Journal of the Neurological Sciences 380 (2017) 242–249

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

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

Aberrant microRNA expression in patients with painful peripheral neuropathies

Leinders Mathias *, Üçeyler Nurcan, Thomann Anna, Sommer Claudia

Department of Neurology, University of Würzburg, Germany

ARTICLE INFO

Article history:
Received 18 January 2017
Received in revised form 25 June 2017
Accepted 26 July 2017
Available online 27 July 2017

Keywords:
miRNA
miR-21
miR-146a
miR-155
Pain
Peripheral neuropathy
Sural nerve biopsy
Skin biopsy

ABSTRACT

Changes in the neuro-immune balance play a major role in the induction and maintenance of neuropathic pain. We recently reported pathophysiologically relevant alterations in skin and sural nerve cytokine expression in peripheral neuropathies of different etiologies. Immune processes and cytokine expression are under tight control of microRNAs (miRNAs). To identify potential master switches in the neuro-immune balance, we aimed at characterizing inflammation-regulating miRNA profiles in patients with peripheral neuropathies. In an unselected patient cohort with polyneuropathies of different etiologies seen at our neuromuscular center between 2014 and 2015, we determined the systemic and local relative expression of miR-21-5p, miR-146a, and miR-155. In white blood cells we found higher miR-21 ($p < 0.001$) and miR-146a ($p < 0.001$) expression and lower miR-155 ($p < 0.001$) expression when compared to healthy controls. In sural nerve, miR-21 ($p < 0.02$) was increased in painful compared to painless neuropathies. In painful neuropathies, skin biopsies from the lower leg had reduced miR-146a ($p < 0.001$) and miR-155 ($p < 0.001$) expression compared to the thigh. Thus, peripheral neuropathies are associated with aberrant miRNA expression in white blood cells, sural nerve, and skin. These miRNA patterns may help to identify factors that determine the painfulness of peripheral neuropathies and lead to druggable targets.

1. Introduction

Chronic pain accompanying peripheral neuropathies is a major health problem. It is still unknown why peripheral neuropathies with apparently similar etiology and pathology can be either painful or painless. Prominent Aδ- and C-fiber involvement, such as in amyloid neuropathies, is commonly regarded as a hallmark of painful neuropathies, however, neuropathies with non-selective fiber loss and those with apparent large fiber loss can be equally painful [1]. Known factors involved in the pathophysiology of pain are cytokine-mediated neuro-immune interactions [2]. In particular, an immune dysbalance with increased expression of pro-inflammatory cytokines or a reduction of anti-inflammatory cytokines in blood, cerebrospinal fluid, and/or nerve tissue may promote pain and hyperalgesia [3–5]. However, due to their pleiotropic and redundant activity, interfering with cytokine function may not result in the desired analgesia. Therefore upstream "master switches" have been implied as potential targets of more efficient analgesics.

microRNAs (miRNA) are small non-coding RNA molecules that possess regulatory functions in multiple cellular systems [6], enabling cross-communication between cellular processes [7]. Recent studies in preclinical neuropathic pain models indicate unique miRNA expression signatures that are characteristic for nerve injury induced pain behavior [8–13]. In addition, first reports hint towards aberrant miRNA expression in blood of patients with distinct chronic pain conditions [12,14–18]. We characterized systemic and local expression profiles of miR-21-5p, miR-146a, and miR-155 in an unselected cohort of patients with polyneuropathies of different etiologies seen at our neuromuscular center within one year. Expression of these miRNAs was found in neuronal and various immune cell types and shown to regulate multiple pathways in inflammation and pain [19–21] and might thus be involved in the pathophysiology of painful conditions, as reviewed before [22,23]. We thus specifically choose these miRNAs, since their expression is also regulated in the same direction in pre-clinical and clinical painful or inflammatory conditions. miR-21 is associated with pain in experimental nerve injury models [23]. It was shown to promote neurite outgrowth [24] and low expression was associated with decreased demyelination in autoimmune diseases [25]. miR-146a, a regulator of the cell death inducers caspase-3 and Fas, was decreased after spinal cord injury [26] and associated with painful conditions in mice [27]. In addition, miR-146 was associated with inflammatory degeneration [28], nerve regeneration after axonal injury [29], and distal axonal outgrowth [30]. miR-155 is ubiquitously expressed in many cell types and tissues including the central nervous system (CNS) [31]. It is critically involved in the

* Corresponding author at: Department of Neurology, University of Würzburg, Josef-Schneider-Straße 11, 97080 Würzburg, Germany.
E-mail address: leinders_m@ukw.de (M. Leinders).

http://dx.doi.org/10.1016/j.jns.2017.07.041
0022-510X/

regulation of inflammation-associated diseases [32], and was shown to be increased in synovial fibroblasts in patients with rheumatoid arthritis [33] and the prefrontal cortex of mice with inflammatory pain [34]. Furthermore, miR-155 was found to be up-regulated in active white matter lesions of patients with multiple sclerosis [35], whereas the absence of miR-155 induced resistance to experimental autoimmune encephalomyelitis in mice [36].

