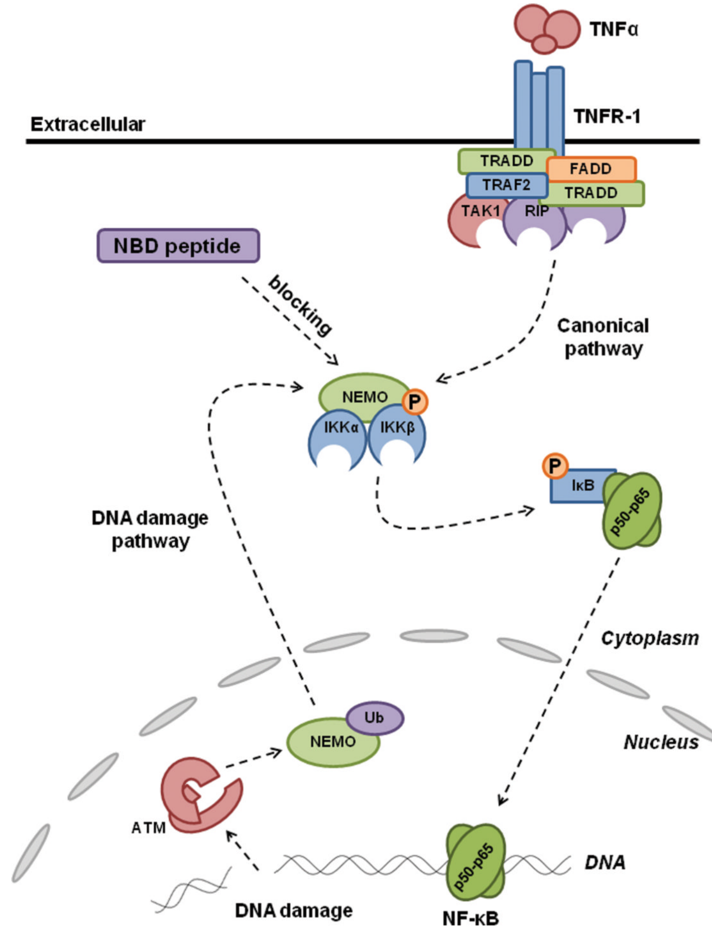

Figure 3. Inhibition of NF-κB activation by the NBD peptide

NF-κB activation: Pro-inflammatory stress (canonical pathway) or stress from unrepaired DNA damage (non-canonical pathway) induces the formation of the IKK complex which leads to the phosphorylation of IκB and its subsequent degradation. NF-κB is released and translocated into the nucleus to activate transcription of its target genes. NBD inhibition of NF-κB: NBD binds to the NEMO protein (also known as IKKγ) and prevents it from associating with the α and β subunits. This prevents the formation of the IKK complex and hence NF-κB activation.

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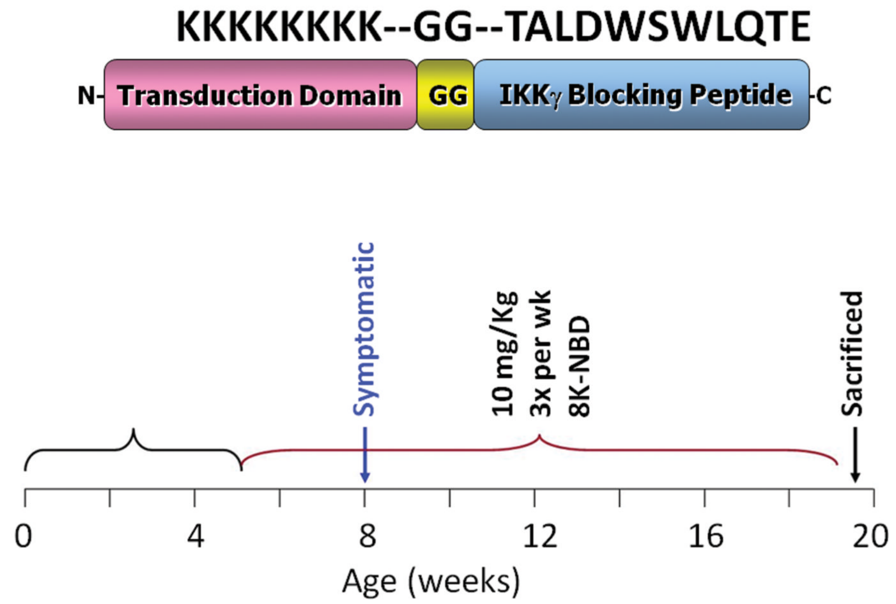

