

# Localized Knee Irradiation Creates Persistent DNA Damage in Chondrocytes and Promotes Joint Hyperalgesia

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**INTRODUCTION:** Osteoarthritis (OA) is the most common form of arthritis and is characterized by progressive loss of articular cartilage which can cause chronic joint pain and loss of mobility. Aging is a significant risk factor for OA and is thought to be a consequence of cumulative DNA damage<sup>1,2</sup>. Recently it was shown that either aged or OA human knee cartilage contained more DNA-damaged chondrocytes relative to young or healthy cartilage<sup>3</sup>. Furthermore, proinflammatory pathways are activated in response to a particularly toxic form of DNA damage, DNA double-strand breaks (DSBs), notably stimulating the nuclear factor  $\kappa$ B (NF- $\kappa$ B) pathway<sup>4</sup>. We previously identified increased NF- $\kappa$ B activity in aged chondrocytes and demonstrated early OA onset in young mice using a model of chondrocyte-specific NF- $\kappa$ B induction<sup>5</sup>. Whether DNA damage could cause proinflammatory signaling in chondrocytes and other neighboring cell types has not been well-characterized. Here, we used highly localized X-irradiation to induce DNA damage in mouse knee joints to examine the hypothesis that accumulation of DNA damage leads to chronic proinflammatory signaling in chondrocytes, pain-related behaviors, and ultimately OA onset.

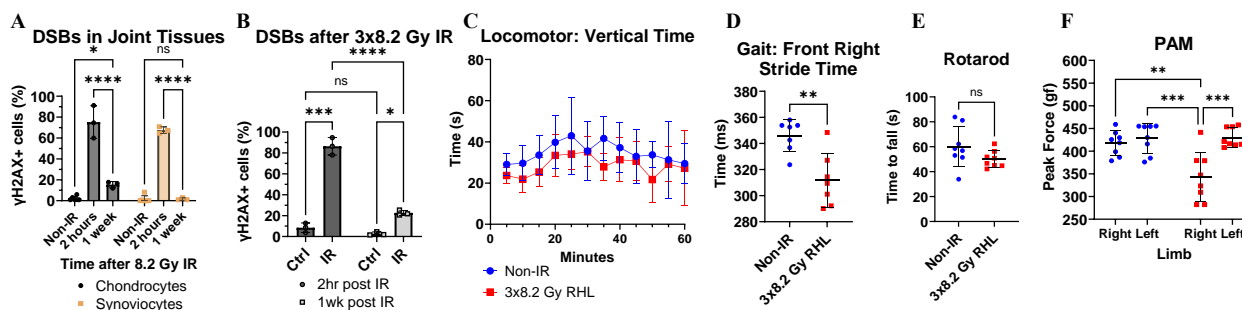
**METHODS:** Male C57BL/6J mice, 4 months of age, were irradiated using the Xtrahl small animal radiation research platform (SARRP). Irradiated mice received either a single 8.2 Gy dose (n=3-6) using a 3x3mm collimator centered on the right hind knee joint or a more clinically relevant dose of 3, 8.2 Gy fractions with a single fraction delivered every 48 hours (n=3-8). Non-irradiated mice and/or non-irradiated contralateral limbs acted as controls. At 6 months after irradiation mouse mobility and pain-related behaviors were assessed by locomotor (open-field), gait, rotarod, and knee pressure application measurement (PAM). Mouse hind limbs were collected 2 hours, 48 hours, or 1 week, after irradiation and subsequently processed for paraffin histology, sections were stained for phosphorylated histone H2AX ( $\gamma$ H2AX) to evaluate the DNA DSB repair response *in vivo*. Semi-quantitative IHC analysis was performed using VisioPharm software. All animal studies were approved by an ethics committee. Statistical software (GraphPad Prism) was used to test significance of results by Students t-test or 2-way ANOVA with post-hoc Tukey's multiple comparisons test.

