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Special Review

Role of MicroRNA in Visceral Pain

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The long-lasting nociceptive transmission under various visceral pain conditions involves transcriptional and/or translational alteration in neurotransmitter and receptor expression as well as modification of neuronal function, morphology and synaptic connections. Although it is largely unknown how such changes in posttranscriptional expression induce visceral pain, recent evidence strongly suggests an important role for microRNAs (miRNAs, small non-coding RNAs) in the cellular plasticity underlying chronic visceral pain. MicroRNAs are small noncoding RNA endogenously produced in our body and act as a major regulator of gene expression by either through cleavage or translational repression of the target gene. This regulation is essential for the normal physiological function but when disturbed can result in pathological conditions. Usually one miRNA has multiple targets and target mRNAs are regulated in a combinatorial fashion by multiple miRNAs. In recent years, many studies have been performed to delineate the posttranscriptional regulatory role of miRNAs in different tissues under various nociceptive stimuli. In this review, we intend to discuss the recent development in miRNA research with special emphases on miRNAs and their targets responsible for long term sensitization in chronic pain conditions. In addition, we review miRNAs expression and function data for different animal pain models and also the recent progress in research on miRNA-based therapeutic targets for the treatment of chronic pain.

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Key Words
Chronic Pain; MicroRNAs; mRNA; Visceral Pain

Introduction

The etiology of chronic visceral pain is complex and the underlying mechanism is difficult to understand. Initially, the pain originates from the dysfunction and/or inflammation of organs including the urinary bladder (interstitial cystitis), hindgut (colitis and irritable bowel syndrome [IBS]), uterus (fibroids and endometriosis), ureter (ureteral calculosis) and prostate (prostatitis).[1-3] In chronic stage, the pain is not restricted to one organ, but often overlaps to other visceral and somatic structures (somatic mechanical and thermal allodynia),[4,5] In general, visceral hypersensitivity can occur due to[2] sensitization of primary sensory afferents innervating the viscera,[1] hyperexcitability of spinal ascending neurons (central sensitization) receiving synaptic input from the viscera, and/or[3] dysregulation of descending pathways that modulate spinal nociceptive transmission. Studies in rodents have documented that pelvic organs including bladder, colon and reproductive organs share a common spinal pathway to transmit signals to supra-spinal sites by (1) peripheral axonal dichot-

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omy,[6-8] and/or (2) common convergence of 2 visceral afferents onto the same spinal neuron (ie, viscero-visceral convergence).[9,10] Animal models that induce a focal irritation or injury to the pelvis may produce cross-sensitization in the functionally comparable afferent pathways of the bowel and bladder. Accumulating evidences also suggest that chronic pelvic pain disorders, such as interstitial cystitis and IBS often overlap as a result of neuronal cross-talk via the convergence of pelvic afferents.[11] With reports emerging about the involvement of various neurotransmitters, receptors and growth factors in the pathophysiology of visceral pain, a detailed understanding of the underlying molecular mechanism of visceral hypersensitivity is essential for the development of effective treatment and therapeutic strategies for the altered sensation in viscera of disorders such as interstitial cystitis, IBS, and ureteric colic.

MicroRNAs (miRNAs) are a class of endogenously expressed noncoding RNAs of 18-25 nucleotides and functionally distinct from but related to small interfering RNA (siRNAs). The mature single stranded miRNAs bind through complementary sequence homology to the 3'-untranslated region (UTR) of target mRNAs and cause a translational repression or mRNA degradation.[12] Over the past decade, miRNAs have emerged as major transcriptional regulators of gene expression for critical biological processes including neuronal development, differentiation and synaptic plasticity in the central nervous system. At the molecular level, deregulation of several neurotransmitters, ion channels and proteins are reported to contribute to the development of central and peripheral sensitization and which is considered as the cause of chronic pain.[13] Therefore, miRNA-mediated posttranscriptional regulation of protein and gene expression could also play a role in pain processing pathway. Indeed, several studies support the involvement of miRNA-mediated gene regulation in pathophysiology of acute and chronic pain.[14] Involvement of miRNAs in inflammatory pain processing has been established recently in mice using conditional Dicer (miRNA synthesizing enzymes) deletion in Nav1.8 positive neurons.[15] Effective strategies have been successfully used for RNA-based targeting in experimental models of neuropathic and inflammatory pain[16] with particular reference to intrathecal (i.t.) administration of siRNA specific for $P2X_3$ gene,[17] μ-opioid receptors,[18] N-methyl-D-aspartate (NMDA) receptor subunit NR2B.[19] Studies also indicate that certain miRNAs can specifically target neuronal mRNAs localized near the synapse and modulate input-specific synaptic protein synthesis and neuronal plasticity.[20,21] However, the involvement of miRNA-mediated transcriptional regulation in visceral pain mechanisms has not been clearly elucidated.

