Experimental Neurology 283 (2016) 276–286

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Research Paper

Increased miR-132-3p expression is associated with chronic neuropathic pain

M. Leinders [a,b], N. Üçeyler [a], R.A. Pritchard [b], C. Sommer [a], L.S. Sorkin [b,*]

[a] Department of Neurology, University of Würzburg, Würzburg, Germany
[b] Department of Anesthesiology, University of California, San Diego, La Jolla, CA 92093, USA

ARTICLE INFO

Article history:
Received 22 February 2016
Received in revised form 21 June 2016
Accepted 22 June 2016
Available online 24 June 2016

Keywords:
Neuropathic pain
miR-132
polyneuropathy
SNI
PEAP
neuroimmune

ABSTRACT

Alterations in the neuro-immune balance play a major role in the pathophysiology of chronic neuropathic pain. MicroRNAs (miRNA) can regulate both immune and neuronal processes and may function as master switches in chronic pain development and maintenance. We set out to analyze the role of miR-132-3p, first in patients with peripheral neuropathies and second in an animal model of neuropathic pain. We initially determined miR-132-3p expression by measuring its levels in white blood cells (WBC) of 30 patients and 30 healthy controls and next in sural nerve biopsies of 81 patients with painful or painless inflammatory or non-inflammatory neuropathies based on clinical diagnosis. We found a 2.6 fold increase in miR-132-3p expression in WBC of neuropathy patients compared to healthy controls ($p < 0.001$). MiR-132-3p expression was also slightly up-regulated in sural nerve biopsies from neuropathy patients suffering from neuropathic pain compared to those without pain (1.2 fold; $p < 0.001$).

These promising findings were investigated further in an animal model of neuropathic pain, the spared nerve injury model (SNI). For this purpose miR-132-3p expression levels were measured in dorsal root ganglia and spinal cord of rats. Subsequently, miR-132-3p expression was pharmacologically modulated with miRNA antagonists or mimetics, and evoked pain and pain aversion were assessed.

Spinal miR-132-3p levels were highest 10 days after SNI, a time when persistent allodynia was established ($p < 0.05$). Spinal administration of miR-132-3p antagonists via intrathecal (i.t.) catheters dose dependently reversed mechanical allodyina ($p < 0.001$) and eliminated pain behavior in the place escape avoidance paradigm ($p < 0.001$). Intrathecal administration of miR-132-3p mimetic dose-dependently induced pain behavior in naïve rats ($p < 0.001$). Taken together these results indicate a pro-nociceptive effect of miR-132-3p in chronic neuropathic pain.

1. Introduction

Neuropathic pain is characteristically severe and persistent and may greatly impair health related quality of life by additionally inducing anxiety, depression, and cognitive impairment (Breivik et al., 2006). There is ample evidence for a potential role of the immune system and particularly of pro- and anti-inflammatory mediators in the pathophysiology of neuropathic pain (Kuner, 2010; McMahon and Malcangio, 2009). Peripheral neuropathies of the same etiology can either be painful or painless (Üçeyler et al., 2007). The mechanism for this discrepancy is unknown.

In recent years, non-coding RNAs have been studied in normal cellular functioning as well as in pathological processes (Huttenhofer and Schattner, 2006; Mattick, 2004). Micro-RNAs (miRNAs) are a family of non-coding RNAs that post-transcriptionally regulate gene expression by inhibiting mRNA translation or inhibiting mRNA and protein degradation (Mattick, 2004). Various diseases, including neuropathic pain

Abbreviation: AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; ANCA, *anti*-neutrophil cytoplasmic antibody; BDNF, brain-derived neurotrophic factor; CIAP, chronic idiopathic axonal polyneuropathy; CIDP, chronic inflammatory demyelinating neuropathy; CCI, chronic constriction injury; CREB, cAMP response element-binding protein; DRG, dorsal root ganglia; EDTA, ethylene-diamine-tetraacetic-acid; ENA, *anti*-nuclear antigen; GCPS, graded chronic pain scale; GFAP, glial fibrillary acidic protein; GLT, 1 glutamate transporter; GluA, AMPA receptor subunit; H&E, Hematoxylin and eosin stain; HPLC, high performance liquid chromatography; Iba1, ionized calcium-binding adapter molecule 1; i.t., intrathecal; LNA, locked nucleic acid; LTP, long-term potentiation; miRNA, microRNA; MOPS, 3-(*N*-morpholino)propanesulfonic acid; NCV, motor nerve conduction velocity; NRS, numeric rating scale; NPSI, neuropathic pain symptom inventory; PEAP, place escape avoidance paradigm; PIAN, progressive idiopathic axonal neuropathy; PNP, polyneuropathy; RNA, ribonucleic acid; Scr, scrambled oligonucleotide; SDS, sodium dodecyl sulfate; SNI, spared nerve injury; WBC, white blood cells.

