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Topical review

MicroRNAs as new players in the pain game

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1. Introduction

MicroRNAs (miRNAs, miRs) and small interfering RNAs (siRNAs) form a class of small noncoding RNAs of 19–25 nucleotides that mediate RNA interference (RNAi) by post-transcriptionally modulating gene expression. While siRNAs have already been addressed in many papers, including pain-related RNAi studies (reviewed in [32]), naturally occurring miRNAs are a just-upcoming field of research. MicroRNAs are involved in several developmental, physiological, and pathophysiological processes where they alter and modulate the expression of different proteins [5]. They silence genes either by initiating the cleavage of their respective target mRNA or by inhibiting gene translation after complete or only partial binding to their target sequence, respectively. Nearly 500 human miRNAs have been described so far [8]. Each of them may target many different genes and, vice versa, many genes are regulated not only by one but by several different miRNAs [12,21]. Owing to their role in the regulation of gene expression, miRNAs are assigned by some authors to epigenetics [24]. With the classical epigenetic mechanisms, DNA methylation and histone acetylation [10], miRNAs are key parts of an apparatus of regulatory mechanisms of gene expression.

2. Formation and function of miRNAs

Other than siRNAs, often originating from exogenous sources such as viruses, miRNAs are coded by specific host genes. During the biosynthesis of miRNAs, the miRNA genes are initially transcribed as long-chain pri-miRNAs that are then further processed into ~70 nucleotides-long precursors (pre-miRNAs) by the RNAIII endonuclease "Drosha" [22]. This also differs from siRNAs as Drosha does not process siRNAs lacking the above precursors. Pre-miRNAs are exported from the nucleus to the cytoplasm by the intracellular transport protein exportin 5 and finally converted into mature miRNAs by another RNAIII endonuclease called "Dicer" [6]. The miRNA guide strand is loaded into the RNA-induced silencing complex, which leads to the conversion of miRNA double strands into single strands with a sequence specificity for the respective target mRNA. Complete binding of the miRNA leads to cleavage of the mRNA, whereas incomplete binding induces translational repression [5] (Fig. 1).

miRNAs are receiving increasing interest as diagnostic markers or therapeutic targets in various pathophysiological conditions, especially in cancer and cardiovascular diseases. For example, several miRNAs can specifically act as tumor suppressors (eg, let-7 or miR-34a), while others are inhibitors of apoptosis (eg, miR-21, miR-155, or miR-214), and in this way, regulate tumor progression [11,14]. miRNAs and their targets are also involved in the etiology and progression of cardiovascular diseases [18]. One impressive example is a study by Thum et al. [33], who showed that miRNA-21 is selectively increased in fibroblasts of the failing heart. Silencing of miRNA-21 by a specific inhibitor (antagomir) suggested a therapeutic potential of such approaches by improving cardiac dysfunction.

As miRNAs are also involved in inflammatory and neurodegenerative diseases [16,30], a role in pain was recognized a few years ago. By their key role in the regulation of gene expression, they are likely to be involved in pain perception and chronification and might become novel targets for analgesic therapies that will act via regulating the expression of pain-relevant genes. In this review, we summarize the current knowledge about the role of miRNAs in pain.

3. miRNAs in the nervous system

miRNAs play an important role in neurogenesis, neuron survival, dendritic outgrowth, and spine formation (reviewed in [20]). Accordingly, a complete knockdown of Dicer in different brain regions leads to apoptosis and neuronal degeneration of neurons in mice [9,29]. Moreover, dysregulation of miRNA-controlled proteins in the nervous system was associated with neurodegenerative diseases such as Alzheimer's disease, Tourette syndrome, or Parkinson disease [16,20]. Less known is the regulation and function of microRNAs in mature neurons. It has been suggested that they might contribute to mechanisms of synaptic plasticity [31]. This is supported by an observation in mice showing that a loss of miRNAs in mature neurons of the forebrain, following inducible knockout of Dicer, leads to enhanced learning and memory associated with an increased expression of plasticity-related genes [19].