Mechanism of MicroRNA Biogenesis

In the nucleus, miRNA is transcribed as a long primary miRNA transcript (pri-miRNA) from miRNA gene by RNA polymerase II (Figure). This pri-miRNA is then processed into a stem loop structure of about 70-80 nucleotides known as precursor miRNA (pre-miRNA) by a microprocessor enzyme comprising of a double-strand (ds)-RNA-specific ribonuclease, Dorsha, along with its partner DGCR8 (DeGeorge syndrome critical region protein 8) also known as Pasha.[22] This pre-miRNA is transported into the cytoplasm by exportin-5-RanGTP dependent mechanism.[23] In the cytoplasm, pre-miRNA is digested by a second dsRNA-specific ribonuclease called Dicer into 18-25 nucleotide mature double-stranded miRNA with the help of TRBP (trans-activation response RNA binding protein) and Argonaute 2 (AgO2).[24,25] The guide strand or mature miRNA is incorporated into a miRNA-induced silencing complex (miRISC), which carries the miRNA strand with sequence complementary to specific target mRNA. The RNA-induced silencing complex is the effector complex of the miRNA function and is comprised of miRNA, AgO2 proteins, and other RNA binding proteins.[26,27]

During biogenesis of miRNAs, loading of the guide strand into the miRISC makes it functional and ready to regulate post-transcriptional gene expression. The miRISC-mediated translational inhibition has been reported to arise from 3 putative mechanisms: (1) site specific cleavage, (2) enhanced mRNA decay, and (3) translational inhibition. The mechanism and the effectiveness of this regulation are dependent on the characteristics of the miRNA and target mRNA interaction. In metazoans, extensive base pairing has been demonstrated to induce mRNA cleavage,[28,29] whereas, in mammals, imperfect binding leads to target repression through translational inhibition and/or mRNA destabilization.[30] In addition, the location of complementary miRNA binding sites on the target mRNA plays an important role in determining the efficacy of the regulation. Although, binding of miRNA has been also demonstrated in the 5'UTR and open reading frame of mRNA, these sites are less effective than those located in 3'UTR.[12,31-38] However, it has been shown that miRNA seeds in coding regions potentiate the effect of 3'UTRs and about a third of predicted miRNA-3'UTR interaction at the synapse also has been reported to involve at least one binding site in the coding region.[39,40] Recently a link between miRNA- mediated poly (A)-tail length shortening and mRNA

