Decoupling Glucocorticoid Benefit from Cartilage/Nucleus pulposus Toxicity via Tau–CXCL12 Axis

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INTRODUCTION: Glucocorticoids (GC) are widely used to treat inflammatory conditions such as rheumatoid arthritis and disc related pain due to their potent anti-inflammatory effects. However, prolonged or high dose exposure has been linked to musculoskeletal toxicities, including bone loss and, in intervertebral disc degeneration (IDD), chondrocyte catabolism and ECM loss. Since the late 1990s, high-dose glucocorticoids have been implicated in the pathogenesis of IDD, encompassing cartilage and nucleus pulposus degeneration. More recently, supratherapeutic GC regimens to manage severe COVID-19 cases, accompanied by low back pain and arthritic symptoms. Local delivery of GC via epidural/intradiscal injection induces transient local concentration spikes and exacerbate IDD. Mechanism-based approaches that decouple efficacy from musculoskeletal harm remain limited. We recently identified Tau as a low affinity GC receptor preferentially engaged at high dose, promoting bone resorption via phosphorylation, distinct from GR-mediated anti-inflammatory signaling (Fu et al., 2025). Given its expression in disc cartilage endplate (CEP), annulus fibrosus (AF), and nucleus pulposus (NP) cells, we investigated whether Tau mediates GC-induced IDD and explored downstream pathways including chemotaxis, metabolism and angiogenesis.

METHODS: Animal procedures were approved by the Yale University IACUC. Human tissue research was approved by the Independent Ethics Committee of The Second Hospital of Shandong University. Lumbar spine instability and caudal disc puncture models were established in mice. Animals received PBS, low dose dexamethasone (Dex; 10 μg/kg) or high dose Dex (10 mg/kg), ± TRx0237 (4 mg/kg), administered daily for 4 weeks as indicated. For *ex vivo* disc culture, Dex was applied at 10 nM or 10 μM. Disc were digested and macrophages were quantified by flow cytometry. CXCL12 and p-Tau level of human AF were correlated with Pfirrmann grade using Pearson's correlation. All animal experiments used sex-balanced cohorts (4 males and 4 females per experimental group). Human samples were also sex-balanced across Pfirrmann grades (I–V), with two male and two female donors per grade (total n=20).

