Activation of Hedgehog Signaling Reverses Intervertebral Disc Degeneration and Associated Pain and Sensitization in Adult Mice

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INTRODUCTION: Chronic low back pain (cLBP) is an increasingly prevalent condition strongly associated with intervertebral disc (IVD) degeneration. However, a better understanding of the molecular mechanisms driving degeneration and the transition to pain is needed to develop therapeutics targeting the root cause at the IVD. Previous research reported that Sonic Hedgehog (SHH) is a key signaling molecule required for postnatal maintenance of IVD structure and function. Moreover, age-related decline in SHH was reported to be associated with naturally occurring IVD pathologies related to pain and sensitization in aging mice. The goal of the current study is to investigate the role of SHH in homeostasis of IVDs in adult mice. To achieve this, we developed several novel conditional genetic mouse models. First, we accelerated IVD degeneration by targeting SHH (Shh-cKO). Next, we employed genetic and pharmacological rescue strategies in the Shh-cKO mouse model to evaluate the therapeutic potential of reactivating the Hedgehog pathway.

METHODS: Male and female mice were used. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) and performed in compliance with NIH guidelines. To specifically knock down SHH in NP cells, we generated tamoxifen-inducible NP-specific Krt19CreERT/+; Shhflx/flx mice (ShhcKO, n=29, 15♂). The effects of Shh-cKO on IVD and DRG were analyzed three and six months later. The tamoxifen-treated Shhflx/flx (WT, n=23, 11♂) littermates served as controls. We achieved rescue of SHH signaling by generating Krt19CreERT/+; tetO7-rShh, rtTA; Shhflx/flx mice (Shh-rescue, rSHH, n=21, 12♂), where tamoxifen administration in adult mice resulted in NP-specific conditional targeting of Shh, and three months later, doxycycline treatment for one month resulted in conditional and transient overexpression of rSHH specifically by NP cells for a month. Tamoxifen and doxycycline-treated tetO7-rShh; Shhf/f; rtTA (n=22, 12♂) served as controls. The effects of SHH-rescue on IVD and DRG were analyzed six months later. All mice from both Shh-cKO and Shh-rescue cohorts underwent behavioral assessments using the open field test, tail suspension test, acetone sensitivity, and capsaicin response at 8-9 months of age (pre-tamoxifen), three months post-tamoxifen, and an additional three month post-Dox treatment. At least two blinded observers recorded behavioral data. DEXA scans, performed both pre- and post-Shh-cKO and rescue, ensured body composition and IVD morphological comparability between groups. DRGs, NP, and AF were micro-dissected L1-L5 IVDs and subjected to RNA-sequencing. Cryosections from L5-S1 were analyzed for morphometric, histopathological, and molecular changes in all components of the IVD. Immunofluorescence on IVD and lumbar DRG from the same mice was used to assess changes in the presence of Nav1.8, Nav1.9, TRPA1, CD31, and PGP9.5. GO and pathway analyses were conducted for RNA-seq data. Statistical analysis was performed in GraphPad Prism version 10.

RESULTS SECTION: Molecular analysis by RNA-seq and histopathological analysis revealed significant degeneration in the IVDs of Shh-cKO mice starting at three months post-tamoxifen treatment, characterized by a significantly higher expression of inflammatory and neurotrophic factors by the NP and AF cells of the Shh-cKO mice compared to WTs. Hence, we chose the three-month post-tamoxifen treatment for rSHH overexpression using doxycycline treatment for the rescue studies. At six-month post-tamoxifen treatment, Shh-cKO mice exhibited substantial changes in IVD structure and behaviors indicative of pain compared to age-matched WT controls. Additionally, immunofluorescence validated increased innervation (PGP9.5) and vascularization (CD31) in the IVDs of Shh-cKO mice, along with an increased prevalence of nociceptive channels, including Nav1.8, Nav1.9, and TRPA1, in their DRGs compared to WTs. Genetic transient overexpression of rShh in the NP led to a significant reduction in pain-associated behaviors in Shh-rescue (following Shh-cKO) mice at the six-month post-tamoxifen treatment time point compared to the Shh-cKO mice. These mice exhibited restored rearing behavior in the open-field test and reduced escape behavior in the tail suspension test compared to untreated Shh-cKO mice. rShh rescue also reduced cold allodynia response to a level not significantly different than controls, and substantially lower than Shh-cKO, suggesting that overexpression of rSHH mitigated downstream nociceptive sensitization. The behavioral changes were associated with the restoration of the IVD structure. DEXA analysis confirmed no significant differences in body composition across cohorts.

DISCUSSION: These findings demonstrate the beneficial effects of Hedgehog signaling in the adult mouse IVD and that it can reverse structural and molecular hallmarks of degeneration and reduce pain-like behaviors. While SHH levels naturally decline with age, concomitant with an increase in disc degeneration and painful pathologies, this study shows that timely restoration of SHH in the NP can prevent or mitigate the onset of disc-related pain phenotypes. These results provide a strong rationale for further exploration of Hedgehog-based therapies in age-related disc degeneration and cLBP.

SIGNIFICANCE/CLINICAL RELEVANCE: cLBP and IVD degeneration are the top global burden of disease, and the development of therapeutics targeting the root cause may result in efficient treatments that may not lead to dependency.

ACKNOWLEDGEMENTS: The research reported in this abstract was supported by the NIA/NIH award R01AG070079, and the OD/NIH award S10OD026763