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Clinical Trial

Disruption of glucocorticoid signalling in osteoblasts attenuates age-related surgically induced osteoarthritis

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SUMMARY

Objective: Aging is a major risk factor for osteoarthritis (OA). Skeletal expression and activity of the glucocorticoid-activating enzyme 11β-hydroxysteroid-dehydrogenase type 1 increases progressively with age in humans and rodents. Here we investigated the role of endogenous osteocytic and osteoblastic glucocorticoid (GC) signalling in the development of osteoarthritic bone and cartilage damage in mice.

Methods: We utilized transgenic (tg) mice in which glucocorticoid signalling is disrupted in osteoblasts and osteocytes via overexpression of the glucocorticoid-inactivating enzyme, 11β-hydroxysteroid-dehydrogenase type 2. Osteoarthritis was induced in 10- and 22-week-old male transgenic mice (tg-OA, $n = 6$/group) and their wildtype littermates (WT-OA, $n = 7–8$/group) by surgical destabilization of the medial meniscus (DMM). Sham-operated mice served as controls (WT- & tg-Sham, $n = 3–5$ and 6 −8/group at 10- and 22-weeks of age, respectively).

Results: Sixteen weeks after DMM surgery, mice developed features of cartilage degradation, subchondral bone sclerosis and osteophyte formation. These changes did not differ between WT and tg mice when OA was induced at 10-weeks of age. However, when OA was induced at 22-weeks of age, cartilage erosion was significantly attenuated in tg-OA mice compared to WT-OA littermates. Similarly, subchondral bone volume (−5.2%, 95% confidence intervals (CI) -9.1 to −1.2%, $P = 0.014$) and osteophyte size (−4.0 mm^2, 95% CI -7.5 to −0.5 mm^2, $P = 0.029$) were significantly reduced in tg-OA compared to WT-OA mice.

Conclusion: Glucocorticoid signalling in cells of the osteoblast lineage promotes the development of surgically-induced osteoarthritis in older, but not younger, male mice. These data implicate osteoblasts and osteocytes in the progression of DMM-OA, via a glucocorticoid-dependent and age-related pathway.

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Introduction

Osteoarthritis (OA) is a common chronic condition, with age as one the major risk factors[1,2]. In line with the aging world population, the prevalence of OA has increased across all ethnicities. The physical health as well as social consequences of OA account for a large proportion of health care expenditure worldwide. This individual and societal socioeconomic burden is accentuated by a lack of effective non-surgical treatments for OA. Thus, symptomatic therapies, including oral or transdermal analgesics or intra-articular injections of glucocorticoids have limited long-term efficacy. More importantly, there are currently no medical interventions that effectively prevent or delay the development of progressive OA structural damage. While intra-articular injections of glucocorticoids at pharmacological doses are commonly used for pain relief and to control inflammation[3–7], the role of *endogenous* glucocorticoids in the pathogenesis of OA is unknown.

Glucocorticoid actions in cells and tissues depend not only on plasma hormone concentrations and receptor expression, but also on local glucocorticoid metabolism within target cells. This 'pre-receptor' regulation of glucocorticoid availability is determined by the interconversion of hormonally active and inactive ligands by two 11β-hydroxysteroid dehydrogenase (11βHSD) isoenzymes[8]. Thus, 11βHSD1 type 1 (11βHSD1) catalyses the conversion of inactive cortisone (in humans) and 11-dehydrocorticosterone (in rodents) to active cortisol and corticosterone, respectively. Stimulation of 11βHSD1 activity therefore leads to increased intracellular levels of active glucocorticoids. Conversely, 11βHSD type 2 (11βHSD2) inactivates cortisol & corticosterone, and thus decreases the intracellular availability of active glucocorticoids[8]. While 11βHSD1 is expressed widely, including in osteoblasts, adipocytes and hepatocytes, 11βHSD2 is only expressed in mineralocorticoid target tissues, such as the kidney, and is not normally present in murine bone cells[9,10].

Interestingly, 11βHSD1 expression and activity increases progressively in osteoblasts with increasing age in both humans and rodents. As a result, the local activation of endogenous glucocorticoids in bone increases significantly with age[11,12]. Allelic variants of *Hsd11b1* (the gene for 11βHSD1) that are associated with low 11βHSD1 activity have been linked to increased bone density and reduced fracture risk in humans[13]. However, it is not clear if the cellular metabolism of endogenous glucocorticoid in osteoblasts and osteocytes modulates OA progression.

To address this question, we made use of a transgenic (tg) mouse model in which expression of the glucocorticoid inactivating enzyme, 11β-hydroxysteroid-dehydrogenase type 2 (11βHSD2), has been targeted exclusively to osteoblasts and osteocytes, using a 2.3 kb collagen type Iα1 promoter (Col2.3-11βHSD2 tg mice). Targeted overexpression of 11βHSD2 represents a highly specific means of disrupting glucocorticoid signalling in osteoblasts and osteocytes. Using the destabilization of the medial meniscus (DMM) surgical mouse model of osteoarthritis, the current study aims to investigate the role of age-associated changes in endogenous glucocorticoid signalling in osteoblasts and osteocytes in osteoarthritis.

Materials and methods

Transgenic mouse model

Col2.3-11βHSD2 tg mice were generated in a CD1 background as described previously[9] and were a gift from Dr Barbara Kream (University of Connecticut, USA). Mice were maintained at the animal facilities of the ANZAC Research Institute, Sydney, in accordance with institutional Animal Welfare Guidelines and all *in vivo* study procedures were approved by the Sydney Local Health District Animal Welfare Committee.

Mouse osteoarthritis model

Individual male wild-type (WT) and *Col2.3-11βHSD2* tg (tg) littermates were assigned to experimental groups (sham, OA) using the random number generating function in Microsoft Excel. Osteoarthritis (OA) was induced in 10- and 22-week-old mice littermates by DMM of the right knee[14]. Briefly, the anterior fat pad was dissected to expose the anterior medial menisco-tibial ligament, which was then severed. The knee was flushed with saline and the incision closed (n = 6–8/group). Mice were anaesthetized by inhalation of isoflurane (3% induction and 2% maintenance) in 1L/min oxygen during surgery. The mice were fully mobile within 3 min following withdrawal of isoflurane. Joints subjected to sham-operation (capsulotomy only, n = 3–8/group) were used as controls. Only male mice were used to avoid sex-related differences in disease severity[15]. Mice were maintained in their pre-operative groups (2–5/cage) and allowed unrestricted cage exercise. For both age groups, mice were humanely killed 16 weeks post-surgery.