RESULTS SECTION: **Tau—a low affinity receptor for GC—is essential for the adverse effects of high dose GC on intervertebral disc.** To define receptor dependency, WT, GR$^{-/-}$, and Tau$^{-/-}$ mice we administered low or high dose Dex under physiological and degenerative conditions. Across histochemistry (SO&FG, H&E, TRAP) and immunohistochemistry, high dose Dex induced significant disc degeneration, marked by reduction disc height index (1.53-fold), NP area (2.13-fold), proteoglycan content (3.92-fold) and endplate score (2.13-fold), along with suppressed anabolic and elevated catabolic markers (**Fig. 1a**). These effects were absent in Tau$^{-/-}$ mice but persisted in GR$^{-/-}$ mice, indicating Tau-specific GC toxicity. In bone marrow chimeras, Tau$^{-/-}$ marrow provided partial protection from Dex-induced degeneration, suggesting a hematopoietic contribution but a dominant role for radioresistant, host-derived disc stromal cells. As predicted, high dose Dex induced Tau phosphorylation in CEP and AF cells, but not in NP cells (**Fig. 1b**). Pharmacological inhibition of Tau phosphorylation with TRx0237 effectively rescued Dex-induced degeneration (**Fig. 1c**). Dex's ability to suppress p65 (RelA) phosphorylation requires GR and remains unaffected by Tau deficiency or TRx0237, demonstrating that blocking Tau, the low-affinity glucocorticoid receptor, does not compromise the intrinsic anti-inflammatory efficacy of Dex (**Fig. 1d**). **CXCL12 is the predominant chemokine induced downstream of Dex-phosphorylated Tau and was strongly associatied with clinical severity.** Given the prominence of GC-related bone resorption and myeloid influx in osteoclastogenesis, we quantified disc-resident macrophages by flow cytometry; high dose Dex increased macrophage abundance 3.29-fold (**Fig. 2a**). Chemokine profiling of primary CEP and AF cells exposed to Dex ± TRx0237 identified CXCL12 as the top intersection candidate—upregulated by Dex and fully reversed by TRx0237 (**Fig. 2b**). Blockage of Tau-downstream NF-κB signaling with andrographolide significantly reduced CXCL12 expression (**Fig. 2c**). In human AF tissues stratified by Pfirrmann grade I–V (n = 4 per grade), Two-photon IF image showed strong co-localization and a positive correlation between p-Tau and CXCL12, with grade V disc exhibited 2.78-fold higher p-Tau and 3.41-fold higher CXCL12 than grade I (**Fig. 2d**). Co-staining of COL1 and CXCL12 with 3D reconstruction localized CXCL12 specifically to AF cells, and the proportion of CXCL12^{+} AF cells increased with disease severity, with no expression in infiltrating immune cells (**Fig. 2e**). **CXCL12 is a central mediator of GC-induced macrophage chemotaxis, metabolic dysregulation, and angiogenesis.** Dex-induced infiltration of macrophages was abolished in CXCL12$^{-/-}$ mice, indicating a nonredundant role for CXCL12 in myeloid chemotaxis (**Fig. 3a**). In ex vivo disc organ culture lacking circulating macrophages, CXCL12$^{-/-}$ disc exhibited restored anabolic and suppressed catabolic markers following Dex exposure, implying that CXCL12 acts beyond chemotaxis to directly modulate metabolism (**Fig. 3b**). To test paracrine effect, a disc–cell Transwell system was used: Dex preconditioned disc were co-cultured with primary CEP or NP cells (**Fig. 3c**). WT disc drove up catabolic markers and downregulated anabolic markers in primary CEP/NP cells, whereas CXCL12$^{-/-}$ disc failed to elicit these changes (**Fig. 3d**). Given that CXCL12 is pro-angiogenic and endothelial cells expressing its receptor are invasive, we examined endothelial responses. DEX-preconditioned WT discs elevated VEGF levels in cell lysates and culture supernatants, and in Transwell assays, attracted endothelial cells (3.41-fold); both effects were abolished with CXCL12$^{-/-}$ discs (**Fig. 3e-g**).

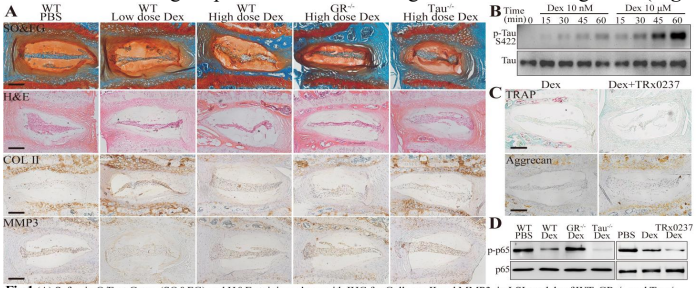

Fig.1 (A) Safranin O/Fast Green (SO&FG) and H&E staining, along with IHC for Collagen II and MMP3, in LSI models of WT, GR$^{-/-}$, and Tau$^{-/-}$ mice systemically treated with low or high dose Dex for 1 month daily. (B) Primary CEP cells stimulated in vitro with low or high dose Dex for 15, 30, 45, and 60 min, followed by detection of Tau phosphorylation levels. (C) TRAP staining and IHC for Aggrecan and ADAMTS5 in LSI WT mice systemically treated with high-dose Dex ± TRx0237. (D) CEP cells stimulated with IL-1β in vitro, then treated with high dose Dex ± TRx0237, followed by detection of p65 phosphorylation levels

