Non-neuronal NGF-TrkA Signaling Contributes to Joint Pain and Inflammation in Experimental Osteoarthritis

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INTRODUCTION: Osteoarthritis (OA) is a painful chronic joint disease, characterized by progressive deterioration of articular cartilage, subchondral bone (SCB) sclerosis, and synovitis. Nerve growth factor (NGF) is a pro-algesic mediator with elevated levels in OA joints. Repeated intra-articular (i.a.) injection of NGF into healthy mouse knees causes hyperalgesia, marked synovitis, and nociceptor sprouting in the joints, similar to the phenotypes observed in surgically induced murine OA (1). NGF is elevated in many OA joint tissues (*e.g.*, cartilage, SCB, synovium), and its high-affinity receptor, tyrosine receptor kinase A (TrkA), is expressed by nociceptors and also by immune cells and chondrocytes (2). The aim of this study was to use inducible conditional knockout mice to explore the role of NGF-TrkA signaling in non-neuronal pathways in joint pain and OA progression.

METHODS: First, we assessed the specificity and efficiency of tamoxifen-induced Cre-loxP recombination in CreERT2;tdTomato mice, which were generated by crossing tdTomato Cre-dependent reporter mice with CreERT2 mice: chondrocyte-specific aggrecan (Agg)-CreERT2, osteoblast-specific osterix (Osx)-CreERT2, and myeloid cell (comprising macrophage and osteoclast)-specific lysozyme M (LysM)-CreERT2. Adult male Agg-CreERT2;tdTomato, Osx-CreERT2;tdTomato, and LysM-CreERT2;tdTomato mice were intraperitoneally (i.p.) injected with 50 µl of 4-hydroxytamoxifen (4-OHTam, 10 mg/ml) or vehicle (corn oil) once a day for 5 consecutive days. Mice were perfused 3 weeks after the last injection. TdTomato signal in the knee joints was visualized in the cryosections (20 µm) with a slide scanner. Next, floxed *Ngf* (Ngf-lx) mice were crossed with CreERT2 mice to generate Agg-CreERT2;Ngf-lx, Osx-CreERT2;Ngf-lx, and LysM-CreERT2;Ngf-lx mice for inducible knockout of *Ngf* in the selected non-neuronal cells. Ten-week-old male CreERT2;Ngf-lx mice were i.p. injected with 4-OHTam for 5 days and subjected to partial meniscectomy (PMX) surgery one week later. Ngf-lx mice without CreERT2 were used as controls. Weightbearing asymmetry was assessed pre- and 12 weeks post-PMX. Floxed *Ntrk1* (TrkA-lx) mice were crossed with Agg-CreERT2 and Osx-CreERT2 mice to generate Agg-CreERT2;TrkA-lx and Osx-CreERT2;TrkA-lx mice, respectively. Ten-week-old male mice were i.p. injected with 4-OHTam for 5 days. NGF i.a. administration (500 ng in 5 µl PBS, twice a week) started one week after the last injection of 4-OHTam and lasted for 4 weeks. TrkA-lx were used as controls. Joint swelling was measured using calipers at baseline and before each injection of NGF.

RESULTS: I.p. injection of 4-OHTam resulted in tdTomato expression in articular and growth plate cartilage in Agg-CreERT2;tdTomato mice (Fig. 1A), and in subchondral trabecular bone surface in Osx-CreERT2;tdTomato mice (Fig. 1B). TdTomato+ signals were distributed in various tissues in LysM-CreERT2;tdTomato mice (not shown). No reporter signals were observed in vehicle-injected mice, indicating specific Cre-loxP recombination achieved by 4-OHTam. We then used the same dose and frequency of 4-OHTam administration in male CreERT2;Ngf-lx and CreERT2;TrkA-lx mice to knockout *Ngf* or *Ntrk1*, respectively. As expected, weightbearing asymmetry occurred 12 weeks after PMX in the controls, with a significant difference between pre- and 12 weeks post-surgery ($p<0.001$, N=13 mice/group) (Fig. 1C, D). In contrast, Agg-CreERT2;Ngf-lx mice were protected from developing weightbearing asymmetry 12 weeks post-PMX (N=4 mice/group) (Fig. 1C), while in Osx-CreERT2;Ngf-lx mice, weightbearing asymmetry existed 12 weeks after PMX ($p=0.036$ *vs.* pre-PMX, N=7 mice/group) (Fig. 1D). In LysM-CreERT2;Ngf-lx mice, weightbearing asymmetry was observed 12 weeks after PMX ($p=0.029$ *vs.* pre-PMX, N=3 mice/group), and there was no difference between LysM-CreERT2;Ngf-lx and NGF-lx mice (not shown). In another set of experiments, knee joint swelling was induced by i.a. injection of NGF in naïve male TrkA-lx mice (one-way ANOVA: $p<0.05$, N=8 mice/group) (Fig. 1E, F). When TrkA was deleted in chondrocytes using Agg-CreERT2;TrkA-lx mice (N=5 mice/group) or in osteoblasts using Osx-CreERT2;TrkA-lx mice (N=4 mice/group) after 4-OHTam administration, the joint swelling in response to NGF stimulation was reduced in both groups of mice compared to TrkA-lx mice (two-way repeated measures ANOVA with Šídák test: $p<0.05$) (Fig. 1E, F).