Tissue collection and micro-CT

After harvesting, knee joints were dissected and fixed in 4% paraformaldehyde/PBS. Micro-CT analyses were performed using a Skyscan 1,172 scanner (SkyScan, Kontich, Belgium) as previously described[16]. The tibiae were positioned firmly into place with low-density foam in a sample holder topped with PBS. Scanning was performed at 60 keV, 167 μA, 1,475 ms without using a filter. In total, approximately 1,300 projections were collected at a resolution of 7.59 μm per pixel. Reconstruction of sections was performed using GPU Accelerated NRecon software (SkyScan, Kontich, Belgium). After reconstruction, samples were analysed using CTAn software (CTAn1.8, Skyscan). A region of interest (ROI) focusing on the subchondral bone in the middle third of the medial tibial plateau (MTP) was selected from the original reconstructed images. Morphologic measurements of the ROI were performed and the bone volume fraction (BV/TV) was obtained.

Histological scoring

Following micro-CT analysis, the knee joints were decalcified with 10% EDTA and embedded in paraffin with the patella down for coronal sections. The entire knee joint was cut in serial sections of 5 μm thickness and every 5th and 6th slide was stained with toluidine blue and Haematoxylin and Eosin (H&E), respectively.

Two observers (JT and CBL) blinded to genotype and treatment group scored cartilage aggrecan loss and structural damage of all four quadrants of the knee joint, i.e., the medial and lateral femoral condyles (LFC), and the medial and lateral tibial plateaus (LTP). Scoring was performed in multiple sections throughout the joint according to the OARSI histopathology initiative recommendations for the mouse[17], as follows: 0, normal; 0.5, loss of toluidine blue without structural changes; 1, small fibrillations without loss of cartilage; 2, vertical clefts down to the layer immediately below the superficial layer and loss of surface lamina; 3, vertical clefts/erosion to the calcified cartilage extending to <25% of the articular surface; 4, vertical clefts/erosion to the calcified cartilage extending to 25–50% of the articular surface; 5, vertical clefts/erosion to the calcified cartilage extending to 50–75% of the articular surface; 6, vertical clefts/erosion to the calcified cartilage extending >75% of the articular surface.

The maturity of osteophytes formed in the MTP was scored as follows: 0, none; 1, predominantly cartilaginous; 2, mixed cartilage

and bone with active vascular invasion and endochondral ossification; 3, predominantly bone.

Subchondral bone sclerosis of the MTP area and synovitis activity in the medial joints (pannus, bone invasion, synovial hypertrophy, sub-synovial cell infiltrate and exudate) were scored as follows: 0, normal; 1, mild; 2, moderate; 3, severe.

All scoring was done on coded digital images in each animal, with the maximal score recorded. The scores generated independently by the two observers had a concordance rate of 82%. The scores were averaged and used as the final score. Kappa statistics for each of the following variables were: for MTP score, kappa = 0.458, SE = 0.098, $P < 0.0001$; for medial femoral condyle (MFC) score, kappa = 0.331, SE = 0.091, $P < 0.0001$; for LTP score, kappa = 0.147, SE = 0.090, $P = 0.112$; for LFC score, kappa = −0.075, SE = 0.109, $P = 0.482$; and for osteophyte maturity, kappa = 0.559, SE = 0.133, $P < 0.0001$.

Histomorphometric analysis

Subchondral bone volume and osteophyte size were measured by histomorphometric analysis using OsteoMeasure XP Version 1.01 (OsteoMetrics. Inc, Atlanta, GA, USA). Three consistent levels of sections from each knee joint were chosen to present anterior, middle and posterior levels of MTP area. Subchondral bone volume and osteophyte size were measured and averaged for each animal.

Immunohistochemistry

Immunolocalization of rat 11βHSD2 transgene was performed on 5 μm paraffin sections using a polyclonal rabbit anti-rat 11βHSD2 primary antibody (RAH23), at concentration of 1 g/ml[9,10,18] after heat-induced citrate buffer antigen retrieval[10]. A biotinylated goat anti-rabbit secondary antibody (1:150 dilution) in combination with the ABC kit and DAB substrate (Vector Laboratories, CA, USA) were used. Samples were counterstained with Harris haematoxylin.

Quantitative RT-PCR

Total RNA was extracted from the tibiae of 2, 6 and 12 months old WT and tg male mice. Bone marrow was flushed from the tibia prior to isolation using Trizol (Invitrogen, Carlsbad, CA, USA) followed by an on-column RNA clean up (NucleoSpin RNA Kit, Machery-Nagel, Düren, Germany). cDNA was generated from RNA samples using SuperScript III (Invitrogen). Real-time PCR was performed for the determination of changes in the expression of 11β-HSD1 (F: 5′-GGAGCCGCACTTATCTGAA-3’; R: 5′-GACCTGGCAGTCAATACCA-3′) and glucocorticoid receptor (F: 5′-GTTCATGGCGTGAGTACCTC-3’; R: 5′-AGAGTTTGGGAGGTGGTCC-3′). 18S (F: 5′-CATGATTAAGAGGGACGGC-3’; R: 5′-TTCAGCTTTGCAACCATACTC-3′) was used for normalisation and analysis was performed using the comparative Ct method.

Statistical analysis

All analyses were performed and graphically presented using GraphPad Prism 7 software (GraphPad Software Inc., La Jolla, CA, USA) and SPSS v.22 (IBM Corporation, New York, NY, USA). *A priori* sample size power calculations based on unbiased, continuous variables (e.g., BV/TV%) in wild-type mice indicated the following: assuming a mean BV/TV (%) of 78.9 ± 5.2 (mean ± SD), a sample size of seven mice per group would provide us with 80% power to detect a difference of 10% in BV/TV (%) between groups, with a two-sided $\alpha = 0.05$. It was not possible for us to perform equivalent power calculations based on histological scores, as they are ordinal variables.

