Role of MRGPRs in substance P signaling, itch, and mast cell activation

Yanek Jiménez-Andrade[1*], Lilah Gmyrek[1], Jamie Harden[2], Jeegar Patel[2], Hans Hofland[2], Jin Mo Park[1], and Ethan A. Lerner[1]

[1]Cutaneous Biology Research Center, Department of Dermatology, Massachusetts General Hospital and Harvard Medical School [2]Evommune, Palo Alto, California, USA

*Presenting author (Email, yjimenezandrade@mgh.harvard.edu)

Introduction

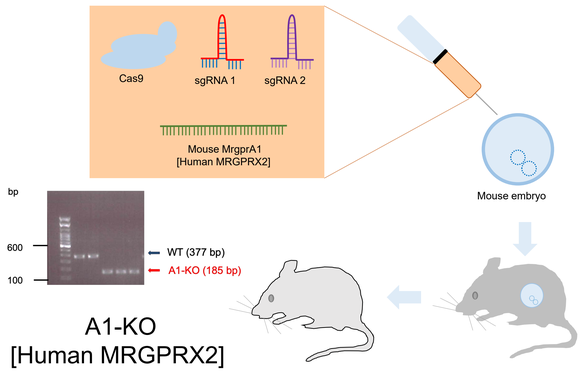
Mas-related G-protein coupled receptor (MRGPR) family proteins function as innate sensors for pruritogens and play a crucial role in itch and neurogenic inflammation[1-3]. Our previous studies demonstrated that the neuropeptide substance P (SP) exerted its physiologic function via MRGPRs, in addition to its conventional receptor, the neurokinin-1 receptor[4-5].

Aim

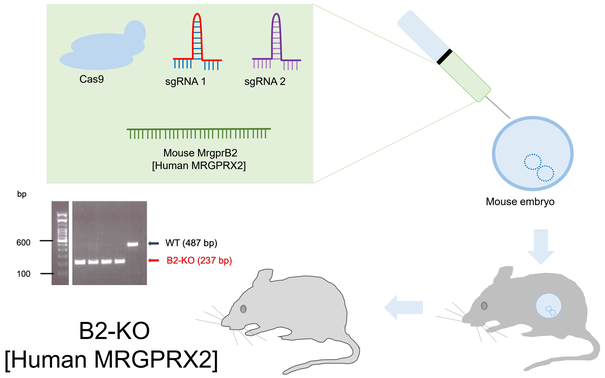
We sought to determine the role of MrgprA1 and MrgprB2, two mouse proteins homologous to MRGPRX2 in humans, in SP-induced itch and mast cell activation.

Material and Methods

Generation of MrgprA1 knock out (A1-KO) mice

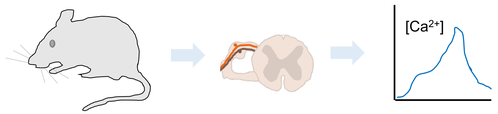


Generation of MrgprB2 knock out (B2-KO) mice



Results

Substance P stimulates dorsal root ganglion (DRGs) WT but not A1-KO neurons



Ligand	WT-DRG neurons	A1-KO DRG neurons
Substance P (1μM)	+	-
Chloroquine (1 mM)	+*	+

Table 1. Activation of cultured DRGs neurons by substance P or chloroquine. Cervical to lumbar dorsal root ganglion (DRGs) from wild-type and MrgprA1-KO mice were dissected and pooled from groups of 4 mice and subjected to collagenase/dispase digestion and mechanical trituration. DRG neurons were incubated as indicated in the table, and activation was determined by ratiometric calcium imaging. + indicate activation. – indicates no activation. *Not determined in this specific study, but well characterized in literature[6].