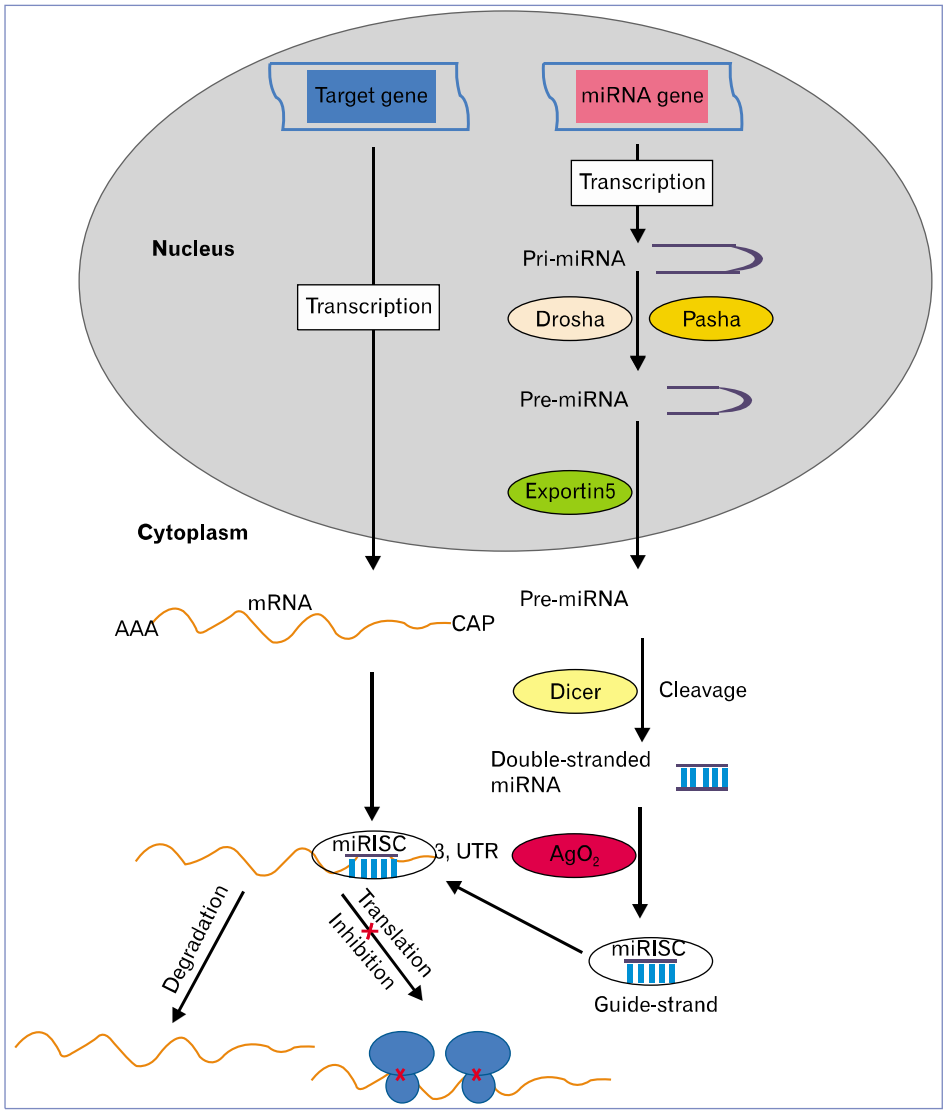

Figure. MicroRNA (miRNA) biogenesis pathway. In the nucleus, miRNAs are expressed as long hairpin RNAs called primiRNAs. Primary miRNAs (pri-miRNAs) are cleaved by Dorsha and Pasha into 70-100 nucleotides hairpin RNAs known as precursor miRNAs (pre-miRNAs). Precursor miRNAs are exported by expotin-5 to the cytoplasm, then another ribonuclease Dicer cleaves them into 18-25 nucleotides long mature double-stranded miRNA. The guide strand of the mature miRNA is then incorporated into a miRNA-induced silencing complex (miRISC). This complex binds to the 3'-UTR of the target gene through partial complementarity and prevents mRNA from translation into protein. If the miRNA carries the exact complementary sequence to an mRNA, it will cleavage the target mRNA.

destabilization has been reported, suggesting another potential mechanism of non-coding RNA-mediated gene regulation.[41] Furthermore, the mRNAs under miRNA-mediated regulation are stored into cytoplasmic particles called processing bodies or glycine-tryptophan bodies.[42] These cytoplasmic structures are free of translational machinery but are enriched in proteins that are required for mRNA inhibition.[43]

Prediction and Validation of MicroRNA Targets

The nucleotide sequences in the target mRNA that are engaged in miRNA binding are called the miRNA recognition element or seed region.[26,44] The composition of this seed region varies but always involves a sequence with conserved Watson-Crick pairing (ie, the hydrogen bonding between guanine-cytosine and adenine-thymine) to the 5' region of the miRNA centered on nucleotides 2-7.[26] This includes canonical sites with a 6- to 8-nucleotide match between miRNA and mRNA, and noncanonical sites that include additional pairing at the 3'end of the miRNA. In addition, AU rich sites have been shown to increase the seed efficacy of miRNA binding.

Other factor includes the position of the seed within the 3'UTR of the target mRNA, with sites at the ends of the 3'UTR being more accessible than those in the middle because long 3'UTRs may form occlusive interactions and this factor greatly reduces miRNA site accessibility. In addition, the presence of multiple sites for the same or different miRNAs on the target

