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Intrathecal Injection of miR-133b-3p or miR-143-3p Prevents the Development of Persistent Cold and Mechanical Allodynia Following a Peripheral Nerve Injury in Rats ☆

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Abstract—In DRG an increase in miR-133b-3p, miR-143-3p, and miR-1-3p correlates with the lack of development of neuropathic pain following a peripheral nerve injury. Using lentiviral (LV) vectors we found that a single injection of LV-miR-133b-3p or LV-miR-143-3p immediately after a peripheral nerve injury prevented the development of sustained mechanical and cold allodynia. Injection of LV-miR-133b-3p or LV-miR-143-3p by themselves or in combination, on day 3 post-injury produced a partial and transient reduction in mechanical allodynia and a sustained decrease in cold allodynia. Injection of LV-miR-1-3p has no effect. Co-injection of LV-miR-1a with miR-133b-3p or miR-143-3p on day 3 post-injury produced a sustained decrease in mechanical and cold allodynia. In DRG cultures, miR-133b-3p and miR-143-3p but not miR-1-3p, enhanced the depolarization-evoked cytoplasmic calcium increase. Using 3′UTR target clones containing a Gaussian luciferase reporter gene we found that with the 3′UTR-Scn2b, miR-133-3p and miR-143-3p reduced the expression while miR-1-3p enhanced the expression of the reporter gene. With the 3′UTR-TRPM8, miR-133-3p and miR-143-3p reduced the expression and miR-1-3p had no effect. With the 3′UTR-Piezo2, miR-133-3p increased the expression while miR-143-3p and miR-1-3p had no effect. LV-miR133b-3p, LV-miR-143-3p and LV-miR1a-3p reduced Scn2b-mRNA and Piezo2-mRNA. LV-miR133b-3p and LV-miR-143-3p reduced TRPM8-mRNA. LV-miR-133b-3p and LV-miR-143-3p prevent the development of chronic pain when injected immediately after the injury, but are only partially effective when injected at later times. LV-miR-1a-3p had no effect on pain, but complemented the actions of LV-miR-133b-3p or LV-miR-143-3p resulting in a sustained reversal of pain when co-injected 3 days following nerve injury.

Key words: microRNAs, dorsal root ganglia, peripheral nerve injury, neuropathic pain.

INTRODUCTION

Despite concerted efforts to study, treat, and prevent chronic neuropathic pain, treatment of this type of pain remains relatively unsuccessful in a large number of patients (Baron, 2006; Macrae, 2008). In humans, chronic post-surgical pain is associated with peripheral nerve

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Abbreviations: 3′UTR, 3′ untranslated region; CL, contralateral; DRG, dorsal root ganglia; i.t. injection, intrathecal injection; IL, ipsilateral; L#-DRG, lumbar#-DRG; LV, lentivirus; miR, microRNA; Piezo2, piezo-type mechanosensitive ion channel component 2; Scn2b, sodium voltage-gated channel beta subunit; Sural-SNI, sural-spared nerve injury; Tibial-SNI, tibial-spared nerve injury; TRPM8, transient receptor potential cation channel subfamily M member 8; TU, transduction units.

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injury (Macrae, 2008), and continual peripheral primary sensory input (DRG-dorsal root ganglia-sensory neurons) appears to be required for the maintenance of central changes that mediate persistent neuropathic pain (Hoffert et al., 1984; Gracely et al., 1992). In animals, various peripheral nerve injury models result in chronic neuropathic pain and in expression changes of hundreds of proteins in the DRG (Costigan et al., 2002; Niederberger et al., 2008). Thus effective prevention and treatment of refractory neuropathic pain resulting from peripheral nerve injury (trauma, surgery, or disease) may require the simultaneous modification of many proteins.

