miR-183 cluster scales mechanical pain sensitivity by regulating basal and neuropathic pain genes

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Nociception is protective and prevents tissue damage but can also facilitate chronic pain. Whether a general principle governs these two types of pain is unknown. Here, we show that both basal mechanical and neuropathic pain are controlled by the microRNA-183 (miR-183) cluster in mice. This single cluster controls more than 80% of neuropathic pain-regulated genes and scales basal mechanical sensitivity and mechanical allodynia by regulating auxiliary voltage-gated calcium channel subunits of neuronal pain transduction in a specific, light-touch–sensitive neuronal type recruited during mechanical allodynia.

nociceptive (basal) pain serves as a protective mechanism to bring awareness of injured tissue. Pain is relayed by nociceptors in the dorsal root ganglia (DRGs) that link skin or deep tissues to the spinal cord dorsal horn. Variation in an individual’s responses to nociceptive pain (1) is partially explained by genetic variation (2). Mechanical allodynia during neuropathic pain—which involves hypersensitivity of nociceptive neurons (3) and recruitment of touch-activated low-threshold mechanoreceptive (LTMR) neurons into the nociceptive network (4)—is governed by changes in gene expression (3) susceptible to influence by microRNAs (miRNAs) (5, 6). Down-regulation of microRNA-183 (miR-183, part of miR-183/96/182 or the miR-183 cluster) in mice and control Wnt1-Cre;miRfl/fl mice for RNA sequencing (table S1). Eighteen up-regulated genes (11 of which were predicted as direct miR-183 cluster targets by TargetScan (15)) and 15 down-regulated genes were identified (Fig. 2A; fig. S6C; table S2). Of the up-regulated genes, Cacna2d2 could affect pain sensitivity, as Cacna2d2 family members encode voltage-gated calcium channel auxiliary subunits α2δ, which affects nociceptor excitability (16–18). miRNAs of Cacna2d1 and Cacna2d2 expressed in both nociceptors (94 ± 3.5% and 51 ± 8.8%, respectively) and TrkB+ nociceptor (98 ± 6% and 98 ± 5%, respectively) (Fig. 2B and fig. S6D) were both significantly increased in Wnt1-Cre;miRfl/fl DRG (Fig. 2C). Consistently, protein levels in both nociceptors and TrkB+ neurons were elevated (Fig. 2D and fig. S6D). The anticonvulsant gabapentin [1-(aminomethyl) cyclohexane acetic acid], which acts through interacting with α2δ subunits (19) and is widely used to treat neuropathic pain (20), did not lead to any behavioral changes between the genotypes in cold, heat, or pinprick sensitivity but reversed the mechanical hyperalgesia in Wnt1-Cre;miRfl/fl mice (Fig. 2E and fig. S6E).

To analyze whether the miR-183 cluster continuously determines basal sensitivity in the adult, we generated Rosa26ΔSer/ERT2+/miRΔR mice and induced deletion of the miR-183 cluster acutely by tamoxifen administration (100 mg/kg). Mechanical threshold measurements in the same animals before and 6 days after tamoxifen administration revealed sensitization similar to that observed in Wnt1-Cre;miRfl/fl mice (Fig. 2F). Tamoxifen administration reduced expression of the miR-183 cluster by about half within 6 days and consequently induced increased expression of Cacna2d1 and Cacna2d2 (Fig. 2, G and H). Thus, loss of miR-183 expression in adult rapidly promotes excess Cacna2d1/2 expression through de-repression. Combined, our results show that the miR-183 cluster continuously suppresses Cacna2d1/2 expression controlling basal mechanical sensitivity in nociceptors without apparent effects on LTMRs.