mRNA increases the miRNA-mediated repression response of the target gene. Moreover, cooperative regulation, where the seed sequences are within 40 nucleotide but with a minimum of 8-nucleotide gap results in stronger repression than the sum of the individual and independent sites.[45] MicroRNA cooperativity is defined as the positive interaction of two or more individual miRNAs, or one individual miRNA acting on multiple seed regions on the same 3'UTR for target repression. In this context, miRNA cooperatively studies on neuronal system demonstrate that, miRNA seed density in synaptic mRNAs is higher than in non-synaptic mRNAs, indicating that synaptic mRNAs may be under stronger miRNA control. Therefore, miRNA cooperativity could be a relevant mechanism in the regulation of synaptic mRNA expression. miRNA silencing efficiency is also regulated by the cellular concentrations and stoichiometric relationships between: (1) the miRNAs, (2) the target mRNAs, and (3) the RISC complexes. MicroRNAs that have multiple targets and are highly expressed are supposed to downregulate individual target mRNAs to a lesser extent than those with a lower number of targets. In addition, highly abundant target transcripts that may act as decoys, dilute the effect of miRNAs under specific conditions.[46]

The manual prediction of miRNA: mRNA interaction is not feasible because of the large and growing number of miRNA species and the enormous number of putative target mRNAs. Computational analysis using algorithms and prediction databases is the common method to identify miRNA targets. Several databases such as TargetScan, miRWalk, miRanda, PICTAR5, and pathway analysis using Ingenuity Software have been utilized to analyze the potential target genes for miRNAs. The target prediction programs are based on seed region recognition tools that basically list all the sites with miRNA-binding properties and rank them accordingly to the kind of seed, conservation across evolution and some of the above mentioned factors known to modulate binding site efficiency.[47] However, despite the advancement of prediction bioinformatics, the percentage of predicted targets that fulfill the experimental validation is not optimal. In line with this observation, recent data suggest that miRNA-target interaction also involves the 3' end of miRNAs and some of these interactions are functional.[48] There are other parameters that are not taken into consideration in this target prediction algorithms could also influence the target site accessibility and its regulation, for instance, the secondary structure of miRNA and its association with RNA binding proteins (RBPs).[49] Several approaches have been used to validate functional interaction between miRNAs and their predicted mRNA targets. The reporter construct carrying the binding site of putative mRNA under the control of luciferase promoter has been used extensively in heterologous in vitro systems to validate a functional interaction between a particular miRNA and its predicted targets. Alternatively, single-stranded modified RNA oligos (referred to as miRNA target protectors) that specifically blocked the interaction of a given miRNA with its target mRNA has been used in several in vitro studies.[50,51] Recently, plasmid-based target protectors have been developed to specifically block the miRNA silencing activity in mammalian system, which may have wide application in miRNA target identification.[52]

Involvement of MicroRNA in Neuronal Activity

Several miRNAs are developmentally regulated and demonstrate tissue-specific expression patterns, including many of them expressed only in the nervous system. These miRNAs may play major roles both in neuronal development and neuronal functions. Recent studies emphasize the involvement of miRNAs and their target genes in neuronal development, from early neurogenesis and cell-fate specification to neuronal differentiation and synaptic development of postmitotic neurons.[53] In the mature neurons, miRNAs are important for maintaining normal neuronal function.[21,54-57] During activity-dependent synaptic plasticity, a single synapse or sets of synapses undergo intense modification in an independent manner for a long period of time.[58] Therefore, local posttranscriptional modification regulated by activity is an important event for the plasticity and maintenance of neuronal connections.[59-61] Experimental evidence demonstrates the presence of specific mRNAs and other translational machineries such as ribosomes and non-coding RNAs at the dendritic regions of the neurons, where they are likely to involve in local protein translation.[62,63] Moreover, some mRNAs are located at the postsynaptic density (PSD) as polyribosome structures and encode proteins such as kinases, which are essential components to mediate synaptic plasticity.[62] Therefore, local translation mechanism could play a major role in synaptic plasticity and certainly contribute to the molecular basis of learning and memory.

MicroRNAs are enriched in the brain and there is evidence of their involvement in local protein synthesis at the synaptic level.[64-67] Several components of the miRNA biogenesis pathway and translation inhibitory machinery have been identified in the isolated synapto-neurosomes,[68] and studies indicate that miRNA

driven posttranscriptional regulation locally at the synapse can either increase or decrease mRNA translation through different mechanisms during synaptic activity and stimulation. In addition to their involvement in physiological conditions, recent studies suggest that miRNAs also play an important role in the pathophysiological mechanisms of neurological diseases such as Parkinson's, Alzheimer's and Huntington's disease and Tourette syndrome.[69,70]