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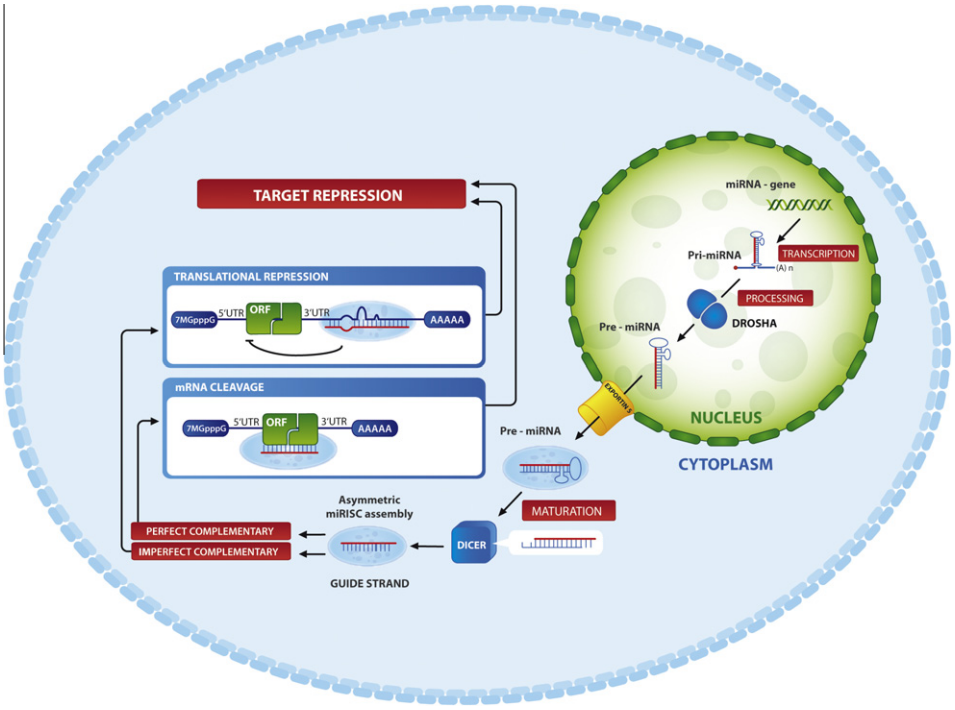

Fig. 1. MicroRNA (miRNA) biogenesis. Primary mRNA (pri-miRNA) is transcribed from miRNA genes and then processed to pre-miRNA by the endonuclease Drosha. After transport to the cytoplasm by exportin 5 a further processing step by Dicer generates mature miRNA. The guide strand is incorporated into RNA-induced silencing complex (RISC) generating single strands that bind to their target mRNA. miRNAs that bind to mRNA targets with imperfect complementarity bind preferentially to the 3′ untranslated regions (3′ UTRs) of the target mRNA genes and block target gene expression at the level of protein translation. Perfect complementary miRNAs generally bind to sites that are found in the coding sequence or open reading frame (ORF) of the mRNA target and induce target-mRNA cleavage. Both mechanisms lead to the repression of target gene expression.

4. miRNAs in pain processing

Altered protein expression is characteristic for chronic pain and contributes to the development of long-term hyperexcitability of nociceptive neurons in the periphery and the central nervous system (peripheral and central sensitization, respectively) [17]. The modifications include expressional changes of signaling molecules, transmitters, ion channels, or structural proteins. As miRNAs are part of ubiquitous mechanisms of gene expression, they are likely to contribute to these changes. First studies investigating the regulation and function of miRNAs in different models of pathophysiological pain support this hypothesis. In addition, particular miRNAs (eg, microRNAs-133 and let-7) have been suggested to play a role in the development of tolerance to morphine antinociception [15,28]. Specifically, morphine was found to upregulate a let-7 family mouse miRNA that repressed μ-opioid receptors. Inhibiting let-7 partially attenuated the tolerance to the antinociceptive opioid effects [15].

4.1. miRNAs in acute and inflammatory nociception

So far, only a few studies have addressed the role of miRNAs in experimental inflammatory pain. One of them was based on a conditional knockout of Dicer in $Na_v1.8$ neurons, which led to a loss or downregulation of more than 60 known or new miRNAs in these damage-sensing neurons [36]. This was associated with the differential expression and regulation of ubiquitously expressed or nociceptor-specific transcripts. Interestingly, although deletion of Dicer resulted in the loss of all mature miRNAs, some sensory neuron-specific transcripts were downregulated, among them CamKII and $Na_v1.8$ itself. Although unexpected, this nevertheless agreed with the significant inhibition of nociceptive responses in the formalin test and carrageenan- or complete Freund's adjuvant (CFA)-induced paw inflammation. In the formalin test, this effect was associated with a reduction of formalin-induced c-FOS-positive neurons in the spinal cord, showing a reduced nociceptive signaling into the central nervous system. Furthermore, isolated sensory neurons from wild-type $Na_v1.8$ mice had an elevated excitability to stimulation with inflammatory mediators, whereas neurons from Dicer-null mutants remained unchanged. In contrast, the acute nociceptive behavior in response to electrical, mechanical, and thermal stimuli was normal in these mice. This showed that pain transmission to the central nervous system by A- or C-fiber-connected nociceptors was overall functional despite the absence of Dicer. In addition, the neuropathic pain behavior after sciatic nerve ligation was unaffected in Dicer-depleted mice, possibly due to an only minor role of $Na_v1.8$ in neuropathic pain [1].

In an inflammatory rat model of CFA-induced muscle pain, after injection of CFA in the masseter muscle, several microRNAs (miR-10a, -29a, -98, -99a, -124a, -134, and -183) were significantly but differentially downregulated in neurons of the ipsilateral trigeminal ganglion [4]. The authors suggested that this decrease in miRNAs allows for an upregulation of "pain-related" proteins and by that, facilitated the development of inflammation and allodynia.