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Fig. 1. Increased basal mechanical sensitivity of nociceptors in miR-183–depleted sensory neurons. (A) Deficiency of the miR-183 cluster in Wnt1-Cre;miRfl/fl mice [miR conditional knock out (CKO)] leads to increased acute nocifensive response to mechanical, but not other, stimuli (n = 8 per genotype; **P < 0.01, Mann-Whitney test). (B) Deficiency of the miR-183 cluster in TrkBCreERT2/+;miRfl/fl mice (miR CKO) leads to no changes in acute pain sensitivity (n = 7 per genotype). (C) Deficiency of the miR-183 cluster in TH-Cre;miRfl/fl mice (miR CKO) leads to increased acute nocifensive response to mechanical, but not other, stimuli (control, n = 8; TH-Cre;miRfl/fl, n = 9; ***P < 0.01, Mann-Whitney test). (D) Increased nocifensive response of Wnt1-Cre;miRfl/fl compared with Wnt1-Cre mice in response to 1.4 g Von Frey stimuli (n = 7 or 6, respectively. *P < 0.05, Mann-Whitney test). (E) Action potential (AP) threshold reduction in the absence of the miR-183 cluster. Current protocol is to evoke the first AP in a representative trace. Wnt1-Cre;miRfl/fl (n = 27) or WT control (n = 13) DRG neurons; P = 0.0016, t test. (F) Increases of Fos+ neurons (NeuN+) in the spinal cords of miR-183 cluster–deficient mice (80 min after 10 min of running on a rotarod). The gamma isoform of protein kinase C (PKCγ) indicates lamina II. Hatched boxes outline the enlarged images below (n = 4 per group; means ± SEM; **P < 0.01, ***P < 0.001, t test; scale bar, 50 μM).

Fig. 2. Control of acute nociceptive mechanical sensitivity through a continuous miR-183-96-182–dependent tuning of auxiliary VGCC expression. (A) MA plot of M (log ratio) and A (mean average) scales to show the log2-fold changes. Red dots indicate genes up- or down-regulated more than 1.8-fold (P < 0.01) in DRGs of Wnt1-Cre;miRfl/fl mice (CKO versus WT, n = 4 or 5, respectively). (B) Cacna2d1 and Cacna2d2 expression in both nociceptors (Scn10a, arrowheads) and TrkB+ neurons (arrows, triple in situ hybridization); 4',6-diamidino-2-phenylindole (DAPI) counterstained. Scale bar, 50 μM. (C) Quantitative polymerase chain reaction (PCR) of Cacna2d1 and Cacna2d2 expression in DRGs of control and Wnt1-Cre;miRfl/fl mice (n = 6 or 6; means ± SEM; **P < 0.01, t test). (D) CACNA2D1 and CACNA2D2 immunofluorescence protein intensity levels in nociceptive and TrkB+ neurons of control and Wnt1-Cre;miRfl/fl mice (n = 3 or 3; means ± SEM; *P < 0.05, **P < 0.01, t test). (E) Relative to Wnt1-Cre mice, gabapentin reverses mechanical hypersensitivity of Wnt1-Cre;miRfl/fl mice (Wnt1-Cre/Wnt1-Cre;miRfl/fl, n = 6 or 8, **P < 0.05, Wilcoxon test; **P < 0.01, Mann-Whitney test). (F) Acute depletion of the miR-183 cluster induces mechanical hypersensitivity. Before (BL) and 6 days after tamoxifen (Tam) (Rosa26CreER/+ littermate controls versus Rosa26CreER/+;miRfl/fl mice; n = 10 or 8; *P < 0.05, Wilcoxon test; ***P < 0.001, Mann-Whitney test). (G) miR-183-96/182 expression in DRGs 6 days after tamoxifen administration. Quantitative PCR, control mice set to 1 (n = 4 or 3; means ± SEM; *P < 0.05, t test). (H) Cacna2d1 and Cacna2d2 de-repression in DRG 6 days after tamoxifen administration. Quantitative PCR, control mice set to 1 (n = 4 or 3; means ± SEM; **P < 0.01, t test).
Human CACNA2D1 and CACNA2D2 genes are predicted targets of the miR-183 cluster (fig. S7A). Profiling DRG expression in a human cohort (n = 214) revealed a significant inverse correlation between all three miRNAs and CACNA2D1 and CACNA2D2 expression (fig. S7B). The negative correlation for both CACNA2D1/2 by the miR-183 cluster in both mice and humans suggest a role of the miR-183 cluster in pain mechanisms across mammalian species.