One of first studies reporting the control of local translation by miRNA at the synapses showed that miR-134 negatively regulates LIMK1 in an activity-dependent manner.[71] LIMK1 is a protein kinase that controls actin filament polymerization through inhibition of actin depolymerization factor cofilin.[71] This regulation could be critical for synaptic transmission and plasticity since majority of the excitatory synapses are formed on denderitic spines, which are actin-rich protrusions from the dendritic shaft.[72,73] Over-expression of miR-134 inhibits local translation of LIMK1 mRNA at the synapse resulting in a negative regulation of the size of the dendritic spines. Involvement of miR-132 as an activity-modulated miRNA has been established in a recent study, where bicuculline-mediated blockages of GABAA inhibitory tone in neurons resulted in a rapid increase in the expression of miR-132 precursor and mature miR-132.[74] Furthermore, this regulation could be attenuated by pretreatment with the selective NMDA receptor antagonist amino-5-phosphonovaleric acid. The involvement of miR-132 in different neuronal activities is evident from the findings that inhibitors for CaM kinase, mitogen activated protein kinase-extracellular signal-regulated kinase (MEK-ERK) or cAMP response element binding protein (CREB), all prevent miR-132 upregulation upon activity enhancement, suggesting that miR-132 is predominantly regulated by these signaling pathways. MicroRNA-132 directly targets p250GAP, a protein known to inhibit Rho family GTPases.[75,76] Overall, miRNA upregulation modulates dendritic morphogenesis and

Table. Involvement of MicroRNAs in Synaptic Plasticity and Pain Mechanisms

miRNA	Synaptic plasticity models/conditions	Tissue	References	miRNA	Pain models/ conditions	Tissue	References
miR-134	BDNF exposure	Hippocampal neuron	71	miRNA-183, -182, -96, -71, -125b, -30d, -379, -103	SNL	DRGs/SDH	105-109
miR-132	Bicuculline/KCl	Hippocampal neuron	74	miRNA-1, -124, -129-1/2, -223, -124a, -21, -137, -181a, -219, and others	SCI	SC/SDH	110, 112, 117
miR-219	NMDA antagonist	Prefrontal cortex/ hippocampus	81	miR-96, -146a, -34c, -125b, -103, Let-7 family	CCI	DRGs/ Hippocampus	111, 113
miR-181a	Cocaine/ Amphetamines	Hippocampal neuron	21	miR-29b, -142, -424, -223, -21, -221, -182, -183, -145, and others	SNC	Sciatic nerves	122
miR-188	LTP	Hippocampus	56	miR-1, -16, -206	SNA	DRG/SDH	115
miR-125b, -132	FNMRP	Mouse brain/ neuron	55	miR-21, -143	SNT	DRGs	119, 120
miR-124	miRNA depletion	Retinal ganglion cell	78	miR-200b, -429	PNL	Nucleus	114
miR-29a/b	Psychoactive drugs	Brain regions/ neurons	82			Accumbens	
miR-124, -181a, Let-7d	Cocaine	Necleous	79	miR-183, -124a, -134, -143, -1, -16, -206	CFA	DRGs/SDH	87, 115, 118, 120
		Accumbens		miR-124a	Formalin/ IL-1β	SDH/spinal microglia	116, 121
miR-212	Cocane	Dorsal Striatum	80	miR-181a	Cystitis	SDH	103
				miR-449b, -500, -320, -199a-5p	BPS	Bladder/cell culture	100, 101
				miR-29a	IBS	Colon/small bowel/ cell culture	99

miRNA, microRNA; BDNF, brain-derived neurotrophic factor; NMDA, N-methyl-D-aspartate; LTP, long-term potentiation; FMRP, fragile x mental retardation protein; SNL, spinal nerve ligation; DRG, dosal root ganglion; SDH, spinal dorsal horn; SCI, spinal cord injury; SC, spinal cord; CCI, chronic constriction injury; SNC, sciatic nerve crush; SNA, sciatic nerve transactivation; SNT, sciatic nerve transaction; PNL, partial nerve ligation; CFA, complete Freund's adjuvant; BPS, bladder pain syndrome; IBS, irritable bowel syndrome.

spinogenesis.[77] Several major miRNAs involved in synaptic plasticity are listed in Table.[21,55,56,71,74,78-82]

