

Systemic Ablation of VEGFA Protects Against Chronic Mechanical Allodynia Independently of Early Intervertebral Disc Degeneration

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INTRODUCTION: Despite its staggering prevalence and the public health costs of low back pain, the molecular factors that drive the development and chronicity of pain remain poorly understood [1]. The degenerating intervertebral disc expresses a milieu of inflammatory factors that may contribute to the pathophysiology of pain. Amongst these, Vascular Endothelial Growth Factor A (VEGFA), plays critical roles in angiogenesis as well as neurogenesis and neural migration, differentiation, proliferation, and guidance, and it is associated in painful behavior [2-3]. Although the constitutive deletion of VEGFA is embryonically lethal, a Cre-ER/loxP driven by tamoxifen-induction enables one to examine the role of VEGFA postnatally [4]. Using a model of painful intervertebral disc degeneration, we sought to understand the effects of VEGFA on intervertebral disc degeneration and chronic low back pain development.

METHODS: All procedures were performed with WUSM IACUC approval. Ubiquitin (UBC)-Cre^{ERT2}; VEGFA^{fl/fl} mice were allocated into groups at age 4-6 months. Surgical intervention exposed the L5/L6 IVD ('Sham') and injured the disc ('Injury') with a 30G needle 3 times through the annulus fibrosis and partially into the nucleus pulposus. On days 3 and 4 post-operatively, tamoxifen (TAM) was given via oral gavage to Cre+;VEGFA^{fl/fl} animals (aka 'VEGFA-null') and Cre-;VEGFA^{fl/fl} littermates ('WT'). **Intervertebral disc degeneration after 3 weeks:** Mice were sacrificed and prepared for frozen histology using 10 µm thick midsagittal sections stained with Safranin O/ Fast Green and imaged at 10x on a Nanozoomer (Hamamatsu, Japan). Mean disc degeneration scores from three independent investigators (n=24 total, n=3/sex/group/surgery) were used for analysis. **Acute (3-week) and chronic (12-week) mechanical allodynia:** Behavioral analysis was performed on a different set of mice (n=47, n=3-8/sex/group/surgery) at baseline, 3 weeks, and 12 weeks. Von Frey filament test (Bioseb, France) assessed the mechanical force needed to elicit a hind paw withdrawal using the "up-down" method [5]. Verification of VEGFA ablation was performed with a VEGFA ELISA (R&D Systems) on tissue culture caudal functional spine units at 12-week sacrifice with TNF-α as an antagonist. Statistical comparisons were done using a linear mixed effect model to assess sex, surgery, genotype, and duration after surgery.

RESULTS: The TNF-α mediated production of VEGFA was blunted by 82% in the VEGFA-null animals, twelve weeks after the TAM administration regimen (data not shown). This efficiency is similar to our prior work showing approximately 80-90% attenuation of VEGFA immediately following TAM delivery. Histopathological scoring revealed greater degeneration in the injured discs (p < 0.0001; Figure 1A,B). Mechanical allodynia measured by the Von Frey filament test showed that there were differences between the animals between 3-weeks and 12-weeks (p=0.035) with an interaction between genotype and week (Figure 1C, p=0.036). The VEGFA-null mice exhibited reduced sensitivity (i.e., greater withdrawal force) compared to WT animals (Figure 1C).

DISCUSSION: The lumbar intervertebral disc injury model consistently causes IVD degeneration that transitions to chronic low back pain [6-7], most commonly defined as persistence of pain behavior for 12-weeks or more after the onset of the injury [8]. Consistent with these models, we observed that the surgical injury to the IVD resulted in consistent degeneration with accompanying acute and chronic low back pain. In animals with post-surgical ablation of VEGFA, degeneration of the IVD was unaffected and mechanical sensitivity after 3 weeks was similar to WT counterparts. However, the mechanical allodynia in the VEGFA-null animals subsided after 12-weeks and averted the transition to chronic pain behavior. The blockade of VEGFA is also known to have analgesic effects [9], although this is unlikely in our model, since the mechanical allodynia is present in the VEGFA-null animals at levels similar to the WT animals. These findings suggest that VEGFA may be a critical mediator of chronic pain behavior independently of intervertebral disc degeneration, although the precise mechanism of action remains to be investigated.

SIGNIFICANCE/CLINICAL RELEVANCE: Loss of VEGFA is protective against mechanical sensitivity and shows no major effect on IVD degeneration after lumbar injury thus providing a possible therapy to prevent lower back pain.

REFERENCES: [1] Hartvigsen et al. Lancet 2018 [2] Lai et al. Spine, 2016 [3] Carmeliet and Almodóvar Cell Mol Life Sci. 2013 [4] Buettermann et al. JBMR 2019 [5] Chaplan et al. Neuro Methods 1994 [6] Leimer et al. Sci. Rep. 2019 [7] Millecamps and Stone Pain 2018 [8] <https://www.ninds.nih.gov/health-information/disorders/back-pain> [9] Sato et al. Spine 2018

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FIGURE 1: A) Disc degeneration score 3 weeks Post-Op B) Representative histological images 3 weeks Post-Op C) Von Frey sensitivity 12 weeks Post-Op