Substance P evokes scratching behavior in WT mice, and A1-KO mice show a reduced response

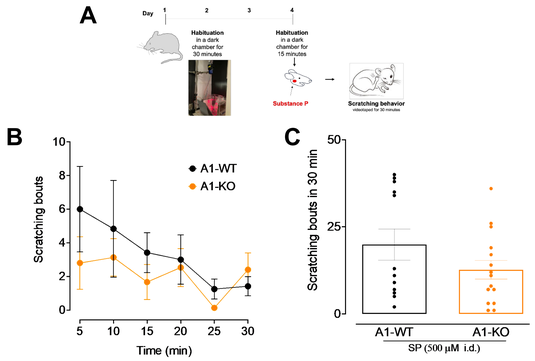


Figure 1. Scratching behavior was evaluated in A1-WT and A1-KO mice. Mouse experiment workflow. Mice received an intradermal injection of 10 μL containing Substance P (500 μmol/L) into the right cheek, delivered by a 31-G needle. The injection site is shown in the red circle (**A**). Time-course of the effect of substance P on scratching behavior over 30 (**B**). The cumulative number of scratching bouts observed for 30 minutes (**C**). Results are means ± SEM (n=12-15 mice/group) combined from three independent experiments.

Substance P evokes scratching behavior in B2-KO mice

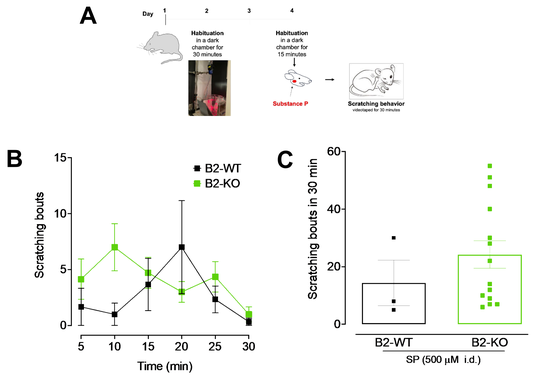


Figure 2. Scratching behavior was evaluated in A1-WT and B2-KO mice. Mouse experiment workflow. Mice received an intradermal injection of 10 μL containing Substance P (500 μmol/L) into the right cheek, delivered by a 31-G needle. The injection site is shown in the red circle (**A**). Time-course of the effect of substance P on scratching behavior over 30 (**B**). The cumulative number of scratching bouts observed for 30 minutes (**C**). Results are means ± SEM (n=3-14 mice/group) combined from three independent experiments.

Conclusions

Our study establishes new molecular mechanisms by which MRGPRs mediate SP signaling and SP-dependent neuroimmune function.

This knowledge supports the development of MRGPR inhibitors for the treatment of itch, dermatitis, and other MRGPR-driven pathologic conditions.

Acknowledgments

This study was supported by the National Eczema Association, grant NEA20-ECRG137 and a fund under a sponsored research agreement with Evommune.

References

1. Akiyama, T., et al., *Roles of glutamate, substance P, and gastrin-releasing peptide as spinal neurotransmitters of histaminergic and nonhistaminergic itch.* Pain, 2014. **155**(1): p. 80-92.
2. Mollanazar, N.K., P.K. Smith, and G. Yosipovitch, *Mediators of Chronic Pruritus in Atopic Dermatitis: Getting the Itch Out?* Clin Rev Allergy Immunol, 2016. **51**(3): p. 263-292.
3. Kuhn, H., et al., *Mas-related G protein-coupled receptor X2 and its activators in dermatologic allergies.* J Allergy Clin Immunol, 2020.
4. Azimi, E., et al., *Substance P activates Mas-related G protein-coupled receptors to induce itch.* J Allergy Clin Immunol, 2017. **140**(2): p. 447-453 e3.
5. Azimi, E., et al., *Dual action of neurokinin-1 antagonists on Mas-related GPCRs.* JCI Insight, 2016. **1**(16): p. e89362.
6. Liu, Q., et al., *Sensory neuron-specific GPCR Mrgprs are itch receptors mediating chloroquine-induced pruritus.* Cell, 2009. **139**(7): p. 1353-65.