Assessing the contribution of estrogen receptor-α loss to cartilage degradation in osteoarthritis

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DISCLOSURES: Nothing to report.

INTRODUCTION: Knee osteoarthritis (KOA) is a common joint disease, and cartilage degradation represents a central pathological change. Currently, the prevalence of KOA among people over the age of 60 in the United States is > 37%, while that among people over the age of 40 in China is as high as 15.6%. Patients with long-term KOA also suffer from sleep disorders, depression, and even disability. At present, non-steroidal anti-inflammatory drugs (NSAIDs), intra-articular injections, artificial joint replacement surgery, and other methods are mainly used as the main means to alleviate or treat KOA, but none of them can effectively delay or reverse the occurrence and development of KOA and associated cartilage degeneration. Previous studies in our lab demonstrated that levels of estrogen receptor-α (ERα) decreased with OA pathogenesis, which is sex and ligand-independent [1,2]. Moreover, when expression levels of *ESR1,* the gene encoding ERα, were suppressed, mechanical loading enhanced hypertrophic and osteogenic transition *in vitro*. We also found out the critical role of ERα in maintaining the health of chondrocytes by inhibiting DNA damage and senescence. However, as of now, the influence of ERα on cartilage degradation and OA pathogenesis has not been examined in animal models.

HYPOTHESIS/AIMS: In this study, we hypothesize that global or cartilage-specific depletion of ERα will accelerate OA progression in mice.

METHODS: Destabilization of the medial meniscus (DMM) surgery was performed to induce OA in mice. With the IACUC approval, DMM surgery was performed in 12-week-old ERα global knockout mice (Esr1$^{-/-}$) and wild type (WT) mice. After surgery, mice were housed for 12 weeks. The knee joints were fixed and embedded. All samples were sectioned at a thickness of 6 μm. Safranin O/Fast green staining and immunohistochemical staining were performed. Cartilage damage was measured using the Osteoarthritis Research Society International score. To exclude the systemic influence due to the global loss of ERα, we are also working on the generation of transgenic mice with cartilage-specific depletion of ERα. We constructed two types of mice. One is HOM HEMI(conditional knock out mice, ERacKO), homozygous for loxERα and hemizygous for Col2CreERT, and the other is HOM NCAR(littermates, LT), homozygous for loxERα and nocarrier for Col2CreERT. Mouse genotyping was conducted using the mice tail DNA. Depletion of ERα in cartilage was induced by tamoxifen intraperitoneal injection once every 24 hours for a total of 5 consecutive days. Tamoxifen was dissolved in corn oil at a concentration of 10mg/ml by shaking overnight at 37℃. The injection dose was determined by mice weight, using approximately 100mg/kg body weight.

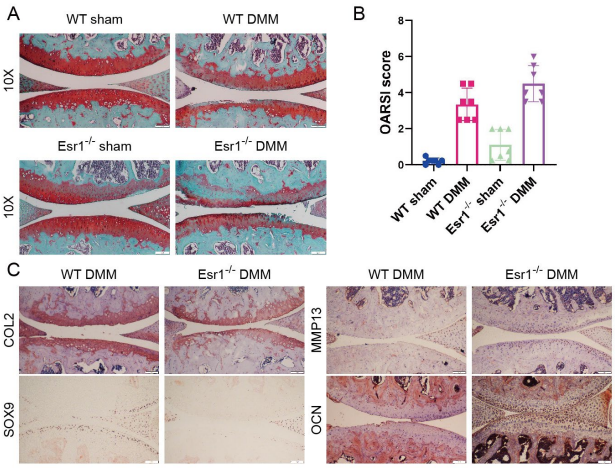

Fig. 1 ERα loss in chondrocyte accelerates OA development in mice. (A) Representative images from Safranin O/Fast Green staining. Wile type (WT) and ERα global knock out (Esr1$^{-/-}$) mice were used. Scale bar=100 μm. (B) The severity of OA-like phenotype 12 weeks after surgery was analyzed by grading histological sections using the Osteoarthritis Research Society International score system. (C) Representative images of immunohistochemical staining for COL2, SOX9, MMP13 and OCN in knee joint of mice. Scale bar=100 μm.

RESULTS: DMM mice exhibited significant cartilage erosion and loss of both proteoglycans and cellularity in the articular cartilage when compared with sham surgery mice (**Fig. 1A, B**). The generation of OA phenotype was further confirmed by a significantly higher Osteoarthritis Research Society International (OARSI) score. Interestingly, Esr1$^{-/-}$ mice undergoing DMM showed a higher OARSI score than WT control (**Fig. 1A, B**). Moreover, levels of matrix metallopeptidase 13 (MMP13) and osteocalcin (OCN) were increased, whereas collagen type II (COL2) and SRY-box transcription factor 9 (SOX9) were significantly decreased in Esr1$^{-/-}$ DMM mice compared with WT DMM mice(**Fig. 1C**). The results demonstrated that global loss of ERα and DMM synergistically increase the hypertrophic and ossific transition of chondrocytes, and accelerate OA progression. To exclude the systemic influence due to the global ERα loss, transgenic mice with cartilage-specific depletion of ERα are also generated. Genotyping data shown in **Fig. 2B** confirmed the successful generation of ERacKO and LT mice. Importantly, the IHC results indicated the ablation of ERa in articular chondrocytes of ERacKO mice (**Fig. 2C**). Currently, we are performing DMM on LT and ERacKO mice (**Fig. 2A**).

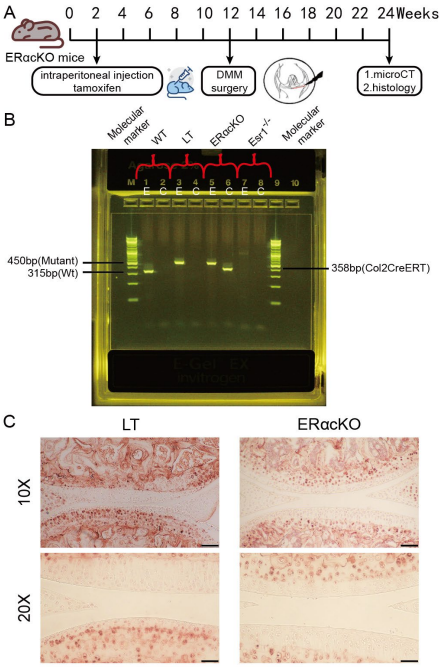

Fig. 2 Generation of cartilage-specific ERα conditional knockout mice. (A)Experimental flow chart. (B) DNA electrophoresis for genotyping wild type mice (WT), global knock out mice (Esr1$^{-/-}$), conditional knock out mice (ERacKO), and their littermates (LT). (C) Representative images of IHC staining of ERα in articular cartilage of conditional knock out mice (ERacKO) and littermates (LT) at 12 weeks old. Scale bars: 100 μm (first row) and 50 μm (second row).

DISCUSSION/FUTURE PLANS: Through the ongoing study, we will finally determine if cartilage-specific deletion of ERα will result in cartilage degradation, as we observed in Esr1$^{-/-}$ mice. In order to verify whether the presence of estrogen will affect the effect of ERα knockout on the OA phenotype, we will conduct a new study by including additional female mice undergoing ovariectomy to remove the influence of endogenous estrogen. Lastly, we will conduct a mechanistic study to define the pathways mediating ERα and pathological changes in chondrocytes.

SIGNIFICANCE/CLINICAL RELEVANCE: Successful project completion will lay the groundwork for the future development of disease-modifying OA drugs that target ERa or its downstream molecules.

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