* Corresponding author at: 9500 Gilman Drive, La Jolla, CA 92093-0818, United States.
E-mail address: lsorkin@ucsd.edu (L.S. Sorkin).

http://dx.doi.org/10.1016/j.expneurol.2016.06.025
0014-4886/

disorders, appear to possess unique miRNA expression signatures. Recent reports on modulation of miRNA function in both neuronal and immune processes predict the therapeutic potential of manipulating miRNAs in diseases affecting the immune system and the brain (O'Connor et al., 2012; Soreq and Wolf, 2011). miRNAs that communicate between the nervous and immune system have been termed "neurimmiRs" and primarily target transcription factors or other regulatory genes, which enable simultaneous cross-communication between neural and immune compartments (Soreq and Wolf, 2011). Thus, miRNAs possibly control cellular pathways in multiple systems and act as "master-switches" (Soreq and Wolf, 2011).

Aberrant expression of several miRNAs has been reported throughout many peripheral and central nervous system loci associated with pain perception (Aldrich et al., 2009; Bai et al., 2007; Imai et al., 2011; Kusuda et al., 2011; von Schack et al., 2011). First reports describing characteristic miRNA expression profiles in blood or cerebrospinal fluid of patients with distinct pain conditions are starting to emerge (Andersen et al., 2016; Beyer et al., 2015; Bjersing et al., 2013; Orlova et al., 2011), however evidence linking specific miRNA expression profiles to specific pain disorders is still insufficient.

miR-132 is abundantly expressed in the brain and is emerging as a regulator of cognition, neuronal plasticity, and memory. It can regulate synapse structure and function (Bredy et al., 2011; Miller et al., 2012; Schratt, 2009; Soreq and Wolf, 2011). Hippocampal miR-132 mediates stress-induced cognitive deficits through suppression of acetylcholinesterase (Haramati et al., 2011) and miR-132 has recently been implicated in neuropathic pain after chronic constriction injury (CCI) (Arai et al., 2013). Similarly, spinal cord miR-132 is now proposed as a mediator of neuropathic pain following spared nerve injury (SNI) (Zhang et al., 2015). However, direct links between pain and miR-132 expression levels in human and/or animal models of neuropathic pain still remain elusive.

The current studies evaluated blood and sural nerve miR-132-3p, a splice variant of miR-132, expression in patients suffering from chronic neuropathic pain accompanying peripheral neuropathy and analyzed the role of miR-132-3p in pain behavior in an animal model of neuropathic pain.

2. Materials and Methods

2.1. Subjects

2.1.1. Patient assessment and diagnostic classification

Patients with neuropathies of different etiologies were recruited at the Department of Neurology, University of Würzburg between 2014 and 2015, where they underwent diagnostic work-up, including sural nerve biopsy. The study was approved by the Würzburg Medical Faculty Ethics Committee and written informed consent was obtained from every study participant before recruitment. The diagnosis of neuropathy was based on characteristic symptoms and signs in the neurological examination and typical findings in the electrophysiological assessment with standard nerve conduction studies in motor and sensory nerves of the upper and lower limbs (Kimura, 2001). Motor nerve conduction velocity (NCV) and evoked compound muscle action potential of the median, tibial and peroneal nerves were measured orthodromically. Sensory conduction velocity and amplitude of the nerve action potential were measured antidromically in the median and sural nerves. Skin temperature in both upper and lower extremities was controlled (> 32 °C) during the examinations. For differential diagnosis detailed laboratory studies included: glucose metabolism (HbA1c, oral glucose tolerance test), whole blood and differential cell counts, erythrocyte sedimentation rate, *C*-reactive protein, serum electrolytes, monoclonal immunoglobulins, vitamin levels (B6, B12), folic acid, renal and liver function tests, thyroid function tests, *anti*-nuclear antigen (ENA), *anti*-neutrophil cytoplasmic autoantibody (ANCA), rheumatoid factor, serology of borreliosis, immunofixation, and serum electrophoresis. In addition, all patients underwent a diagnostic lumbar puncture and cerebrospinal fluid was checked for pathological cell counts and protein levels. Diagnostic subgroups and definition of neuropathies are summarized in supplemental patient diagnostic criteria.