We have previously reported that pain is associated with an immune dysbalance, favoring the action of pro-inflammatory cytokines and their algesic effects on nociceptive neurons [37]. All three miRNA candidates investigated in our study possess master-regulatory functions throughout different stages of inflammation. Thus, by balancing for example the expression of pro-inflammatory and anti-inflammatory cytokines, miRNAs might directly contribute to pain generation. We thus characterized the expression of these miRNA candidates in white blood cells, sural nerve, and skin punch biopsy specimens in biomaterial of our patient cohort as available. We aimed at identifying the profiles of these miRNAs to better understand potential factors that differentiate painful from painless neuropathies and to potentially pave the way for new druggable targets.

2. Materials and methods

2.1. Patient assessment and diagnostic classification

We included all available data and biomaterial collected within one year (2014–2015) from patients with neuropathies of different etiologies that were seen at the Department of Neurology, University of Würzburg for diagnostic work-up. This included blood withdrawal, sural nerve, and skin biopsy as needed. The study was approved by the Würzburg Medical Faculty Ethics Committee and written informed consent was obtained. The diagnosis of neuropathy was based on characteristic symptoms and signs in the neurological examination and typical findings in the neurophysiological studies. For differential diagnosis, detailed laboratory screening was performed in all patients as described before [4]. Moreover, all patients underwent complete electrophysiological assessment with standard nerve conduction studies in motor and sensory nerves of the upper and lower limbs, as part of the routine work-up as needed [38]. Table 1 summarizes the diagnostic criteria applied. Neuropathies were classified as painful if the patients reported neuropathic 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”). For standardized pain assessment the Graded Chronic Pain Scale (GCPS) [39] with 4 weeks recall and the Neuropathic Pain Symptom Inventory (NPSI) [40,41] with 24 h recall were used. The control group consisted of healthy and age-matched volunteers without a neurologic disorder, infectious disease or pain at study inclusion (see Table 2).

2.2. Blood withdrawal

Venous blood withdrawn in EDTA-containing tubes between 8:00 and 9:00 AM after over-night fasting was used for the extraction of the total white blood cell fraction (WBC). WBCs were re-suspended in RNA-cell protective reagent (QIAGEN, Hilden, Germany) and stored at −80 °C until further processing. Additionally, we collected blood samples from healthy, age- and gender-matched volunteers (see below) without neurologic disorders, infectious diseases or pain at study inclusion.

2.3. Sural nerve biopsy

Diagnostic sural nerve biopsy was performed if the etiology of the neuropathy remained unclear after thorough clinical, laboratory, and electrophysiological assessment following a standard procedure at the Department of Neurosurgery, University of Würzburg [42]. For study purposes, 4 mm of the biopsy specimen was separated and stored in RNA-later (Qiagen, Hilden, Germany) over night at +4 °C; on the following day RNA-later was removed and the specimen was frozen at −80 °C before further processing.

Table 1
Overview of the diagnostic criteria.

Diagnosis	Additional information	Reference
Chronic inflammatory demyelinating neuropathy (CIDP)	Based on the INCAT criteria (inflammatory neuropathy cause and treatment)	[76]
Chronic idiopathic axonal polyneuropathy (CIAP)	Sensory-motor, neurophysiologically axonal neuropathy, slow onset and progress; axonal histology without inflammation; normal CSF, no effect of steroid treatment	[77]
Progressive idiopathic axonal neuropathy (PIAN)	Acute or subacute presentation with slow progression of sensory-motor symptoms; axonal histology with inflammation and neurophysiology; increased CSF and positive response to steroid treatment	[77]
Hereditary neuropathy	According to a combination of positive family history, genetic and neurophysiological data	
Neuropathy in systemic vasculitic and non-systemic vasculitis of the peripheral nervous system (NSVN)		[78]
Other origin	Cases of definite other etiology, i.e. neuropathy due to amyloidosis, paraneoplastic neuropathy or vitamin deficiency	
Unknown etiology	Cases in whom a definitive diagnosis as detailed as above was not possible at the time point of examination	
Idiopathic small fiber neuropathy (SFN)		[79]
Multifocal acquired demyelinating sensory and motor neuropathy (MADSAM)		[80]

Abbreviations: *CIAP* chronic idiopathic axonal polyneuropathy, *CIDP* chronic inflammatory demyelinating polyneuropathy, *CSF* cerebrospinal fluid, INCAT Inflammatory Neuropathy Cause and Treatment Group, *MADSAM* multifocal acquired demyelinating sensory and motor neuropathy, *NSVN* non- systemic vasculitic neuropathy, *PIAN* progressive idiopathic axonal neuropathy, SFN small fiber neuropathy.

2.4. Skin punch biopsy

Two 5-mm skin punch biopsies (lateral lower leg and upper thigh) were obtained from patients under local anesthesia. One third of the skin specimen that remained from routine work-up was used for qRT-PCR and was stored in RNA-later over night at +4 °C; on the following day RNA-later was removed and the specimen was frozen at −80 °C before further processing.

2.5. MiRNA expression analysis

For the generation of miRNA-specific first strand cDNA 5 ng of total RNA extracted from WBC, nerve, and skin was reverse transcribed

Table 2
Summarized healthy control demographics.

Demographics of healthy controls for systemic miRNA expression (N = 30)	
Male, female	26, 4
Male median age (range), y	60 (38–69)
Female median age (range), y	49 (41–53)
Median disease duration (range), y	NA
Current pain (at study inclusion)	0

Abbreviations: N: Number; y: years.