DISCUSSION: We used genetically engineered mice to target NGF or TrkA in non-neuronal cells. In chondrocytes, knockout of *Ngf* attenuated weightbearing asymmetry after PMX, and knockout of *Ntrk1* reduced NGF-induced joint swelling. In osteoblasts, deletion of *Ngf* did not reduce pain on weightbearing after PMX, but deletion of *Ntrk1* markedly decreased joint swelling in response to NGF stimulation. Ongoing studies are assessing the neuroplasticity and joint histopathology in these mice to address underlying mechanisms.

SIGNIFICANCE**:** These findings imply that NGF-TrkA signaling in chondrocytes and osteoblasts may differentially contribute to OA pain and joint inflammation and merit further exploration of the role of this pathway in articular tissues.

REFERENCES: 1. Obeidat AM, *et al*. bioRxiv, 2025. 2. Bracci-Laudiero L, Manni L. Handbook of neurotoxicity, 2014.

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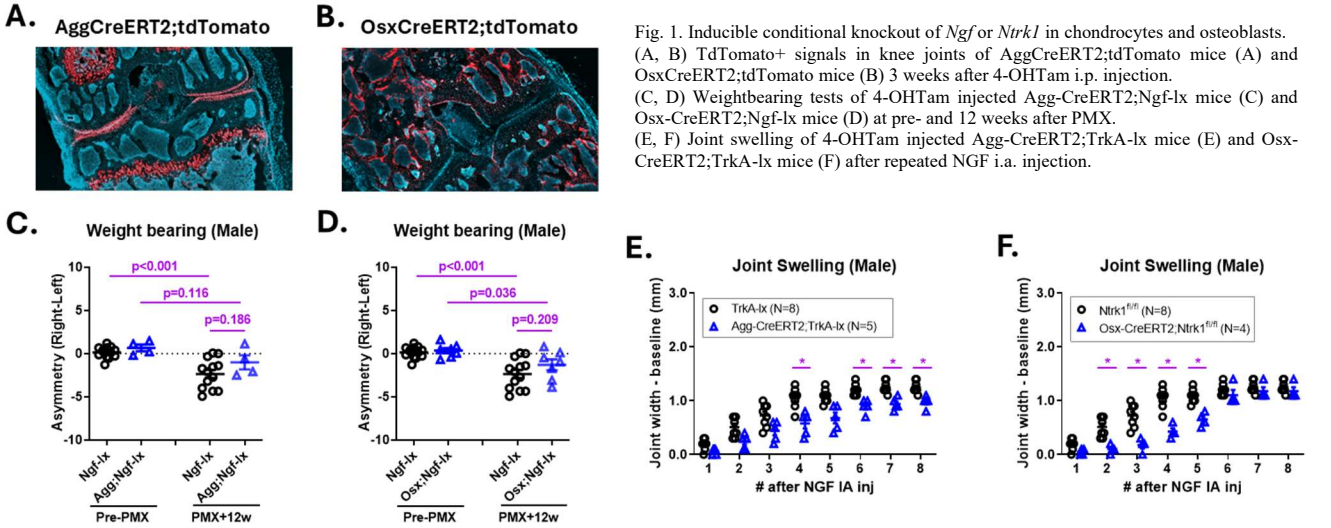

Fig. 1. Inducible conditional knockout of *Ngf* or *Ntrk1* in chondrocytes and osteoblasts.
(A, B) TdTomato+ signals in knee joints of AggCreERT2;tdTomato mice (A) and OsxCreERT2;tdTomato mice (B) 3 weeks after 4-OHTam i.p. injection.
(C, D) Weightbearing tests of 4-OHTam injected Agg-CreERT2;Ngf-lx mice (C) and Osx-CreERT2;Ngf-lx mice (D) at pre- and 12 weeks after PMX.
(E, F) Joint swelling of 4-OHTam injected Agg-CreERT2;TrkA-lx mice (E) and Osx-CreERT2;TrkA-lx mice (F) after repeated NGF i.a. injection.