All patients were specifically asked for details regarding symptoms and signs that may have been associated with other sources of pain, any patient reporting other sources of pain or ongoing infection was excluded. Neuropathies were classified as painful if the patients reported pain with an intensity of 3 or more on a numeric rating scale (NRS) ranging from 0 to 10 (0 meaning "no pain" and 10 "worst pain imaginable"), as previously reported (Üçeyler et al., 2015; Üçeyler et al., 2007). The Graded Chronic Pain Scale (GCPS) (Von Korff et al., 1992) for 4 week recall and the Neuropathic Pain Symptom Inventory (NPSI) (Bouhassira et al., 2004) for the last 24 h recall were also used to assess pain. The control group consisted of healthy and age-and sex-matched (to the neuropathy patients shown in Fig. 1A) volunteers without infectious disease or pain at study inclusion.

2.1.2. Blood withdrawal for miRNA expression analysis

To reduce circadian variations, venous blood was collected from 30/81 patients and 30 healthy controls between 8:00 and 9:00 AM. For quantitative real-time PCR (RT-PCR), 9 ml whole blood was withdrawn in EDTA-containing tubes and the total white blood cell fraction (WBC) isolated. Isolated WBCs were re-suspended in RNA-cell protective reagent (QIAGEN, Hilden, Germany) and stored at −80 °C until further processing.

2.1.3. Sural nerve biopsy

Diagnostic sural nerve biopsy was performed in 67/81 patients at the Department of Neurosurgery, University of Würzburg (Dyck et al., 2005). For miRNA expression analysis, approximately 4 mm of the biopsy specimen was separated and stored in RNA-later overnight at 4 °C; on the following day RNA-later was removed and the specimen was frozen at −80 °C.

2.1.4. PCR amplification of miRNA

Peripheral nerve specimens as the basis of major pathology were obtained from patients and rats. Isolation of miRNAs was performed on all samples (WBCs, nerve, DRGs and spinal cord) using the miRNEASY kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. For the generation of miRNA-specific first strand cDNA, 5 ng of total RNA was reverse transcribed using the Universal cDNA Synthesis kit II (Exiqon, Vedbaek, Denmark) following manufacturer's recommendations. For each reaction, 4 μl of diluted (1:80) cDNA was PCR amplified applying the corresponding miRNA and reference primer sets, using the miCURY LNA™ Universal microRNA PCR (Exiqon). The expression levels of miR-132-3p (5′-3′ UAACAGUCUACAGCCAUGGUCG, MIMAT0000426) and its splice variant miR-132-5p (5′-3′ ACCGUGGCUUUCGAUUGUUACU, MIMAT0004594) were normalized to the expression of endogenous 5 s RNA (5 s RNA, V00589). For individual target normalization, we tested different endogenous controls (U6, snord48, snord44, and 5sRNA) of which 5sRNA (housekeeping gene) was the most stable and thus, was used for both human and rat tissue. Each miRNA was amplified in triplicate and threshold cycle (Ct) values were obtained. Fold changes in miRNA expression among groups were calculated using interplate calibrators (a standard sample that was run on each PCR plate) by means of the delta-delta Ct method.

2.1.5. miRNA target validation

We set out to analyze the role of AMPA-receptor subunit GluA1, in an animal model of neuropathic pain. We performed a comprehensive target prediction analysis of miR-132-3p by employing four databases: TargetScan (Friedman et al., 2009), microRNA.org (Betel et al., 2008), miRTarBase (Hsu et al., 2011), and DIANAmicroT (Paraskevopoulou et al., 2013). GluA1 was identified as a potential downstream target by at least two of the four prediction algorithms. To further narrow down