We asked whether the miR-183 cluster also affects neuropathic pain through gene regulation. Wild-type (WT) mice in the spared-nerve injury (SNI) neuropathy model (21) at 14 days (table S1) revealed 70 up-regulated and 2 down-regulated genes compared with unlesioned controls (Fig. 3A and table S2). In Wnt1-Cre;miRfl/fl mice, more genes showed changes in expression (189 up-regulated and 35 down-regulated) (Fig. 3B and table S2). We used the STRING database to analyze functional protein association networks (22) (fig. S8, A to F). Several pain-inducing and pain-preventing genes (known from the Pain Gene Database (23) and manual interrogation) showed regulatory changes in both WT and Wnt1-Cre;miRfl/fl mice. Various up-regulated genes were candidate direct targets of miR-183 cluster regulation (genes circled with a green ring in fig. S8, A to F).

Many of the regulated genes attributed to cell signaling, cell adhesion, ubiquitination, neuropeptide modulation, and Ca2+ signaling were centered around transcription factors Atf3 and Jun in WT mice with neuropathy. In Wnt1-Cre; miRfl/fl mice with neuropathy, a larger transcriptional network—including Atf3, Jun, Cmrp1, Nfil3, Ets2, Sox11, and Cited2—passed threshold criteria. Indeed, expression of these transcription factors was also increased for WT mice with neuropathy. Furthermore, Atf3, Cmrp1, Ets2, Sox11, and Cited2 are potential direct miR-183 cluster targets (Fig. 3, C and D). Thus, in addition to directly targeting genes, the miR-183 cluster may affect the global neuropathic pain gene–regulatory network indirectly through targeting core neuropathy-induced transcription factors.

More genes become up-regulated in miR-183 cluster–deficient mice with neuropathy than in WT mice with neuropathy (189 versus 70), whereas only 18 up-regulated genes are controlled by the miR-183 cluster in normal mice. These findings suggest that neuropathy-induced genes in WT mice might be further de-repressed in Wnt1-Cre; miRfl/fl mice with neuropathy. If the miR-183 cluster regulates genes that are unrelated to genes increased during neuropathic pain in WT mice, the proportion of shared genes between the two genotypes should decrease with increasing relaxation of the fold-change criteria. Maintaining P < 0.01 but lowering fold-change criteria from 2.0-fold to 1.8-fold or 1.6-fold resulted in a disproportionate increase of genes shared between Wnt1-Cre;miRfl/fl and WT mice with neuropathy and maintenance of close to 80% shared genes regardless of fold-change threshold (Fig. 3, E and F). Consistently, we observed up-regulation of individual known pain genes (fig. S8, G and H). Thus, the miR-183 cluster suppresses expression changes of the neuropathy-regulated genes. Absent the miR183 cluster, gene expression induced during neuropathy is further elevated.

With neuropathy, the miR-183/96/182 cluster was rapidly down-regulated (fig. S9A), consistent with pain gene overexpression. Mice with neuropathy that were deficient in the miR-183 cluster in all neurons or deficient only in TrkB+ neurons, but not in mice deficient in nociceptors, showed enhanced mechanical sensitization (Fig. 4, A to C, and fig. S9, A to D). Thus, this enhancement of neuropathic pain was mediated through loss of miR-183–activity in TrkB+ neurons. The NF1 class of TrkB+ neurons are Aδ LTMRs conveying touch sensation (29). We asked if TrkB+ neurons can transduce pain. Optogenetic excitation of TrkB+ neurons in TrkBCreERT2/−, ChR2−/− mice (fig. S9E) did not result in a nocifensive response (Fig. 4D and fig. S10A). However, light activation of TrkB+ neurons reduced mechanical threshold (Fig. 4E and fig. S10B). In contrast to naïve animals, light-activation of TrkB+ neurons in animals with neuropathy produced a nocifensive response (Fig. 4F). Cacna2d1 but not Cacna2d2, increased in WT mice during neuropathy; however, the miR-183 cluster suppressed both Cacna2d1 and Cacna2d2 expression (fig. 4G and fig. S10C), and gabapentin completely reversed nocifensive behavior in both neuropathic WT and Wnt1-Cre;miRfl/fl mice (Fig. 4H and fig. S10, D and E). Painlike behavior initiated by TrkB+ neurons during neuropathy requires auxiliary a62 subunits, because gabapentin completely prevented nocifensive responses of optogenetically excited TrkB+ neurons in mice with neuropathy (Fig. 4I and fig. S10F). Thus, during neuropathic pain, the miR-183 cluster targets the majority of pain-regulated genes and attenuates sensitization in the most sensitive hairy skin mechanoreceptors. The TrkB+ neurons (22), which contribute to mechanical allodynia during painful neuropathies. However, although Cacna2d2/2 de-repression is critical once TrkB+ neurons are recruited into the nociceptive network in mice with neuropathy, sensitization was not observed after Cacna2d1/2 de-repression in TrkB+ neurons of uninjured miR-183 cluster–deficient animals. Cacna2d1/2 de-repression in these neurons is therefore, by itself, insufficient to cause mechanical hypersensitivity.

