CRISPR Epigenome Editing Abolishes Osteoarthritic Cartilage Induced Neuron Sensitization

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DISCLOSURES: Joshua D. Stover (N), Robby D. Bowles, (N), Alejandro Almarza (N)

INTRODUCTION: Osteoarthritis (OA) of the knee and temporomandibular joint (TMJ) can both be characterized by degeneration of articular cartilage and pain **[1,2].** OA joints contain a milieu of inflammatory factors, such as interleukin-1 beta, IL-1β, that may contribute to OA pain by sensitizing nociceptive neurons innervating **[3,4].** The knee is innervated by sensory neurons whose cells bodies reside in the dorsal root ganglia (DRG), while the TMJ is innervated from the trigeminal ganglia (TG). Our hypothesis, is that direct interactions between OA cartilage and nociceptive neurons will alter neuronal responses to nociceptive stimuli and that CRISPR epigenome editing of IL1R1 to decrease expression in peripheral neurons will mediate this sensitization. To test this hypothesis, we measured DRG and TG neuron responses to thermal stimuli when seeded on OA and healthy cartilage and tested the ability of CRISPR epigenome editing of IL1R1 expression in TG and DRG neurons to regulate IL-1β and OA cartilage induced neuron sensitization.

METHODS: *OA and Healthy Cartilage*: OA cartilage tissue was obtained from patients (n=3) undergoing total knee replacement for the treatment of knee OA and pain. Healthy cartilage tissue was obtained from the knees of cadaveric donors (n=3). Tissue biopsy punches (5mm diameter) of cartilage were obtained from both types of tissue, washed 3 times in sterile PBS, and stored at -80 °C until time of experiments. All protocols were approved by the University of Pittsburgh Human Research Protection Office. *Neuron Cell Culture:* DRG neurons (n=3 animals) or TG neurons (n=3 animals) were isolated from adult (4-6 month old) female Wistar rats (following an IACUC approved protocol) and seeded onto cartilage tissue (OA or healthy) or glass-bottomed tissue culture dishes (TCD) and cultured in SATO- media with NGF (10ng/mL) for 24 hours. qPCR Experiments: TG neurons were transduced with either non-targeting or IL1R1 CRISPR epigenome editing vectors. Seven days later, gene expression was measured via qPCR. *Inflammatory Cytokine Exposure Experiments:* Neurons on TCDs were exposed to IL-1β for 24 hours prior to imaging. *Imaging of Neuron Calcium Transients:* One hour prior to imaging, cells were loaded with calcium dye (Rhod2-AM, 3μM) and incubated in the dark for one hour. Cultures were incubated at 37 °C for 15 minutes to obtain a baseline calcium signal and then exposed to temperatures (37 - 44°C) while imaging using a confocal microscope (excitation 552nm, emission 581nm, 1.96fps). Neurons were considered to exhibit heat induced calcium transients if the ΔF/F for the cell was 3 standard deviations greater than the mean baseline value at 37°C **[5]**. *Statistics:* Data were analyzed by one way ANOVA (gene expression) or two-way ANOVA on repeated measures (responding neurons).

RESULTS: Transduction of TG neurons with CRISPR epigenome editing vectors targeting the IL1R1 gene promoter (**Figure 1A**) resulted in high transduction efficiency (**Figure 1B**) and significantly downregulated expression of IL1R1 expression in TG neurons (**Figure 1C**). The percentage of nontarget edited DRG neurons on TCD exposed to thermal stimuli in the presence of IL-1β exhibiting heat induced calcium transients was significantly elevated over baseline; however, the percentage of IL1R1 edited DRG neurons exposed to thermal stimuli in the presence of IL-1β exhibiting heat induced calcium transients similar to no IL-1β exposure (**Figure 1D**). Additionally, CRISPR epigenome editing of IL1R1 expression in TG neurons on TCDs abolished IL-1β induced elevated percentages of TG neurons responding to thermal stimuli (**Figure 1E**). The percentage of neurons exhibiting heat induced calcium transients in nontarget edited DRG neurons (**Figure 1F**) and TG neurons (**Figure 1G**) seeded on OA cartilage tissue was significantly elevated over nontarget neurons seeded on healthy cartilage tissue. Of note, the IL1R1 edited DRG neurons were desensitized to OA cartilage, but the TG neurons were not.