Prior to analysis, the distributions of continuous variables were checked for normality by analysis of Q–Q plots. To examine the relationships between skeletal *Hsd11b1* mRNA expression, age and genotype, we used a General Linear Model. Log_2-transformed skeletal *Hsd11b1* mRNA expression was included as the dependent variable, age was included as a fixed factor (coded as three levels, 2M, 6M and 12M), and genotype was included as a dichotomous covariate (WT, tg). The effects of age, genotype and age × genotype interactions on skeletal mRNA expression were reported. This analysis was also performed for skeletal *Nc3r1* (glucocorticoid receptor) mRNA, which was normally distributed.

Histopathology scores between groups were analysed using non-parametric ranked Mann–Whitney tests. When continuous variables (BV/TV (%) and osteophyte size) were normally distributed, differences between genotypes were compared using unpaired Student's *t*-tests. In all cases, a two-tailed $P < 0.05$ was significant. Unless stated otherwise, data is presented as mean ± 95% confidence intervals (CI).

Results

Expression of 11βHSD1 in bone increase with age in mice

We firstly examined the mRNA expression levels of *Hsd11b1*, encoding 11βHSD1, the glucocorticoid-activating enzyme, in bones obtained from young and aged WT and Col2.3-11βHSD2 transgenic mice. Similar to previous findings[11,12], we observed a significant age-related increase (95% CI of difference between 2 and 12 months: 2.27- to 8.49-fold) in skeletal *Hsd11b1* mRNA expression, independent of genotype (95% CI of difference between wild-type and tg mice: 0.67- to 2.75-fold, Fig. 1(A)). This model explained ~65% of the variation in skeletal *Hsd11b1* expression, and there was no significant age × genotype interaction. In contrast, the expression of glucocorticoid receptor (*Nr3c1*) mRNA was maintained at similar levels in bone during aging (95% CI of difference between 2 and 12 months: 0.15- to 1.19-fold, relative to 2 month-old mice), although there was a significant effect of genotype, with lower expression overall in tg mice (95% CI of difference: 0.08–0.67-fold of wild-type). 11βHSD2 transgene expressions in knee joints following DMM were also determined by immunohistochemistry. 11βHSD2 was exclusively expressed in bone cells of subchondral bone at similar levels in both age groups of the tg mice, but not detectable in chondrocytes of articular cartilage [Fig. 1(C)].

Surgically induced osteoarthritis in 10-week-old mice

Sixteen weeks after DMM surgery, both WT and tg mice developed proteoglycan loss and cartilage structural damage in the MFC and MTP, with more severe cartilage degradation observed in the MTP [Fig. 2(A)–(C)]. In contrast, there was little change seen in cartilage of the LFC or LTP [Fig. 2(A)]. Subchondral bone sclerosis was observed in the MTP of both WT- and tg-OA mice, as evaluated by micro-CT analysis [Fig. 2(D)]. In addition, the MTP of both WT- and tg-OA joints displayed osteophyte formation. Notably, in these younger animals, differences in cartilage degradation, subchondral bone changes and osteophyte formation between WT and tg mice were not significant. Mean differences (and their 95% confidence intervals) were as follows: for MFC, mean difference in OA = 0.10 (−1.91 to 2.11); for MTP, mean difference in OA = 0.003 (−1.008 to 1.014); for subchondral bone of MTP, mean difference in OA = 0.981 (−4.238 to 6.201); for osteophyte size, mean difference in OA = −0.34 mm^2 (−6.82–6.14 mm^2); and for osteophyte maturity score, mean difference in OA = 0.25 (−0.34 to 0.84). These results are shown in Fig. 2(B)–(F).