In a murine peritonitis model of self-limiting acute inflammation, miRNAs (miR-21, miR-146b, miR-208a, miR-203, miR-142, miR-302d, and miR-219) could be counter-regulated by administration of resolvin D1, an antiinflammatory lipid mediator [26]. These effects were associated with modifications in the extent, duration, and resolution of the inflammatory response. Since resolvins E1 and D1 have been described as important mediators in the resolution of inflammation and inhibited the nociceptive behavior in different models of inflammatory pain in mice [35], it seems possible that these antiinflammatory and antihyperalgesic effects had been mediated at least partially by regulation of specific miRNAs.

4.2. miRNA in neuropathic pain

In a rat model of traumatic spinal cord contusion injury (SCI), the expression of more than 250 mature miRNAs was regulated at 4 hours, 1 day, or 7 days after surgery [23]. Subsequent bioinformatic analysis identified several regulated target genes known to play important roles in the pathogenesis of SCI by altering inflammatory processes, oxidation, or apoptosis in neurons. Interestingly, some miRNAs regulating antiinflammatory and antiapoptotic proteins were upregulated, while some miRNAs controlling the expression of proinflammatory and proapoptotic proteins were downregulated. This suggested that miRNAs contribute to the pathogenesis of SCI. However, the in vivo relevance of miRNAs in SCI remains to be determined.

In a rat model of peripheral nerve injury (L5 spinal nerve ligation [SNL]), miRNAs of the 183 family (miR-96, miR-182, miR-183), abundantly expressed in tissues and organs involved in sensory perception, were found in high quantities in large myelinated and small nonmyelinated neurons in the dorsal root ganglia (DRG) and significantly reduced in the ipsilateral DRG neurons after SNL [2]. It has been suggested that the miRNA-183 family in the DRG is responsible for regulation of several genes important for the unique function of nociceptive and mechanosensitive primary afferent neurons. Since the regulation of miR-183 has also been shown in the above model of inflammatory muscle pain [4], its general role in several different classes of chronic pain seems reasonable. Besides expressional changes, the authors hypothesized that miRNA activity is altered after nerve injury as several miRNAs were translocated to the cellular periphery after SNL and colocalized with a marker for stress granules (TIA-1), which assemble after nerve injury. However, the detailed functional coherence of this observation has not been clarified. Bioinformatic analysis showed a possible regulation of several pain-relevant miRNA target genes including sodium channels, neurotrophic factors, kinases, and neuropeptides [2]. A further study showed that the peripheral myelin protein 22 was decreased after sciatic nerve crush injury, which might be functionally related to an upregulation of miR-29, indicating that this miRNA is involved in the response to nerve injury and neuronal demyelination [34]. Peripheral myelin protein 22 is an integral membrane protein that is a major component of myelin in the peripheral nervous system. Mutations of this gene are causes of hereditary moto-sensory neuropathy syndromes [25].

4.3. miRNA in painful diseases

Alterations of miRNA expression have been described in patients with rheumatoid arthritis, osteoarthritis, systemic lupus erythematosus, and the Sjögren syndrome [3,13]. Differences in miRNA expression profiles have also been shown between the endometrium of women with and of those without painful endometriosis in the early secretory phase [7]. Similarly, miRNA expression changes have been reported from patients with chronic bladder pain syndrome. It has been suggested that an increased expression of distinct miRNAs (miR-449b and -500) in the bladder tissue of these patients may induce a downregulation of neurokinin receptors. This mechanism might also be important in other painful diseases related to neurokinin receptor modulation, such as cancer or pancreatitis [27].

These findings show that miRNAs play an important role in the regulation of gene expression in several human diseases associated with pain. They might therefore constitute novel biomarkers for the respective disease or serve as novel targets for specific miRNA antagonists. However, this hypothesis must be proven in the future by human studies with antagomirs, which will show whether or not inhibitors of specific miRNAs are effective as new types of analgesics.

5. Conclusion

miRNAs have been discovered only recently and gain increasing interest as research tools, biomarkers, and potential new drug targets for several diseases. Although most studies are still descriptive, showing only the regulation at the miRNA level, it is conceivable that their significance in pain will rise in the next years. miRNAs are, for example, pivotal in altering activation thresholds and excitability of sensory $Na_v1.8$ nociceptors [36]. This may provide a tool to treat patients with chronic inflammatory pain. Naturally, due to the ubiquitous role of miRNAs in the regulation of translational processes, a complete loss of all miRNAs by a deletion of Dicer is likely to interfere with other physiological functions and produce side effects. However, interfering with single specific miRNAs, yet to be completely identified, may reduce inflammatory pain by modulating the excitability of the nociceptive system. Future investigations on the regulatory role of miRNAs in processing and development of pain might therefore characterize miRNAs as potential and specific drug targets to relieve pain.

Conflict of interest statement

The authors declare no conflict of interest.

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