Our results show that the miR-183 cluster continuously prevents elevation of basal mechanical sensitivity in nociceptors and enhancement of mechanical allodynia in TrkB+ LTMRs.
Neuropathies, TrkB+ neurons may be equally or more important. We propose a unifying concept for scaling mechanical sensitivity through expression of the miR-183 cluster. Thus, somatic mechanosensitivity is a dynamic system able to respond and adapt to various intensities of stimulation.

Ongoing activity of TrkB+ neurons also potentiates mechanical transduction from nociceptors in normal mice. Nociceptors have been a focus for development of analgesic drugs for painful neuropathies, TrkB+ neurons may be equally or more

**Fig. 4. Pain signaling through TrkB+ LTMRs recruited by neuropathy is suppressed by the miR-183 cluster.** (A) Enhanced SNI-induced mechanical allodynia in Wnt1-Cre;miRfl/fl (n = 8 or 8; BL, base line; *P < 0.05, **P < 0.01, Mann-Whitney test). (B) Enhanced SNI-induced mechanical allodynia in TrkBCreERT2+/−;miRfl/fl mice (n = 7 or 7; *P < 0.05; **P < 0.01, Mann-Whitney test). (C) No effect on SNI-induced mechanical allodynia in TH-Cre;miRfl/fl mice (n = 8 or 9; *P < 0.01, Mann-Whitney test). (D) No response by optogenetic excitation of TrkB+ LTMRS increases sensitivity to mechanical stimuli (light + von Frey) (n = 6; one stimuli train; *P < 0.05, Wilcoxon test). (E) Excitation of TrkB+ neurons produces pain after SNI. Optogenetic activation (58 mW) of TrkB+ LTMRS before (BL) and after SNI in littermate controls and TrkBCreERT2+/−;ChR2+/− mice (n = 6 or 6). (G) Quantitative PCR of Ca2+1 and Ca2+2 expression in wild-type and Wnt1-Cre;miRfl/fl mice after SNI (n = 3 or 3; means ± SEM; *P < 0.05; **P < 0.01, t test). (H) Reversal of neuropathic allodynia by gabapentin in both Wnt1-Cre;miRfl/fl and Wnt1-Cre mice (n = 6 or 6, respectively, *P < 0.05 **P < 0.01, Mann-Whitney test; *P < 0.05, Wilcoxon test). (I) Reversal by gabapentin of nociceptive pain-like behavior through optogenetic excitation (58 mW) of TrkB+ neurons (TrkBCreERT2+/−;ChR2+/−) and littermate controls, n = 6 or 6.

**References and Notes**


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MicroRNAs in functional and dysfunctional pain
Pain serves the useful purpose of alerting us to danger. Chronic pain, however, can arise from dysfunctional responses. Peng et al. found that a cluster of microRNAs regulates the gene networks behind both physiological and dysfunctional pain (see the Perspective by Cassels and Barde). The recruitment of genes that regulate a subset of the light-touch mechanoreceptors found in hairy skin was critical to the generation of dysfunctional pain. Science, this issue p. 1168; see also p. 1124