Online Proceedings

POSTER SESSION 4

MGH 206, Easel 165

4:00 PM to 6:00 PM

CRISPR Based Behavioral Screening for Genes Affecting Nociception in Zebrafish

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Chronic pain affects millions of people worldwide, and current treatments are often ineffective and come with unwanted side effects. Modern research focuses on studying the mechanisms of pain sensation in the nervous system, with the goal of using our improved knowledge to develop more efficient analgesics. Our research focuses on neurons located in the Trigeminal and Dorsal Root Ganglia (TRG and DRG), as they are known to play an important role in the sensation of touch and pain, and on genes regulating the development and function of these neurons. Recent advances in gene editing methods, particularly the CRISPR/Cas9 system, allow us to mutate specific genes of interest a living animal and monitor the effect of such mutations within a matter of days. To this end, we have developed a behavioral assay which determines the effect of CRISPR induced mutations on pain sensation in zebrafish larvae. CRISPR injected larvae are exposed to noxious stimuli such as AITC (mustard oil) and harmful temperatures. The CRISPR treated larvae's response to the painful stimuli determines the impact of the specific gene we targeted on nociception. Through this assay we have identified two genes, zmat4b and zbtb7b, as potentially important to the sensation of noxious stimuli. Further experiments are now required to determine the precise mechanisms through which these genes play a role in the sensation of pain.

POSTER SESSION 4

MGH 206, Easel 166

4:00 PM to 6:00 PM

Fusion Proteins: An Approach to Distinguishing "Itch" and "Pain" Neurons

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Contrary to the previous belief that itch (pruritis) is simply a less intense form of pain, recent findings suggest that itch and pain are distinct sensations mediated via independent neuronal circuits. Nevertheless, itch and pain share similar molecular machinery. In mammals, itch sensations are the product of the coupling of pruritic receptors, usually GPCRs, and TRP ion channels such as TRPA1, nociceptors that normally encode noxious, painful stimuli. We identified an itch selective compound, imiquimod (IMQ), which was found to mediate pruritic responses via direct activation of TRPA1. However, other TRPA1 agonists such as allyl isothiocyanate (AITC) specifically evoke nociceptive responses. We found that IMQ is a weak agonist of TRPA1 and specifically activates itch selective neurons. These itch selective neurons are primed to respond to TRPA1 agonists while having no effect on TRPA1-expressing nociceptors. We hypothesized that the differences in sensitivity of itch-selective versus nociceptive sensory neurons to TRPA1 agonists could be caused by differences in the amount of TRPA1 channels they contain, the activation mechanism of TRPA1, or the trafficking of TRPA1 following activation. Thus, we developed a strategy to visualize TRPA1 by creating fusion proteins that tether various indicators to TRPA1. We have created a fusion construct of TRPA1 and green fluorescent protein (GFP) by using overlap polymerase chain reactions (PCR) to amplify segments of DNA and tethering the two pieces together via direct, rigid, and flexible linkers. This experiment will lead to the fusion of TRPA1 to other molecules, such as genetically-encoded calcium indicators, GCaMP and CaMPARI. These fusions will give us the ability to visualize the localization, expression, activation, and trafficking of TRPA1. These studies will help elucidate the molecular mechanisms underpinning itch versus pain sensation and show how a single ion channel can mediate distinct sensations via differential activation.