CellPress

Small RNAs, but Sizable Itch: TRPA1 Activation by an Extracellular MicroRNA

Tayler D. Sheahan,[1] Junichi Hachisuka,[1] and Sarah E. Ross[1,2,*]
[1]Department of Neurobiology and the Pittsburgh Center for Pain Research, University of Pittsburgh, Pittsburgh, PA 15213, USA
[2]Department of Anesthesiology, University of Pittsburgh, Pittsburgh, PA 15213, USA
*Correspondence: saross@pitt.edu
https://doi.org/10.1016/j.neuron.2018.07.040

Chronic itch is a major symptom of cutaneous T cell lymphoma (CTCL). In this issue of *Neuron*, Han and colleagues (2018) provide evidence that one of the itch mediators in CTCL is an extracellular miRNA that directly activates TRPA1 on sensory neurons.

Some types of T cells home to the skin to protect against cutaneous infection. If these T cells become cancerous, rashes and itch can be presenting symptoms. For instance, a very common type of cutaneous T cell lymphoma (CTCL) is *Mycosis Fungoides*, which is associated with itchy, ringworm-like lesions that worsen with disease progression. Although itch from this and other types of CTCL can be debilitating, the underlying causes are unknown (Ahern et al., 2012). In this issue of *Neuron*, Han and colleagues provide evidence for the involvement of a novel and unexpected mechanism of CTCL-mediated itch: direct activation of transient receptor potential ankyrin 1 (TRPA1) by the extracellular microRNA (miRNA) miR-711 (Figure 1).

TRPA1 is an irritant sensor that is found on the plasma membrane of several types of sensory neurons (Bautista et al., 2013). Opening of this channel results in current influx that drives neuronal activity, including action potentials that signal pain or itch, as well as local peptide release in the periphery that can contribute to neurogenic inflammation. The structure of TRPA1 has been solved (Paulsen et al., 2015), and most of the effectors that drive channel activation do so from the *intracellular* side. Specifically, reactive chemicals, such as those found in mustard, onions, and wasabi, modulate TRPA1 through covalent bonding at cysteine residues on the N-terminal cytosolic ankyrin repeats (Bautista et al., 2013). Inflammatory mediators that act via G protein-coupled receptors also modulate TRPA1's activity on the inside of the cell, in this case via phosphorylation as a consequence of second messenger signaling. Now it appears that TRPA1 can also be activated by an *extracellular* effector by way of RNA-protein interactions.

Though miRNAs were originally studied as a cell-autonomous mechanism for the regulation of mRNA translation and degradation, there is now a growing body of evidence that these non-coding RNAs are also found in the extracellular environment where they may serve as a biomarker for diseases, such as cancer and Alzheimer's disease (Sohel, 2016). Previous work from the Ji lab was among the first to describe an unconventional role for circulating extracellular miRNAs. Specifically, Park et al. (2014) found that miR-let-7b acts on sensory neurons to cause pain (Park et al., 2014). Given that upregulation of miRNAs has been reported in patients with itchy cutaneous lymphomas (Ralfkiaer et al., 2011), in this issue of *Neuron*, Han and colleagues investigated the possibility that extracellular miRNAs might likewise act on sensory neurons to cause itch.

Using clinical observations to guide their study, Han et al. first tested whether any of the five miRNAs that were found to be upregulated in the skin of lymphoma patients (Ralfkiaer et al., 2011) evoke either itch or pain when injected into mice. Interestingly, they found that only one—miR-711—evoked itch, and none evoked pain. TRP channels are frequently required for responses to itchy chemicals, so Han et al. used knockout mice to test their involvement. Indeed, miR-711-induced scratching was attenuated in mice lacking TRPA1, but not TRPV1, suggesting that miR-711 evokes itch via TRPA1. In addition to its role in mediating itch, activation of TRPA1 by agonists such as allyl isothiocyanate (AITC), the spicy component of mustard and wasabi, has been shown to cause pain and neurogenic inflammation (Dong and Dong, 2018). Therefore, Han et al. further tested whether miR-711 produces a similar array of effects. In contrast to AITC, injection of miR-711 into the hindpaw failed to elicit neurogenic inflammation or pain-like behavior, again suggesting an exclusive role for miR-711 in itch.