DISCUSSION: There results demonstrate CRISPR epigenome editing of IL1R1 expression in DRG and TG neurons mediates IL-1β induced neuron sensitization to nociceptive stimuli. In addition, the lack of thermal response of DRG neurons on OA cartilage with decreased IL1R1 expression from our CRISPR edits suggest that IL-1β is a major signaling cytokine. However, the continued thermal response of TG neurons on OA cartilage suggest other cytokines are the primary drivers to sensitization.

SIGNIFICANCE/CLINICAL RELEVANCE: These results suggest CRISPR epigenome editing of IL1R1 expression in nociceptive neurons as a treatment strategy for OA pain.

REFERENCES: [1] Vina et al., Curr Opin Rheumatol. 29: 462-82, 2015 **[2]** Derwich et al. Int J Environ Res Public Health 17(8):2923, 2020 **[3]** Juan et al. J Dent Sci. 18(3): 959-971, 2023**[4]** Eitner et al. Front Mol Neurosci 10:349, 2017 **[5]** Stover et al., Mol Ther. 25(9): 2014-2027, 2017

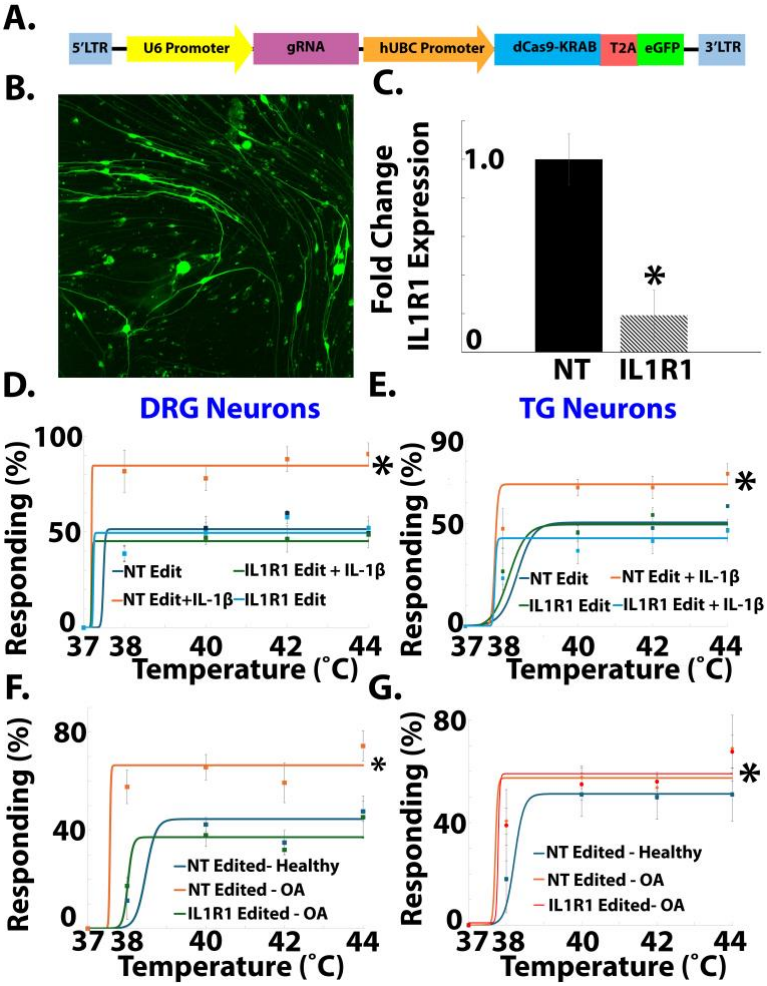

Figure 1. A) Vector Map of Lentivirus Vectors. B) Transduction of TG with IL1R1 epignome editing vectors C) significantly downregulated IL1R1 expression (n=3, *p<0.05). IL-1β induced sensitization of D) DRG and E) TG neurons to thermal stimuli was abolished by CRISPR epigenome editing of IL1R1 expression. OA cartilage sensitization of F) DRG neurons was abolished by IL1R1 epigenome editing; however, G) epigenome editing of TG neurons did not reduce OA induced TG neuron sensitization (n=3, *p<0.05)