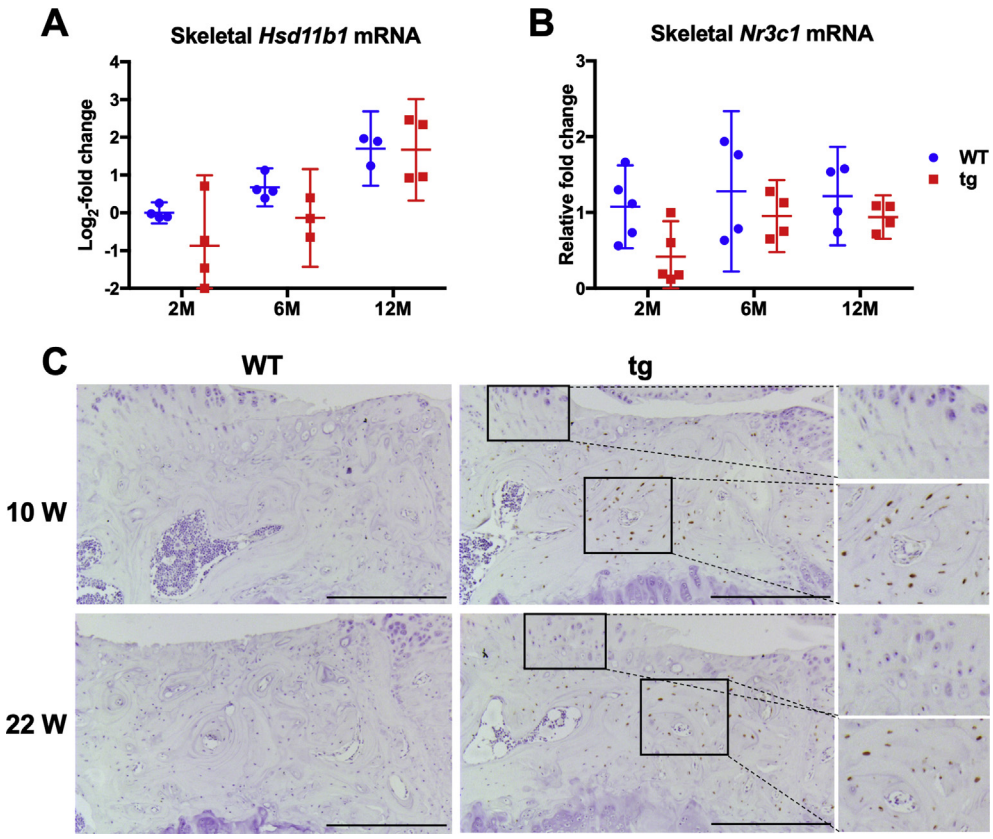

Fig. 1. Age-associated expression of 11®-HSD1, GR and 11®-HSD2. A-B, Gene expression analysis of *Hsd11b1* (encoding 11®-HSD1) and *Nr3c1* (encoding GR) of the bone shafts of the tibiae harvested from 2, 6 and 12 months WT and tg male mice. Genes of interest have been normalised with a housekeeper (18S) and expressed relative to 2M WT mice. Scatter plots display values for each mouse and mean (horizontal bar) ± 95% confidence intervals (CI). For skeletal *Hsd11b1* mRNA, the effect of age was significant ($F_{(2,21)} = 19.469$, $p < 0.0001$), but the effect of genotype was not ($F_{(1,21)} = 3.882$, $p = 0.064$). For skeletal *Nr3c1* mRNA expression, the effect of genotype was significant ($F_{(1,25)} = 7.394$, $p = 0.013$), but the effect of age was not ($F_{(2,25)} = 2.247$, $p = 0.13$). **C**, Expression of 11βHSD2 transgene in WT- and tg-OA joints of 10-week and 22-week detected by immunohistochemistry staining. Scale bars, 500 μm.

Surgically-induced osteoarthritis in 22-week-old mice

When DMM surgery was conducted in 22-week-old WT and tg mice, the cartilage erosion, subchondral bone sclerosis and osteophytosis were clearly observed 16 weeks post-surgery.

Cartilage damage

In contrast to mice operated at 10 weeks of age (Fig. 2), proteoglycan loss and cartilage structural damage in mice undergoing DMM at 22 weeks of age developed to the same extent in the MFC and MTP in OA mice [Fig. 3(A)–(C)]. However, while vertical clefts and erosion extended to the calcified cartilage layer and included >75% of the articular surface in WT mice [Fig. 3(A)–(C)], this was significantly attenuated in tg-OA mice (in the MFC ($P = 0.008$) but not MTP ($P = 0.10$); [Fig. 3(A)–(C)]. Similar to mice having surgery at 10 weeks, there was no significant cartilage damage observed in the LFC ($P = 0.26$) or LTP ($P = 0.46$) of WT or tg mice [Fig. 3(D)&(E)]. These results indicate that disruption of skeletal glucocorticoid signalling protects the older mice from DMM-induced cartilage damage.

Subchondral bone sclerosis

The middle third of the subchondral bone of the MTP was selected as the ROI for micro-CT analysis [Fig. 4(A)]. When compared with WT-OA mice, subchondral bone sclerosis was significantly attenuated in tg-OA mice, in which glucocorticoid signalling was disrupted in the cells of the osteoblast lineage [Figs. 4(C) and 95% CI of the difference: −0.37 to −10.07%, $P = 0.014$]. Similar results were obtained by histomorphometric analysis of the same ROI of the MTP [Fig. 4(B)], with the subchondral bone volume reduced in tg-OA compared to WT-OA mice [Fig. 4(D), $P = 0.007$]. This difference was statistically significant when specimens were evaluated by quantitative analysis, but not when subchondral bone sclerosis was assessed using a semi-quantitative histological score (Fig. 4(E), $P = 0.58$), despite the pattern between group differences being similar to that seen with micro-CT [Fig. 4(C)].

Osteophyte formation

Osteophytes developed on the anterior-medial aspect of the tibial plateau following DMM surgery and were significantly smaller (95% CI of the difference: −1.28 to −6.69 mm^2, $P = 0.029$) and more mature ($P = 0.021$) in tg-OA than in WT-OA mice [Fig. 5(A)–(C)].

Synovial activity

Despite the severe osteoarthritic changes observed in both cartilage and subchondral bone, DMM surgery resulted in only mild synovitis in both genotypes, compared to sham-operated mice [Fig. 6(A)]. Pannus ($P = 0.23$), bone erosion ($P = 0.59$), synovial hypertrophy ($P = 0.73$) and sub-synovial cell infiltrates ($P = 0.46$)