To address how miR-711 influences TRPA1 channel activity, Han et al. used a combination of electrophysiological recordings and Ca^{2+} imaging. Through these efforts, they provide further evidence that AITC and miR-711 activate TRPA1 in somewhat different ways. First, using single-channel recordings, the authors showed that miR-711 acts on the extracellular face of TRPA1, whereas AITC acts on the intracellular face. Further, TRPA1 had a shorter open time and lower Ca^{2+} permeability when activated by miR-711 compared to AITC. Intriguingly, though miR-711 acts via TRPA1, it only activated a subset of TRPA1-expressing sensory neurons. Thus, whereas roughly 25% of primary afferents responded to AITC, only about 5% responded to miR-711. Since the miR-711-sensitive afferents also responded to histamine and chloroquine, these may represent an itch-selective population.

How might miR-711 interact with TRPA1? To address this question, Han et al. performed a series of elegant experiments that provide evidence that miR-711 binds extracellularly to cause activation of TRPA1 and in turn itch. The authors first identified an evolutionarily conserved

core sequence that was found to be necessary and sufficient for miR-711-evoked itch in mice. This core sequence was then used in molecular dynamic computer simulations, which predicted that miR-711 binds extracellularly to TRPA1, in contrast to other TRPA1 agonists. Then, mutagenesis analysis was used in combination with electrophysiology to validate the prediction that functional interactions between TRPA1 and miR-711 occur at extracellular TRPA1 residues. Finally, Han et al. provided evidence of the physiological relevance of the miR-711/TRPA1 interaction to acute itch. A blocking peptide that covers the relevant TRPA1 extracellular residues prevented miR-711-induced itch but did not affect itch evoked by other pruritogens such as chloroquine. These observations suggest that interaction with TRPA1 is required for miR-711-induced itch.

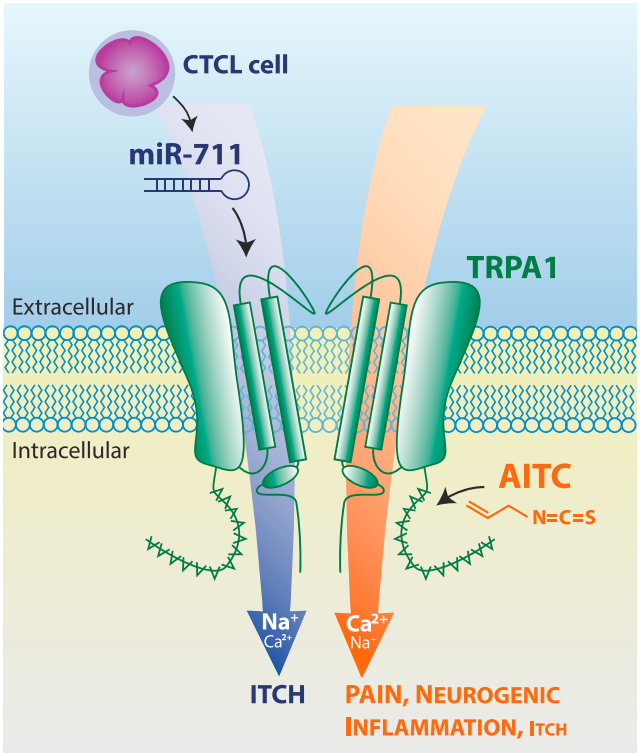

Figure 1. Distinct Modulation of TRPA1 by miR-711 and AITC
Irritating compounds such as AITC, the pungent component of mustard and wasabi, interact with the intracellular domain of TRPA1, leading to pain, neurogenic inflammation, and to a lesser extent, itch. In the current issue of *Neuron*, Han et al. demonstrate that the miRNA miR-711, which is released by cutaneous T cell lymphoma (CTCL) cells, binds to extracellular TRPA1 residues and selectively causes itch, possibly due to differential effects on TRPA1's ion permeability. TRPA1, transient receptor potential ankyrin 1; AITC, allyl isothiocyanate.