An approach that has gained attention as a way to target multiple proteins is via the modulation of microRNA (miRNA) expression (Klein et al., 2005). Different approaches have been taken to select potentially neuropathic pain-relevant miRNAs. Some groups have selected miRNAs that target molecules involved in inflammatory processes at the spinal cord level. Intrathecal injections of some of those miRNAs (miR-195, miR-146a-5p, miR-221, miR-155, miR-19a, miR-124) can decrease neuropathic pain following lumbar-spinal nerve ligation or sciatic nerve injury (Willemen et al., 2012; Shi et al., 2013; Lu et al., 2015; Tan et al., 2015; Wang et al., 2015; Xia et al., 2016). Another approach has been to select miRNAs that target ion channels. Daily intrathecal injections of miR-103, a miRNA that targets L-type calcium channels, decreased neuropathic pain following spinal nerve ligation (Favereaux et al., 2011). Single intrathecal injections of miR-183 (Lin et al., 2014) or miR-96 (Chen et al., 2014), and injection of miR-7a into the DRG (Sakai et al., 2013), miRNAs that target sodium channels at the DRG, were shown to decrease neuropathic pain in the spinal nerve ligation or the chronic constriction sciatic nerve models. These approaches showed efficacy for hours or up to 16 days. In contrast to all the previous studies that detected miRNA changes associated with nerve injury-induced hypersensitivity, we used a complementary approach that enabled the detection of miRNA changes associated with recovery from nerve injury-induced neuropathic pain. The approach consisted in the parallel assessment of two variants of the spared nerve injury (SNI) model; the sural-SNI which develops chronic neuropathic pain and tibial-SNI which does not develop chronic neuropathic pain (Lee et al., 2000; Norcini et al., 2014). Using this approach we identified seven miRNAs whose upregulation in the DRG correlated with the lack of development of chronic pain in tibial-SNI (Norcini et al., 2014). We chose to study the effects of miR-133b-3p, miR-143-3p, and miR-1a-3p because they showed the largest change in expression in L4 DRG at day 23 post-injury (Norcini et al., 2014). We hypothesized that these three miRNAs act as 'recovery' miRNAs for peripheral nerve injury-induced neuropathic pain. We tested this hypothesis using lentiviral (LV) vectors to induce the up-regulation of miR-133b-3p, miR-143-3p or miR-1a-3p.

Here we show that a single intrathecal injection of either LV-miR133b-3p or miR-143-3p immediately after a peripheral nerve injury prevents the development of persistent mechanical and cold allodynia, while when injected on day 3 post-injury they produced a sustained reversal of cold allodynia but only a transient decrease in mechanical allodynia. An injection of LV-miR1a-3p did not affect the level of mechanical or cold allodynia. However, a single injection 3 days post injury of LV-miR-1a-3p with LV-miR-133b-3p or LV-miR-143-3p produces a sustained reversal of both mechanical and cold allodynia. This is the first study to show that a single intrathecal injection of a miRNA is sufficient to prevent the development of persistent mechanical and cold allodynia following a peripheral nerve injury, and that a combination of two miRNAs can fully reverse the mechanical and cold allodynia once they have developed following a peripheral nerve injury.

EXPERIMENTAL PROCEDURES

Plasmid purification

The plasmids used to produce the lentivirus (LV) were purchased from SBI (System Biosciences, Palo Alto, CA, USA): mouse precursor scramble negative control construct (cat#MMIR-000-PA-1), mouse pre-microRNA expression construct miR-133b (cat#MMIR-133b-PA-1), mouse pre-microRNA expression construct miR-143 (cat#MMIR-143b-PA-1), and mouse pre-microRNA expression construct miR-1 (cat#MMIR-1a-1-PA-1). For the purification of our transfection-grade plasmids we used QIAfilter Plasmid Maxi kit (Qiagen, Mansfield, MD, USA, cat#12262). All plasmids express the fluorescence protein GFP which was used to check the transfection efficiency of the lentiviral vectors.

Lentivirus and animals

All the steps involved in the lentiviral (LV) vector production and usage were approved by the Institutional Biosafety Committee of New York University (NYU) Langone Medical Center and followed BSL2 containment protocols. Adult male Sprague–Dawley rats (250–400 g) were used following the guidelines approved by the NYU Langone Medical Center Institutional Animal Care and Use Committee (IACUC) and in accordance with the National Institute of Health Guide for the Care and Use of laboratory Animals (NIH Publication No. 80-23). All efforts were made to minimize the number of animals used and their suffering. DRG primary neuronal cultures were generated using lumbar DRG from adult male Sprague–Dawley rats as previously described (Castillo et al., 2011).

Cell lines

The 293TN Human Kidney Producer Cell Line (SBI, cat# LV900A-1) was used for packaging and tittering. The HEK293 cell line (ATCC, Manassas, VA, USA) was used transfection with 3′UTR-plasmids.

Lentivector packaging

LV-miR vectors (LV-miR00, LV-miR-133b, LV-miR-143, from systembio.com) were produced by co-transfecting