Role of DNA damage in aging-associated intervertebral disc degeneration: genotoxic stress accelerates disc matrix loss in a mouse model of human progeria

INTRODUCTION. Biological ageing is defined as the loss of functional reserve due to time-dependent accumulation of cellular and molecular damage. Loss of disc extracellular matrix (ECM) is invariably linked with aging. Deficiency in DNA repair induces rapid aging in humans and animals. Thus we hypothesize that DNA damage, when not repaired, is an important type of cellular damage which can promote disc aging and matrix loss. This is consistent with the fact that aging and smoking, both of which accrue DNA damage, are two major risk factors for disc degeneration. To test our hypothesis, we challenged wild-type mice and their DNA repair deficient Ercc1-Δ mice with the chemotherapeutic agent mechlorethamine (MEC) to induce DNA damage. If our hypothesis is correct, the prediction is that exposure to MEC will accelerate age-related disc ECM loss and degenerative changes, and in Ercc1-Δ to a greater extent than wild-type mice.

METHODS. Ercc1-Δ mice (n=6) and their wild-type littermates were chronically exposed to genotoxic stress by administration of a subtoxic dose of the chemotherapeutic agent mechlorethamine (MEC). Beginning at 8 wks of age mice were injected subcutaneously with 8 μg/kg MEC once per week for 6 weeks. At 20 wks of age they were euthanized and spines were collected for analysis. Paraffin embedded spine sections were stained with safranin O to assess proteoglycan (PG) content and with Masson’s Trichrome stain for collagen. Disc cryosections were analyzed by immunohistochemistry (IHC) to detect aggrecan (Millipore, ab1031), ADAMTS4 (Millipore, ab19165), and ADAMTS-generated G1 aggrecan fragments terminating in NITEGE-373 sequence using Ab1320 antibody. PG synthesis was measured by 35S-sulfate incorporation using freshly isolated discs in organ culture.

RESULTS. Histological staining revealed substantial reduction of collagen in the discs of MEC-exposed animals (Fig 1A). Safranin O staining revealed similar results for proteoglycans. In particular, large unstained extracellular gaps in the nucleus pulposus (NP) tissue were observed in MEC-treated Ercc1-Δ, suggesting loss of matrix (Fig 1A, small arrows). Cell number in the endplate was also severely reduced in MEC treated Ercc1-Δ mice (Fig 1A, large arrow). Aggrecan IHC signal (Fig 1B, brown) was reduced in the NP of MEC-exposed mice, but the effect was much more pronounced in Ercc1-Δ strain. MEC treatment drastically increased IHC detection of ADAMTS4 and NITEGE-373 containing aggrecan proteolytic fragment, suggesting enhanced disc matrix breakdown (Fig 1C). On the other hand, disc PG synthesis was reduced 2-3 fold in mice exposed to MEC (Fig 3), indicating that disc PG matrix synthesis is also compromised in the treated animals.

DISCUSSION. Chronic exposure to genotoxic stress decreased disc matrix PGs and collagen in adult wild-type and Ercc1-Δ mice. The effect was exaggerated in the DNA repair deficient Ercc1-Δ mice, implicating DNA damage as a contributor to disc aging and degeneration. The rapid loss of disc matrix in these mice is most likely due to a combination of MEC-mediated enhanced matrix breakdown and reduced synthesis. MEC-induced aggrecan proteolysis could be mediated by ADAMTS4 as its expression is greatly induced by MEC. These results support the conclusion that DNA repair plays a vital role in maintaining disc ECM homeostasis and preventing disc degeneration. Ercc1-Δ mice, a novel and rapid murine model of age-related disc degeneration, are useful for exploring the molecular mechanisms by which DNA damage promotes age-related disc matrix loss and degeneration.

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REFERENCES
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Fig 1: Aggrecan immunodetection (A) and Masson’s Trichrome staining of collagen (B). Insets, untreated controls.

Fig 2: Immunodetection of ADAMTS4 (right) and aggrecan proteolytic products (left).

Fig 3: Suppressive effect of MEC on disc proteoglycan synthesis.