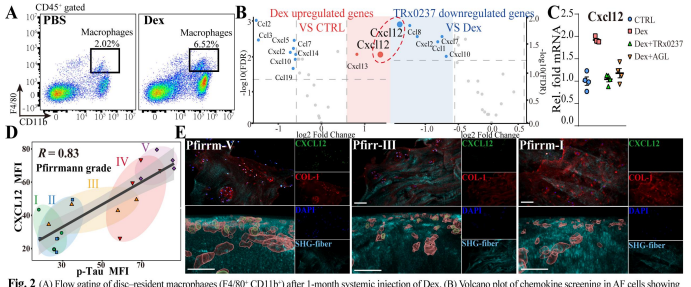

Fig. 2 (A) Flow gating of disc–resident macrophages (F4/80^{+} CD11b^{+}) after 1-month systemic injection of Dex. (B) Volcano plot of chemokine screening in AF cells showing fold change and FDR. Red dashed outline marks Dex-induced genes versus WT (pink region) and TRx0237 downregulated genes versus Dex (cyan region) intersection. (C) AF cells treated in vitro for 24 h with Dex ± TRx0237 or AGL. (D) Human AF tissue graded I–V by Pfirrmann. Mean fluorescence intensities of p-Tau and CXCL12 are correlated. (E) Disc across Pfirrmann grades stained for COL1 and CXCL12; collagen fibers visualized by two-photon second harmonic generation (SHG) with 3D reconstruction.

DISCUSSION

High dose GCs activate a low-affinity Tau–CXCL12 pathway in AF/CEP cells, distinct from canonical GR signaling. This axis coordinates macrophage chemotaxis, CEP/NP metabolic reprogramming, and pro-angiogenic responses culminating in IDD. Genetic loss of Tau or CXCL12 abrogated GC-induced IDD. Co-administration of FDA-proved Tau phosphorylation inhibitor preserved Dex's anti-inflammatory benefit while preventing Cartilage/Nucleus pulposus degeneration. Future studies will test clinically relevant dosing, CXCR4 dependence, and circulating biomarkers.

SIGNIFICANCE / CLINICAL RELEVANCE

This work defines a GC-triggered Tau–CXCL12 mechanism for musculoskeletal disease and proposes a practical co-therapy that maintains anti-inflammatory efficacy while limiting adverse effects, enabling safer GC use. The correlation of p-Tau/CXCL12 with imaging severity in human disc supports translational potential and motivates validation as clinical biomarkers.

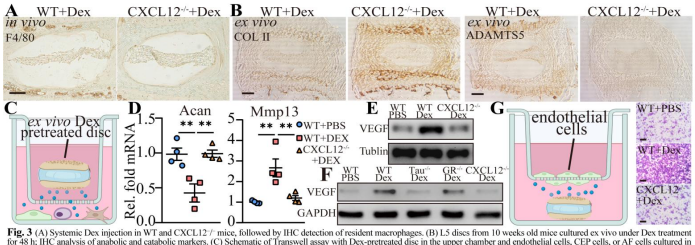

Fig. 3 (A) Systemic Dex injection in WT and CXCL12$^{-/-}$ mice, followed by IHC detection of resident macrophages. (B) L5 discs from 10 weeks old mice cultured ex vivo under Dex treatment for 48 h; IHC analysis of anabolic and catabolic markers. (C) Schematic of Transwell assay with Dex-pretreated disc in the upper chamber and endothelial cells, CEP cells, or AF cells cultured in the lower chamber. (D) qPCR analysis of anabolic and catabolic markers in CEP cells. (E) Western blot analysis of VEGF expression in endothelial cells. (F) Western blot detection of VEGF levels in endothelial cell culture supernatants. (G) Transwell assay with endothelial cells seeded in the upper chamber and Dex-pretreated disc in the lower chamber; migrated endothelial cells stained with crystal violet.