Exploring the Role of GPR63 and GPR153 as Novel Modulators of Opioid-Induced Antinociception via Glial Cell Modulation in a Mouse Model of Neuropathic Pain

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Treatments for chronic pain disorders are currently dominated by opioid drugs which lose efficacy over time and yield various undesirable side effects. Thus, the need for identifying novel targets for modulating pain is critical. In this work, we have identified two potential targets for the development of non-opioid analgesics – the orphan GPCRs GPR63 and GPR153. This was achieved by *in vivo* transfection of targeted CRISPR-Cas9 DNA constructs with a universal promoter in the spinal cords of adult male and female CD-1 mice and evaluating changes in morphine response in a neuropathic pain model. Chemotherapy-induced peripheral neuropathy (CIPN) was induced by 2 mg/kg paclitaxel via intraperitoneal injection resulting in the development of mechanical allodynia. This was followed by administration of 3.2 mg/kg morphine SC and a three-hour von Frey time course to evaluate changes in opioid-induced antinociception. Another cohort of animals received a non-targeted universal negative control CRISPR construct. Receptor knockdown ablated the analgesic effect of morphine in this model whereas there was no effect of knockdown on opioid-induced antinociception in an acute tail flick pain model. These findings suggest that these receptors are not involved in direct neurotransmission of pain signals but instead play roles in the neuropathology of chronic pain and/or in altering cellular responses to opioids in chronic pain states. To further investigate this finding, we performed RNAScope *in situ* hybridization in the spinal dorsal horn to localize RNA transcripts of mouse *Gpr63* and *Gpr153* with immunohistochemical markers for microglia (Iba1) or astrocytes (GFAP), cell types believed to contribute to the development and/or maintenance of neuropathic pain. We found that *Gpr63* and *Gpr153* are expressed in ~50-60% of microglia and astrocytes. Lastly, we began exploring the biological mechanism of these receptors and how their knockdown ablates opioid-induced antinociception by repeating the previously described behavioral assay using glial cell-specific receptor knockdown. This was done by replacing the universal promoter of our original CRISPR-Cas9 constructs with the *Aif1* or *Gfap* promoters to restrict expression of the sgRNA to microglia or astroctyes, respectively. Together this work identifies completely novel pain modulators potentially acting through glial cells which could be exploited to develop new pain therapies.

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