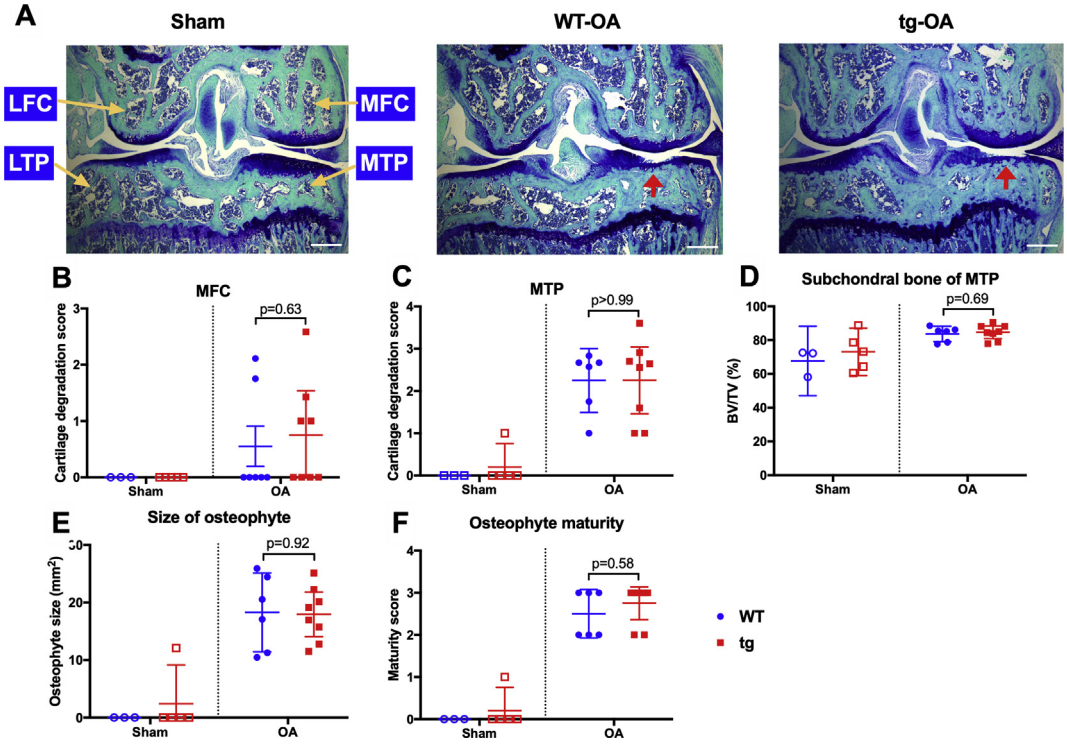

Fig. 2. Destabilization of the medial meniscus (DMM) conducted in 10-week-old mice: joint pathology 16 weeks post-surgery. A, Toluidine blue/Fast green stained representative sections of knee joints from sham-operated ("sham") joints and after medial meniscus destabilization (DMM) induced osteoarthritis ("OA") in WT and tg mice. Scale bars, 500 μm. **B&C**, Histological score of cartilage aggrecan loss and structural damage in medial femoral condyle (MFC) (**B**) and medial tibial plateau (MTP) (**C**) of WT and tg mice. **D**, Micro-CT analysis of the MTP subchondral bone volume (BV/TV) from sham and OA joints in WT and tg mice. **E**, Histomorphometric analysis of the size of osteophytes. **F**, Histological score of osteophyte maturity. MFC, MTP, lateral femoral condyles (LFC) and lateral tibial plateau (LTP) are indicated in the sham joint section. Red arrows indicate sites of cartilage degradation. Scatter plots display values for each mouse and mean (horizontal bar) ± 95% CI. Comparisons between groups connected by line with exact *p* values for each comparison above line.

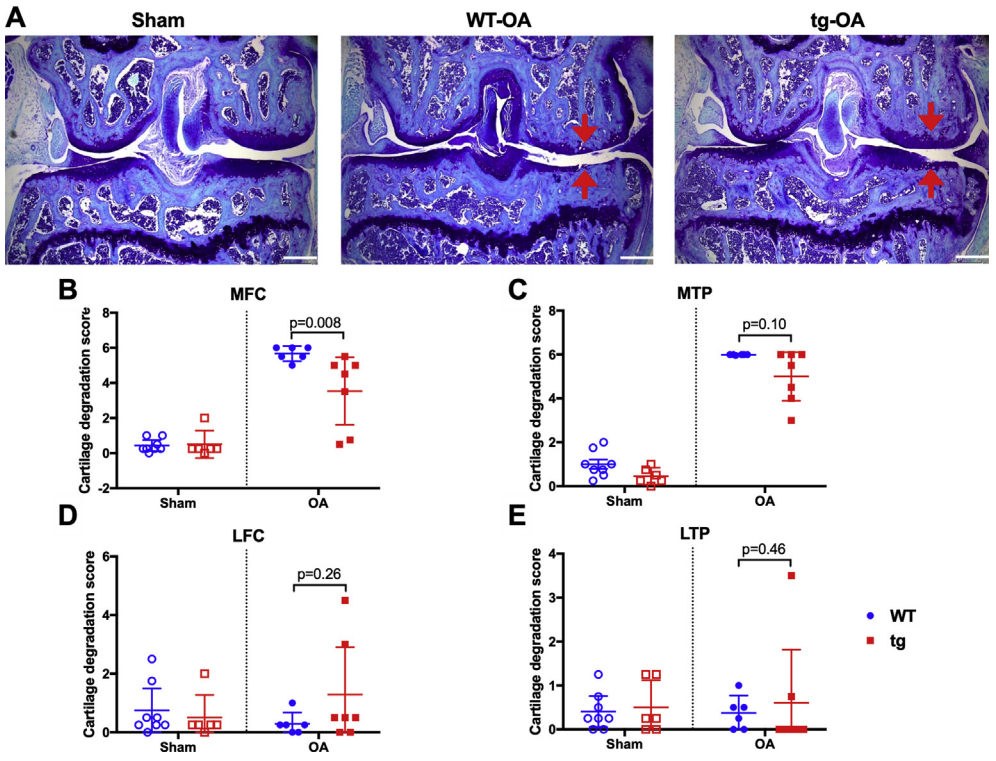

Fig. 3. DMM conducted in 22-week-old mice: cartilage damage of the knee joints 16 weeks post-surgery. A, Toluidine blue stained representative sections of articular cartilage from sham-operated ("sham") joints and after medial meniscus destabilization induced osteoarthritis ("OA") in WT and tg mice. Scale bars, 500 μm. **B-E**, Histological score of cartilage aggrecan loss and structural damage in MFC, MTP, LFC and LTP of WT-sham, tg-sham, WT-OA and tg-OA mice. Red arrows indicate sites of cartilage degradation. Semi-quantitative results are presented as mean ± 95% CI. Scatter plots display values for each mouse and mean (horizontal bar) ± 95% CI. Comparisons between groups connected by line with exact *p* values for each comparison above line.