**RESULTS:** Shortly after radiation treatment by a single dose of 8.2 Gy X-rays, nearly 75% of articular chondrocytes were  $\gamma$ H2AX+. Despite significant repair of DNA DSBs, irradiation induced DNA DSBs which persisted in about 15% chondrocytes at least 1 week after treatment. Notably, synoviocytes completely repaired from a single dose within 48 hours suggesting a unique response to DNA damage from chondrocytes (Fig. 1A). After a more clinically relevant dosing regimen of 3, 8.2 Gy fractions, DNA DSBs were significantly more abundant one week after irradiation compared to contralateral controls (Fig. 1B). Mice that received 3 fractionated doses 6 months prior did not display differences in locomotor behavior with only a slight decline in the rearing behavior of irradiated mice (Fig. 1C). However, gait analysis revealed several significant aberrations including: decreased front right average stride time, decreased front track width, and a decrease in all step sequence counts (Fig. 1D). Irradiation had little effect on time to fall during rotarod tests, but irradiated mice did have lower trending times (Fig. 1E). Peak force PAM thresholds were significantly lower in irradiated limbs, indicating knee hyperalgesia in only irradiated limbs (Fig. 1F).

**DISCUSSION:** Localized irradiation of mouse knee joints induced persistent DNA DSBs as long as a week after irradiation and knee hyperalgesia several months after treatment. These results highlight the importance of chondrocyte DNA damage response to our understanding of the pathogenesis of OA in aging. Surprisingly, DNA DSBs persisted at least a week after initial X-radiation treatment compared to synoviocytes which completely repaired within 48 hours potentially due to their propensity for proliferation. Although we observed little effect on mouse mobility by locomotor and rotarod behavioral tests, we recorded a surprising compensation effect from the front right limbs of irradiated mice and consistently lower pain thresholds by PAM. This could indicate effects like inflammation in synovium before mobility is substantially affected by degeneration of articular cartilage in this irradiation model. We are currently assessing effects up to 9 months after knee joint irradiation on joint tissue structures by  $\mu$ CT and histology as well as the using IHC methods to better understand the DNA damage response of chondrocytes, synoviocytes, and osteocytes.

**SIGNIFICANCE/CLINICAL RELEVANCE:** Localized irradiation of mouse knee joints induced hyperalgesia and gait abnormalities highlighting the importance of understanding the consequences of DNA damage in joint tissues and serving as a foundation for a potential future model for age-related OA development. These data are supported by published findings which suggest that humans who underwent radiotherapy to treat prostate cancer were more likely to develop hip osteoarthritis in the 10 years following their treatment<sup>6</sup>.

**REFERENCES:** 1. Loeser, *Curr Opin Rheumatol*, 2011; 2. Yousefzadeh et al., *eLife*, 2021; 3. Copp et al., *Aging Cell*, 2022; 4. Dunphy et al., *Mol Cell*, 2018; 5. Catheline et al., *Sci Signal*, 2021; 6. Rasmussen et al., *Adv Radiat Oncol*, 2021.



**Figure 1:** A) IHC quantification of  $\gamma$ H2AX+ chondrocytes and synoviocytes at either 2 hours or 1 week after mice received a single 8.2 Gy dose of X-rays localized to the right hind knee. B-F) Data are from mice that received 3x8.2 Gy X-ray treatment localized to the right hind limb (RHL) knee joint. B) IHC quantification of  $\gamma$ H2AX+ chondrocytes 2 hours or 1 week after 3x8.2 Gy X-ray dose with contralateral (Ctrl) controls. Six months after treatment, behavior of the mice was evaluated by: C) Locomotor testing of mice displaying the total time spent in a vertical position in 5-minute increments, D) Gait analysis showing front right stride time, E) Rotarod which displays average time to fall over a 5-day trial period, and F) knee hyperalgesia was measured by PAM of each mouse's hind limbs and displays peak force at pain threshold.