One of the most exciting findings in this study was that Han et al. were able to show a role for miR-711 in CTCL-mediated itch, suggesting that miR-711 is an endogenous itch mediator. To do so, the authors developed a novel mouse model that successfully recapitulated human symptoms of CTCL: mice displayed robust tumor growth, chronic itch, and elevated serum levels of human miRNAs including miR-711, which was also highly expressed in the affected skin. Of particular interest, skin lymphoma of CTCL mice received sensory innervations, raising the possibility that itch-sensitive nerve fibers expressing TRPA1 may be well positioned for activation via miR-711 upon its release from skin lymphoma. This new model of chronic itch was used along with pharmacological and genetic approaches to support a role for miR-711 in the regulation of CTCL-induced chronic itch. Specifically, inhibition of either miR-711 or TRPA1 (or blocking their interaction with a peptide) decreased CTCL-induced itch. Collectively, these results suggest that miR-711 mediates CTCL-induced itch through TRPA1.

One of the fascinating questions raised by this study is why some irritants cause itch and others cause pain, even though they appear to be acting on the same receptors. Indeed, most aversive chemicals can cause pain or itch, depending on how they are applied (Ross, 2011). Here, the authors speculate that miR-711 and AITC cause predominantly itch and pain, respectively, due to their distinct effects on TRPA1—activation with miR-711 is more transient and less Ca^{2+} permeant. These findings fit with the observation that AITC, but not miR-711, causes neurogenic inflammation, which occurs in response to Ca^{2+} influx into primary afferents, raising the possibility that AITC-induced pain is largely secondary to afferent-induced flare. An alternative possibility is that a weaker TRPA1 stimulus (i.e., miR-711) is only sufficient to activate primary afferents with high TRPA1 expression (putative itch-specific), whereas a stronger TRPA1 stimulus (i.e., AITC) is able to activate a broader population of sensory neurons, thereby occluding itch and causing pain (Esancy et al., 2018). This issue of coding is surprisingly elusive, so for now we will simply have to wait, itching (or aching?) for future insight.

REFERENCES

Ahern, K., Gilmore, E.S., and Poligone, B. (2012). Pruritus in cutaneous T-cell lymphoma: a review. J. Am. Acad. Dermatol. *67*, 760–768.

Bautista, D.M., Pellegrino, M., and Tsunozaki, M. (2013). TRPA1: a gatekeeper for inflammation. Annu. Rev. Physiol. *75*, 181–200.

Dong, X., and Dong, X. (2018). Peripheral and central mechanisms of itch. Neuron *98*, 482–494.

Esancy, K., Condon, L., Feng, J., Kimball, C., Curtright, A., and Dhaka, A. (2018). A zebrafish and mouse model for selective pruritus via direct activation of TRPA1. eLife *7*, e32036.

Han, Q., Liu, D., Convertino, M., Wang, Z., Jiang, C., Kim, Y.H., Luo, X., Zhang, X., Nackley, A., Dokholyan, N.V., and Ji, R.-R. (2018). miRNA-711 binds and activates TRPA1 extracellularly to evoke acute and chronic pruritus. Neuron *99*, this issue, 449–463.

Park, C.-K., Xu, Z.-Z., Berta, T., Han, Q., Chen, G., Liu, X.-J., and Ji, R.-R. (2014). Extracellular microRNAs activate nociceptor neurons to elicit pain via TLR7 and TRPA1. Neuron *82*, 47–54.

Paulsen, C.E., Armache, J.P., Gao, Y., Cheng, Y., and Julius, D. (2015). Structure of the TRPA1 ion channel suggests regulatory mechanisms. Nature *520*, 511–517.

Ralfkiaer, U., Hagedorn, P.H., Bangsgaard, N., Løvendorf, M.B., Ahler, C.B., Svensson, L., Kopp, K.L., Vennegaard, M.T., Lauenborg, B., Zibert, J.R., et al. (2011). Diagnostic microRNA profiling in cutaneous T-cell lymphoma (CTCL). Blood *118*, 5891–5900.

Ross, S.E. (2011). Pain and itch: insights into the neural circuits of aversive somatosensation in health and disease. Curr. Opin. Neurobiol. *21*, 880–887.

Sohel, M.H. (2016). Extracellular/circulating microRNAs: release mechanisms, functions and challenges. Achiev. Life Sci. *10*, 175–186.