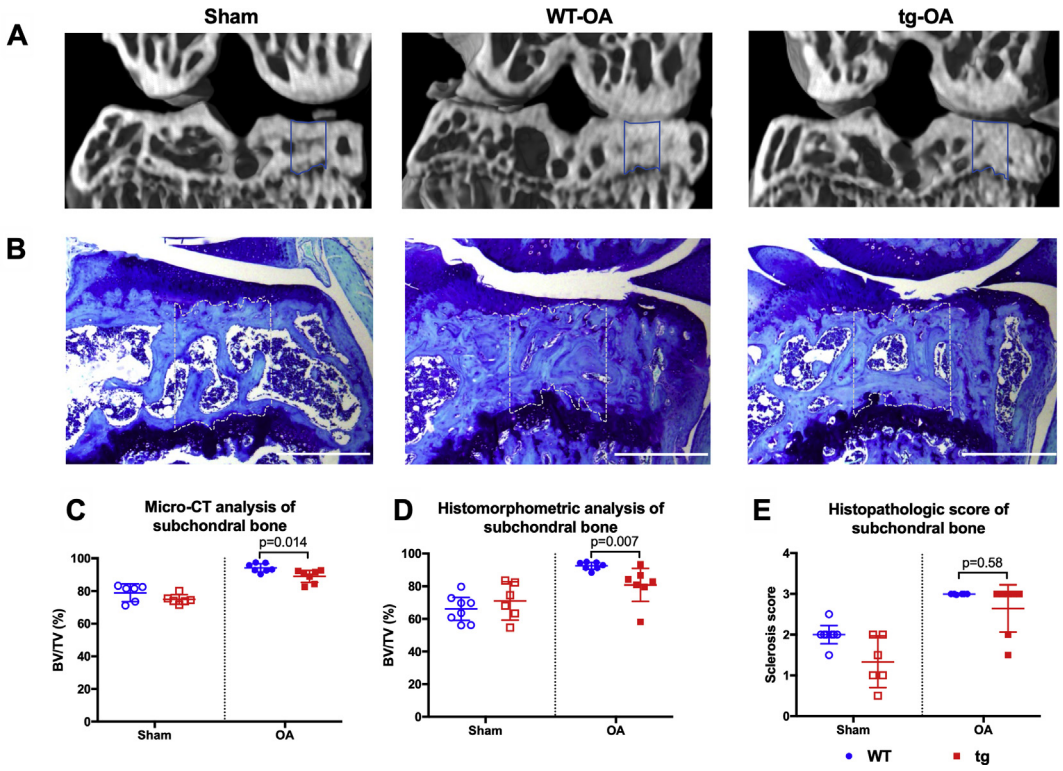

Fig. 4. DMM conducted in 22-week-old mice: subchondral bone sclerosis on the anterior-medial aspect of the tibial plateau 16 weeks post-surgery. A, Representative reconstructed images from micro-CT scan of sham-operated ("sham") joints and after medial meniscus destabilization induced osteoarthritis ("OA") in WT and tg mice. **B**, Toluidine blue stained representative sections of MTP areas from Sham and OA joints in WT and tg mice. Scale bars, 500 μm. The rectangles indicate region of interest (ROI) for subchondral bone analysis. **C**, Micro-CT analysis of the MTP subchondral bone volume (BV/TV) from Sham and OA joints in WT and tg mice. **D**, Histomorphometric analysis of the MTP subchondral bone volume from Sham and OA joints in WT and tg mice. **E**, Histological score of MTP subchondral bone volume from sham and OA joints in WT and tg mice. Scatter plots display values for each mouse and mean (horizontal bar) ± 95% CI. Comparisons between groups connected by line with exact *p* values for each comparison above line.

were not significantly different between WT- and tg-OA mice [Fig. 6(B)–(E)].

Discussion

Our results indicate that disruption of endogenous glucocorticoid signalling in mature osteoblast and osteocytes attenuates the development of global joint pathology in OA, including cartilage damage, subchondral bone sclerosis and osteophyte formation. Importantly, disruption of endogenous glucocorticoid signalling in osteoblasts and osteocytes resulted in attenuated joint pathology only when DMM surgery was conducted in 22-week-old mice, but not in 10-week-old mice. We and others[11,12] have observed that the skeletal expression of 11βHSD1, the enzyme that activates

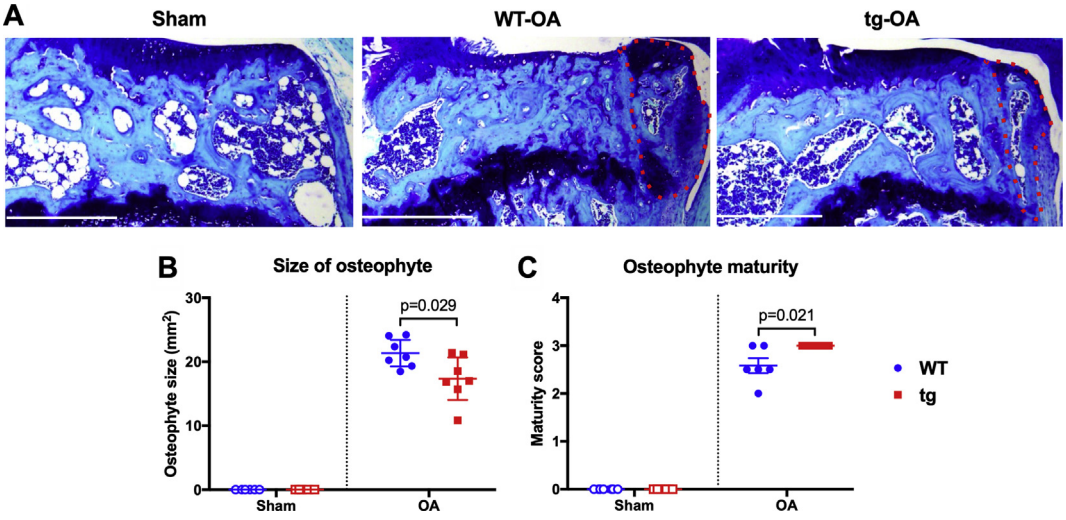

Fig. 5. DMM conducted in 22-week-old mice: osteophyte formation on the anterior-medial aspect of the tibial plateau 16 weeks post-surgery. A, Toluidine blue stained representative sections of MTP areas from sham-operated ("sham") joints and after medial meniscus destabilization induced osteoarthritis ("OA") in WT and tg mice. Scale bars, 500 μm. **B**, Histomorphometric analysis of the size of osteophytes. **C**, Histological score of osteophyte maturity. Red circles indicate the size of osteophytes. Scatter plots display values for each mouse and mean (horizontal bar) ± 95% CI. Comparisons between groups connected by line with exact *p* values for each comparison above line.

