Discovery and validation of a new long noncoding RNA as a novel target for neuropathic pain

HYPOTHESIS:

Nerve injury-specific long noncoding RNA (*NIS-IncRNA*), as a potential therapeutic target, in dorsal root ganglion contributes to peripheral mechanisms underlying neuropathic pain through promotion of DRG FUS-controlled *Ccl2* gene transcription

PROJECT DESCRIPTION (Include design, methodology, data collection, techniques, data analysis to be employed and evaluation and interpretation methodology)

Chronic neuropathic pain is a major public issue due to its high incidence in the general population, associated social burden, and limited efficacy of current treatments. Opioids are the last option for pharmacological treatment of this disorder, but they cause devastating adverse effects including addiction and overdose-induced death. Therefore, the development of new, effective, and non-addictive approaches is urgently needed. Peripheral nerve injury-induced changes in gene expression in the dorsal root ganglion (DRG) contribute to neuropathic pain genesis. Long non-coding RNAs (lncRNAs) regulate gene expression. Many lncRNAs are dysregulated in the DRG following peripheral nerve injury. However, the roles of the majority of these dysregulated lncRNAs in neuropathic pain are still elusive, although we previously reported *Kcna2* antisense RNA in the injured DRG as one of the endogenous triggers for neuropathic pain genesis. Discovery and validation of how lncRNAs contribute to neuropathic pain may provide novel, non-opioid therapeutic strategies for effective treatment of this disorder.

We recently identified a large, native, full-length lncRNA with two splice variants: variant 1 (V1; exons 1 and 2) and variant 2 (V2; exons 1 and 3). Because their expression is significantly increased in response to peripheral nerve injury, but not to inflammation, in the DRG, we named them nerve injury-specific lncRNA V1 and V2 (*NIS-IncRNA V1 and V2*). Mimicking their increases through DRG overexpression of *NIS-IncRNA V1* and *V2*, respectively, in naive mice led to neuropathic pain symptoms. Blocking their increases through DRG knockdown/knockout of *NIS-IncRNA V1* and *V2*, respectively, ameliorated chronic constriction injury (CCI)/spinal nerve ligation (SNL)-induced pain hypersensitivity. *NIS-IncRNA* likely participated in the nerve injury-induced increase in the level of C-C chemokine ligand 2 (CCL2) through recruiting the FUS (Fused sarcoma, a DNA-binding protein) into the *Ccl2* gene promoter (Figs. 10-16). Given that DRG CCL2 is a key player in neuropathic pain genesis and that FUS is required for transcription initiation through its binding to gene-specific transcription factors, we hypothesized that DRG *NIS-IncRNA*, as a potential therapeutic target, contributes to peripheral mechanisms underlying neuropathic pain through promotion of DRG FUS-controlled *Ccl2* gene transcription. Our hypothesis will be tested by the following specific aims:

**Aim 1. To validate *NIS-IncRNA* as a therapeutic target in neuropathic pain models.** First, we will characterize the chemically modified long-acting antisense oligonucleotides (ASOs) that specifically knock down *NIS-IncRNA V1 and V2* (V1 ASO and V2 ASO), respectively, delivered intrathecally for their *in vivo* stability and efficacy in the CCI model, as intrathecal (IT) delivery of the modified ASO has been approved by the FDA for clinical therapy. Next, we will validate their efficacy in other neuropathic pain models, as the mechanism underlying neuropathic pain may vary with different etiology. Effects of IT V1 ASO and V2 ASO on spinal nerve ligation (SNL)-, chemotherapy- (paclitaxel (PTX))- or diabetic- (streptozotocin (STZ))- induced neuropathic pain
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will be examined. Finally, to further confirm the role of DRG NIS-lncRNA in neuropathic pain, we will examine the effect of genetic knockdown/knockout of DRG NIS-lncRNA on CCI-induced pain hypersensitivity through microinjection of AAV5-Cre into the DRG of NIS-lncRNA<sup>fl/fl</sup> mice or cross-breeding of the NIS-lncRNA<sup>fl/fl</sup> mice with conditional adhillin<sup>cre</sup> mice.

Aim 2. To determine how blocking the increased NIS-lncRNA in the injured DRG produces anti-nociceptive effects in neuropathic pain. We hypothesize that nerve injury-induced increase of NIS-lncRNA expression in the injured DRG contributes to neuropathic pain via promotion of DRG FUS-controlled Ccl2 gene transcription. To test our hypothesis, we will first validate the expression pattern of NIS-lncRNA in the DRG after CCI. We will then examine whether blocking the CCI-induced increase in DRG NIS-lncRNA inhibits DRG CCL2 upregulation, CCL2-mediated DRG neuronal hyper-excitability, and spinal cord dorsal horn central sensitization after CCI. Finally, we will define whether the CCI-induced increase in DRG NIS-lncRNA promotes the binding of FUS with the Ccl2 gene promoter and subsequently increases FUS-controlled CCL2 expression in the injured DRG after CCI.

Aim 3. To validate the role of NIS-lncRNA in human DRG. First, we will verify the expression of NIS-lncRNA in human DRG and identify the neuronal subtypes of expression. We will then observe the changes in NIS-lncRNA expression and validate the therapeutic targeting of NIS-lncRNA by examining its role in CCL2 expression and neuronal hyper-excitability in viable human cultured DRG neurons exposed to paclitaxel.

Given that the ASO strategy is FDA-approved and that IncRNAs are therapeutic targets in Phase I/II clinical trials of cancer treatment, completing this proposal will advance neuropathic pain management by providing a novel non-opioid target for this disorder.

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Project Description

Please explain Heart, Lung, Blood relevance

THIS PROJECT INVOLVE RADIOISOTOPES? ☐

THIS PROJECT INVOLVES THE USE OF ANIMALS ☑
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THIS PROJECT IS SUITABLE FOR:
UNDERGRADUATE STUDENTS ☐ ENTERING FRESHMAN ☐
SOPHMORES ☐ ALL STUDENTS ☑

THIS PROJECT IS WORK-STUDY: Yes ☑ or No ☐

THIS PROJECT WILL BE POSTED DURING ACADEMIC YEAR
FOR INTERESTED VOLUNTEERS: Yes ☑ or No ☐

WHAT WILL THE STUDENT LEARN FROM THIS EXPERIENCE?

1. Understanding molecular and cellular mechanisms underlying chronic neuropathic pain
2. NIS-IncRNA is a key player in neuropathic pain genesis and may be a potential target for the management of this disorder.
3. Different animal models of neuropathic pain will be used to mimic neuropathic pain patients with distinct etiology.
4. Combining study of animal experiments with in vitro human cell cultured experiments.