Silencing neuropathic pain

By C. Simone Fishburn, Senior Editor

The seemingly intractable nature of neuropathic pain has led drug developers to look beyond classical receptor and transporter protein targets and explore the emerging field of epigenetics to find new options. Now, researchers at **The Johns Hopkins University School of Medicine** have found a potential target based on the discovery that nerve injury upregulates a long noncoding RNA that silences voltage-gated potassium channel Kv1.2 exclusively in damaged neurons.¹

Neuropathic pain develops after nerves are damaged by injury, viral infection or disease, which set up the nervous system's controls to go awry. Under these conditions, neurons spontaneously fire signals to the brain, sending pain messages even when there is no physical stimulus.

Yuan-Xiang Tao, an associate professor of anesthesiology and critical care medicine at The Johns Hopkins University School of Medicine, told

SciBX that his team began looking for epigenetic causes of the condition five years ago as data emerged on the importance of microRNA and noncoding RNA in diseases of the nervous system.²

In 2009, the group performed proteomic studies to explore how nerve injury alters gene expression in the dorsal root ganglia (DRG),³ which are nodules that house the cell bodies of peripheral nerves. Now, the team has honed in on noncoding RNAs and discovered a 2.5 kb long noncoding RNA (lncRNA) that is upregulated in damaged neurons.

Interestingly, the lncRNA sequence was overlapping with and complementary to the coding region of the voltage-gated *potassium channel Kv1.2 (KCNA2)*, suggesting it could decrease the expression of KCNA2 and affect the axonal membrane potential, leading to increased neuronal firing (see Figure 1, "Making antisense of *Kcna2* in pain").

KCNA2 is expressed in the brain and spinal cord. It is a potential therapeutic target for epilepsy because of the high seizure rate observed in *Kcna2*^{-/-} knockout mice.⁴

The Johns Hopkins team showed that *Kcna2* lncRNA reproduced symptoms of neuralgia in rats when it was overexpressed in DRG neurons. Conversely, *Kcna2* mRNA alleviated symptoms of neuralgia in rat models for neuropathic pain when delivered to the DRG.

Furthermore, the upregulation of *Kcna2* lncRNA that occurred following nerve injury was accompanied by a decrease in *Kcna2* mRNA and protein levels in individual DRG neurons. That finding suggests *Kcna2* lncRNA may act at least in part by directly neutralizing *Kcna2* mRNA.

Figure 1. Making antisense of *Kcna2* in pain. Pain sensation involves transmission of signals by sensory nerves through the spinal cord to motor nerves, which control the physiological motor response.

(I) In normal neurons, *potassium channel Kv1.2 (Kcna2)* mRNA [a] is produced in sensory nerve cell bodies that lie in the dorsal root ganglia (DRG), leading to expression of *Kcna2* voltage-gated potassium channels [b]. The resulting potassium channel conductance counterbalances the sodium influx, stabilizing the neuronal membrane and enabling normal neuronal firing [c].

(II) In injured nerves, antisense *Kcna2* lncRNA is upregulated [a] and silences normal expression of *Kcna2* voltage-gated potassium channels [b]. The reduced potassium conductance alters the balance of ion fluxes, which decreases the threshold for action potential firing compared with that in uninjured nerves and creates hyperexcitable neurons [c].

Data were reported in *Nature Neuroscience*.

According to Jim Barsoum, CSO at epigenetics drug discovery company **RaNA Therapeutics Inc.**, one of the most interesting aspects of the study is that the lncRNA is upregulated in response to nerve damage.

Although antisense lncRNAs are commonly expressed at considerably lower levels than their corresponding sense RNAs, and their upregulation has been associated with several chronic diseases and genetic disorders, it is rare to see such a rapid and significant increase in an lncRNA caused by injury.

Barsoum thinks the antisense lncRNA likely works not only by blocking the sense strand but also by recruiting other epigenetic modulators that control the transcription of the channel.

Sensing a therapy

Turning the Johns Hopkins findings into a therapeutic would likely involve inactivating or eliminating the increase in *KCNA2* lncRNA.

Barsoum thinks this would most likely require using small interfering RNA or a single-stranded oligonucleotide to block the interaction between the antisense and sense transcripts or cause degradation of the lncRNA.

According to Barsoum, single-stranded oligonucleotides might represent the best option, as they have been successfully delivered to several tissues including neuronal cells.^{5,6} They display long half-lives inside the CNS and thus might have an effect that lasts long enough to avoid the need for frequent dosing.

By contrast, double-stranded oligonucleotides require lipid-based nanoparticle formulations that often have toxicity problems.⁷ Additionally, strategies to boost sense RNA would be complicated by the need to deliver the precise dose for sufficient—but not excess—expression of *KCNA2* channels.

Charles Cohen, VP of biological sciences at clinical genetics company **Xenon Pharmaceuticals Inc.**, said a product targeting *KCNA2* lncRNA would most likely need to be delivered intrathecally to achieve sufficient concentrations in the DRG. Cohen previously developed compounds for neuropathic pain at **Vertex Pharmaceuticals Inc.**

Cohen said intrathecal delivery has been employed successfully in treating pain, citing Prialt ziconotide (SNX-111) from **Jazz**

Pharmaceuticals plc. He did note that a narrow therapeutic index limits more widespread use of the drug and that a *KCNA2* lncRNA-targeted therapy might need to have a better safety profile than ziconotide to be competitive.

Cohen also said the widespread distribution of *KCNA2* channels throughout the brain could give rise to side effects and noted that preclinical studies can be misleading because of significant differences in distribution between rodents and primates.

However, the *Kcna2* lncRNA transcript was only upregulated in damaged neurons, and no changes were observed in uninjured DRG neurons. Thus, it is possible that a therapy targeting *KCNA2* lncRNA would not affect *KCNA2* activity in normal cells.

At least four companies have preclinical programs involving lncRNAs. These include RaNA; **Isis Pharmaceuticals Inc.**; Opko-Curna, a unit of **Opko Health Inc.**; and **TransSINE Technologies Co. Ltd.**

The findings of the study have not been patented. The authors said they are exploring collaborations for clinical studies.

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COMPANIES AND INSTITUTIONS MENTIONED

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The Johns Hopkins University School of Medicine, Baltimore, Md.
Opko Health Inc. (NYSE:OPK), Miami, Fla.
RaNA Therapeutics Inc., Cambridge, Mass.
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