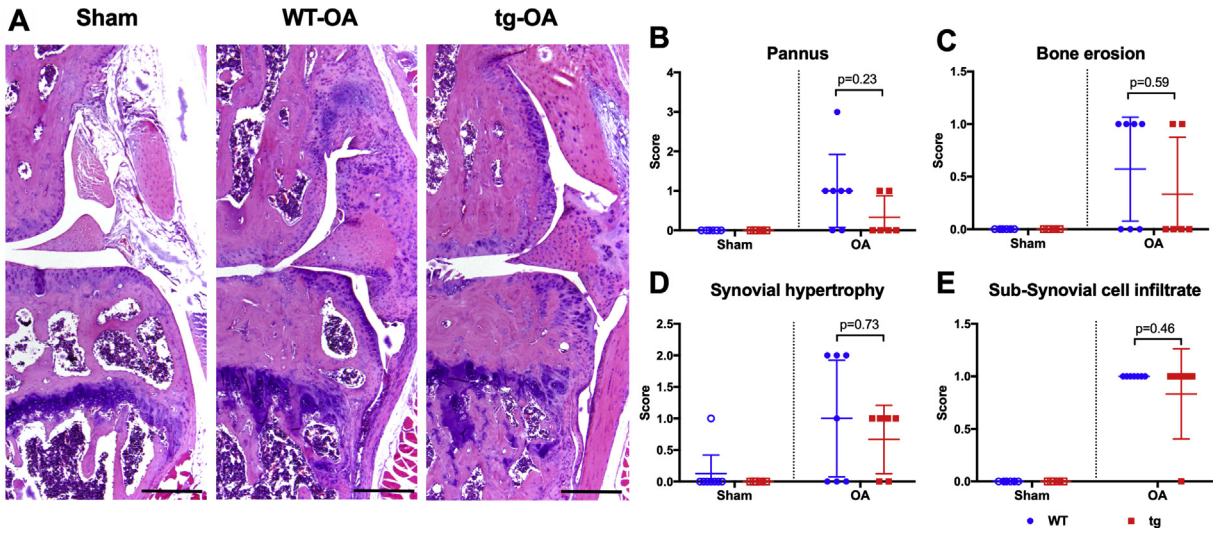

Fig. 6. DMM conducted in 22-week-old mice: synovial activity of the joints 16 weeks post-surgery. A, Haematoxylin and eosin stained representative sections of sham-operated ("sham") joints and after medial meniscus destabilization induced osteoarthritis ("OA") in WT and tg mice. Scale bars, 500 μm. **B-E**, Histological score of synovial activity around the sham-operated ("Sham") joints and after medial meniscus destabilization induced osteoarthritis ("OA") in WT and tg mice. This score was focused on pannus (**B**), bone invasion (**C**), synovial hypertrophy (**D**), and sub-synovial cell infiltrate (**E**). Scatter plots display values for each mouse and mean (horizontal bar) ± 95% CI. Comparisons between groups connected by line with exact *p* values for each comparison above line.

glucocorticoids, increases with age. These findings indicate that the elevated skeletal activity of 11βHSD1 in aging mice leads to increased local glucocorticoid generation, and that this age-dependent enhancement in glucocorticoid signalling in cells of the osteoblast lineage is critical to the development of DMM surgery-induced OA.

In line with others' findings[19,20], we have also observed that DMM surgery resulted in more severe cartilage loss, greater subchondral bone thickening, and bigger osteophyte formation in older mice (22 weeks at DMM surgery) compared to young mice (10 weeks at DMM surgery) (Supp Fig. 1(A)–(C)). Thus, endogenous glucocorticoid signalling in bone cells mediating the DMM-OA development may not be only related to age but also to the severity of lesions. A time point later than 16 weeks post-surgery in young mice could potentially lead to more severe OA phenotypes, and enable us to see the protective effects of the transgene, if present. Of note, consistent with our previous studies[10,21,22], endogenous glucocorticoid signalling was only blocked in mature osteoblasts and osteocytes in the tg mice, as 11βHSD2 was exclusively expressed in bone cells of subchondral bone, but not in chondrocytes of articular cartilage in both age groups [Fig. 1(C)]. Despite this, there was amelioration of cartilage, as well as bone pathology following DMM in older mice, indicating that the cells of the osteoblast lineage can regulate, or at least influence OA progression not only in subchondral bone sclerosis. Irrespective of the molecular mechanisms, the age-specific effects of 11βHSD1 elevation highlights the importance of age when considering the results of OA studies using animal models. The current study, as with others that have used genetically-modified mice to study the role of specific genes in OA[23], indicates the functions of genes and pathways in the OA process may change with age and may be missed if only young animals are used.

Our current study indicates that endogenous glucocorticoid signalling in bone cells plays a role in mediating the adverse effects of DMM-OA development in aged mice. This raises the question as to the appropriacy of the use of intra-articular glucocorticoid injections in the treatment of OA. Intra-articular glucocorticoids have been used for pain relief and functional improvement, however, the effectiveness and safety of these treatments remains controversial[3–7]. Jüni *et al.* conducted a systematic review of the effects of intra-articular corticosteroids on pain, function, quality of life, and safety compared with sham injection or no treatment in people with knee OA. However, they failed to draw a conclusion based on the generally highly discordant results across studies, which was mainly based on small studies of low quality[24]. A recent 2-year randomized controlled trial indicated that among patients with symptomatic knee OA, repeated intra-articular triamcinolone injections resulted in significantly greater cartilage volume loss with no significant difference in knee pain, compared to intra-articular saline[25]. In line with the results from this clinical trial, our findings suggest that endogenous glucocorticoids, which are present and act at much lower concentrations than therapeutic glucocorticoids, promote the development of osteoarthritic joint damage in a model of mechanical joint failure.

A limitation of this study is that despite identifying that age-associated increases in skeletal glucocorticoid signalling plays a role in OA progression, we assessed the phenotype of mice only at one time point (16 weeks post-DMM induction). Earlier time points may give insights into the mechanism that leads to the attenuated phenotype in our tg-OA mice and will be the subject of further investigation.

In conclusion, our results indicate that glucocorticoid signalling in mature osteoblasts and osteocytes promotes the development of OA in a mouse model of surgically-induced osteoarthritis. As the role of local skeletal glucocorticoid signalling in the pathogenesis of osteoarthritis has never been recognised before, our findings may open new avenues for innovative approaches to an unsolved problem of great medical and social significance.

Author contributions

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Jinwen Tu had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study conception and design: Tu, Zhou, Seibel, Dang; acquisition of data:

Tu, Ji, Zhang Li, Kim; analysis and interpretation of data: Tu, Ji, Henneicke, Kim, Swarbrick, Little, Zhou, Seibel; manuscript writing: Tu, Zhou, Kim, Swarbrick, Seibel, Little.