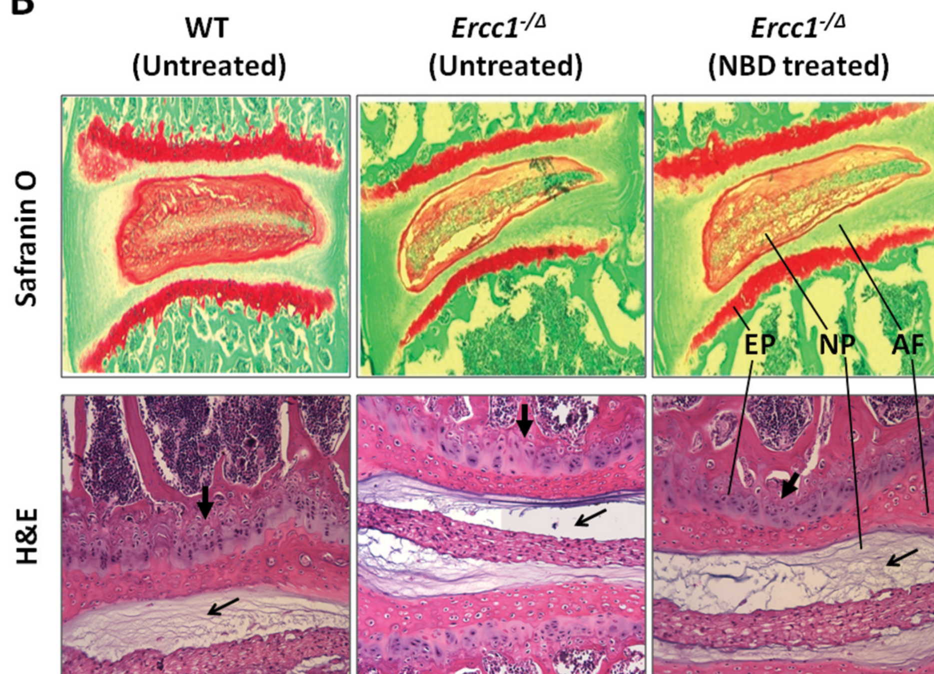

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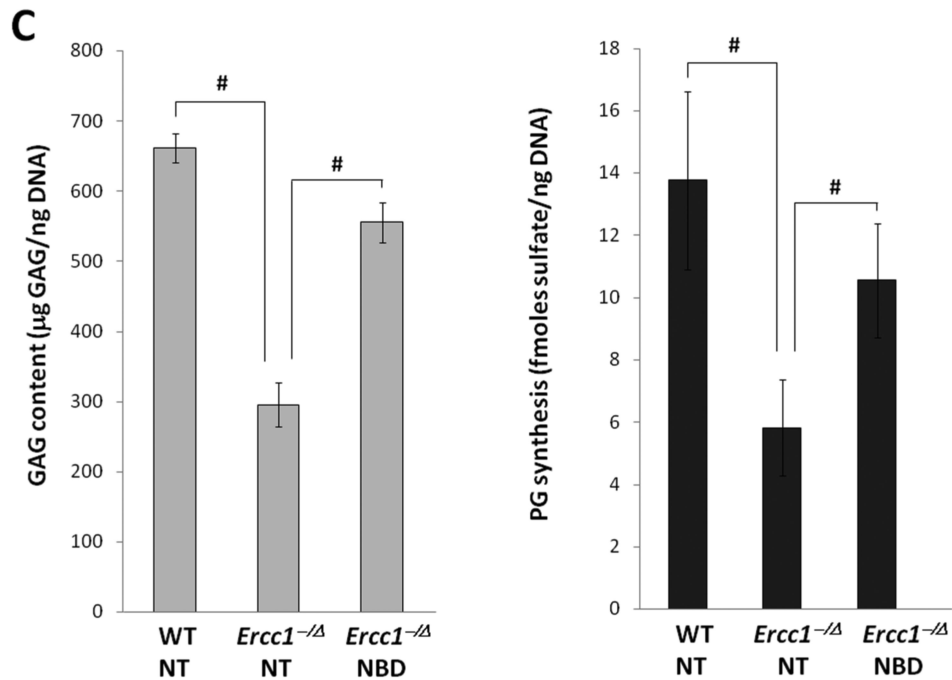

Figure 4. Pharmacologic suppression of IKK/NF-κB activation ameliorates age-associated disc proteoglycan loss and histopathologic changes

A, 8K-NBD peptide structure and amino acid sequence (top) and 8K-NBD treatment regimen (bottom). *Ercc1*$^{-/\Delta}$ mice were treated with 10 mg 8K-NBD per kg body weight three times per week intraperitoneally starting from 5 wks of age until 20 wks of age. **B**, Safranin O and H&E histological staining of disc sections of 8K-NBD-treated *Ercc1*$^{-/\Delta}$ mice and untreated *Ercc1*$^{-/\Delta}$ mice and WT control. Endplate (EP), nucleus pulposus (NP), and annulus fibrosus (AF) are indicated. Red, safranin O staining of proteoglycan. EP cellularity (thick arrow) and NP matrix density (thin arrow) are indicated. **C**, DMMB assay for total GAG of NP tissue (left graph) and proteoglycan synthesis as measured by ^{35}S incorporation by disc organotypic culture (right graph). NT, not treated. # denotes $p < 0.05$.