Conflict of interests

The author(s) declare that they have no competing interests.

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Supplementary data

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References

1. Bouaziz W, Funck-Brentano T, Lin H, Marty C, Ea HK, Hay E, *et al*. Loss of sclerostin promotes osteoarthritis in mice via beta-catenin-dependent and -independent Wnt pathways. Arthritis Res Ther 2015;17:24.
2. Murray CJ, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, *et al*. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380(9859):2197–223.
3. Hollander JL. Intra-articular hydrocortisone in arthritis and allied conditions; a summary of two years' clinical experience. J Bone Jt Surg Am 1953;35-A(4):983–90.
4. Flanagan J, Casale FF, Thomas TL, Desai KB. Intra-articular injection for pain relief in patients awaiting hip replacement. Ann R Coll Surg Engl 1988;70(3):156–7.
5. Friedman DM, Moore ME. The efficacy of intraarticular steroids in osteoarthritis: a double-blind study. J Rheumatol 1980;7(6):850–6.
6. Lambert RG, Hutchings EJ, Grace MG, Jhangri GS, Conner-Spady B, Maksymowych WP. Steroid injection for osteoarthritis of the hip: a randomized, double-blind, placebo-controlled trial. Arthritis Rheum 2007;56(7):2278–87.
7. Wernecke C, Braun HJ, Dragoo JL. The effect of intra-articular corticosteroids on articular cartilage: a systematic review. Orthop J Sports Med 2015;3(5). 2325967115581163.
8. Stewart PM, Krozowski ZS. 11 beta-Hydroxysteroid dehydrogenase. Vitam Horm 1999;57:249–324.
9. Sher LB, Woitge HW, Adams DJ, Gronowicz GA, Krozowski Z, Harrison JR, *et al*. Transgenic expression of 11beta-hydroxysteroid dehydrogenase type 2 in osteoblasts reveals an anabolic role for endogenous glucocorticoids in bone. Endocrinology 2004;145(2):922–9.
10. Zhou H, Mak W, Kalak R, Street J, Fong-Yee C, Zheng Y, *et al*. Glucocorticoid-dependent Wnt signaling by mature osteoblasts is a key regulator of cranial skeletal development in mice. Development 2009;136(3):427–36.
11. Cooper MS, Rabbitt EH, Goddard PE, Bartlett WA, Hewison M, Stewart PM. Osteoblastic 11beta-hydroxysteroid dehydrogenase type 1 activity increases with age and glucocorticoid exposure. J Bone Miner Res 2002;17(6):979–86.
12. Weinstein RS, Wan C, Liu Q, Wang Y, Almeida M, O'Brien CA, *et al*. Endogenous glucocorticoids decrease skeletal angiogenesis, vascularity, hydration, and strength in aged mice. Aging Cell 2010;9(2):147–61.
13. Hwang JY, Lee SH, Kim GS, Koh JM, Go MJ, Kim YJ, *et al*. HSD11B1 polymorphisms predicted bone mineral density and fracture risk in postmenopausal women without a clinically apparent hypercortisolemia. Bone 2009;45(6):1098–103.
14. Little CB, Barai A, Burkhardt D, Smith SM, Fosang AJ, Werb Z, *et al*. Matrix metalloproteinase 13-deficient mice are resistant to osteoarthritic cartilage erosion but not chondrocyte hypertrophy or osteophyte development. Arthritis Rheum 2009;60(12):3723–33.
15. Ma HL, Blanchet TJ, Peluso D, Hopkins B, Morris EA, Glasson SS. Osteoarthritis severity is sex dependent in a surgical mouse model. Osteoarthritis Cartilage 2007;15(6):695–700.
16. Tu J, Henneicke H, Zhang Y, Stoner S, Cheng TL, Schindeler A, *et al*. Disruption of glucocorticoid signaling in chondrocytes delays metaphyseal fracture healing but does not affect normal cartilage and bone development. Bone 2014;69:12–22.
17. Glasson SS, Chambers MG, Van Den Berg WB, Little CB. The OARSI histopathology initiative - recommendations for histological assessments of osteoarthritis in the mouse. Osteoarthritis Cartilage 2010;18(Suppl 3):S17–23.
18. Smith RE, Li KX, Andrews RK, Krozowski Z. Immunohistochemical and molecular characterization of the rat 11 beta-hydroxysteroid dehydrogenase type II enzyme. Endocrinology 1997;138(2):540–7.
19. Huang H, Skelly JD, Ayers DC, Song J. Age-dependent changes in the articular cartilage and subchondral bone of C57BL/6 mice after surgical destabilization of medial meniscus. Sci Rep 2017;7:42294.
20. Loeser RF, Olex AL, McNulty MA, Carlson CS, Callahan MF, Ferguson CM, *et al*. Microarray analysis reveals age-related differences in gene expression during the development of osteoarthritis in mice. Arthritis Rheum 2012;64(3):705–17.
21. Brennan-Speranza TC, Henneicke H, Gasparini SJ, Blankenstein KI, Heinevetter U, Cogger VC, *et al*. Osteoblasts mediate the adverse effects of glucocorticoids on fuel metabolism. J Clin Investig 2012;122(11):4172–89.
22. Buttgereit F, Zhou H, Kalak R, Gaber T, Spies CM, Huscher D, *et al*. Transgenic disruption of glucocorticoid signaling in mature osteoblasts and osteocytes attenuates K/BxN mouse serum-induced arthritis in vivo. Arthritis Rheum 2009;60(7):1998–2007.
23. Miller RE, Malfait AM. Can we target CCR2 to treat osteoarthritis? The trick is in the timing!. Osteoarthritis Cartilage 2017;25(6):799–801.
24. Juni P, Hari R, Rutjes AW, Fischer R, Silletta MG, Reichenbach S, *et al*. Intra-articular corticosteroid for knee osteoarthritis. Cochrane Database Syst Rev 2015;(10):CD005328.
25. McAlindon TE, LaValley MP, Harvey WF, Price LL, Driban JB, Zhang M, *et al*. Effect of intra-articular triamcinolone vs saline on knee cartilage volume and pain in patients with knee osteoarthritis: a randomized clinical trial. J Am Med Assoc 2017;317(19):1967–75.