Activity-dependent Regulation of MicroRNAs in Pain Processing

In pain mechanism, selected miRNAs have been implicated in multiple cellular processes, including neuronal plasticity and neurogenesis, nociceptor excitability and chronic pain condition. A recent study proposed brain derived nerve growth factor (BDNF)-driven upregulation of miR-132 in cortical neurons.[83] Since BDNF is recently identified as a modulator of nociception, it is conceivable that BDNF-induced upregulation of miR-132 would increase dendritic morphogenesis and arborization in the nociceptive pathway, resulting in an increase in pain signal transmission. The role of miRNAs in dendritic spine remodeling and synaptic plasticity has been documented by Lippi et al.[82] In this study adults mice exposed to psychoactive drugs like nicotine or cocaine demonstrated over-expression of miR-29a/b consistently in different brain regions. The protein Arpc3 involved in actin branching during dendritic spine maturation was identified as the target for miR-29a/b, suggesting that these miRNAs regulate the activity-dependent structural plasticity associated with psychoactive exposures. Several studies identified a deregulation of miR-29a/b in pain conditions such as in spinal dorsal horn-induced muscle inflammation, complex regional pain syndrome (CRPS), and chronic constriction injury.[84,85] However, the target proteins of miR-29a/b and their subsequent regulatory mechanisms in pain processing are not clearly defined.

One of the first demonstrations of the role of miRNAs in chronic pain came from the study by Zhao et al,[15] where conditional knockout of Dicer (enzyme essential for mature miRNA synthesis) in Nav1-8-positive nociceptors in the dorsal root ganglia (DRG) resulted in significant attenuation of inflammatory pain. This finding suggests that miRNAs-mediated regulation of this subpopulation of sensory neurons is necessary for inflammation-induced chronic pain condition. The direct targeting of miRNA let-7 on mu opioid receptors during opioid tolerance clearly emphasizes an integral role of miRNA-mediated regulation in chronic pain.[86] In an inflammatory rat model of CFA-induced muscle pain, several miRNAs including miRs-10a, -29a, -98, -99a, -124a, -134, and -183 were significantly downregulated in neurons of the ipsilateral trigeminal ganglion.[87] This downregulation of specific miRNAs was considered to increase the expression of several "pain-related" proteins and therefore, facilitated the development of inflammation and allodynia. On the other hand, upregulation of miR-155 and miR-223 in the prefrontal cortex has been reported following carrageenan-induced facial inflammation in mice. This study also demonstrated an inverse relationship between miR-155 and one of its potential targets, the transcription factor, CCAAT/enhancer binding protein β in this inflammation model.[88] In a murine peritonitis model of self-limiting acute inflammation, increased levels of miRs (-21, -146b, 208a, -203, -142, -302d, and -219) could be counter-regulated by administration of resolvin D1, an anti-inflammatory lipid mediator.[89] Since resolvin D1 has been reported as an important mediator of anti-inflammatory and anti-nociceptive behaviors, this finding indicates that specific miRNAs are the posttranscriptional regulators of the inflammatory pain condition.[90] In the neuropathic pain models, a downregulation of miR-181a or other members of the miR-181 family, namely miR-181b/c/d has been documented.[91,92] In this context, deregulation of miR-181a expression in various brain regions has been reported following chronic exposure to drug of abuse.[21]

MicroRNAs as Modulators of Neuro-immune System

Noxious stimulations have been reported to deregulate the expression of miRNAs in pain circuitry from primary afferents to brain regions associated with emotional components of pain perception. Several miRNAs (termed as NeurimmiRs) such as miR-124 and miR-132 can act within both the nervous and immune systems by primarily targeting several transcription factors and other regulatory genes which may simultaneously modulate both immune and neuronal processes through alterations of neuron-glia or brain-peripheral signaling.[93] These findings indicate that miRNAs control multiple cellular pathways by regulating multiple gene products including several cellular enzymes, trophic factors, neurotransmitters, ion channels, many of them are individually studied as drug targets. miRNAs are frequently deregulated and differentially expressed in disease tissues with neurogenic pain such as in CRPS.[85] The deficiency of anti-inflammatory cytokines in patients with CRPS and the beneficial effect of therapy with glucocorticoids support the association between pathohysiological mechanisms and neuro-immune dysfunction.[94] Various studies also identified the mediators of neuro-inflammation as critical component of diabetic neuropathy,[95] in addition to cellular mechanisms that include the classical changes of the diabetic milieu.[96] Besides the role of intracellular miRNAs

as the posttranscriptional regulator of gene expression, the role of extracellular miRNAs as signal regulators for membrane receptors activation have recently been established.[97] The extracellular miRNA-let-7 is identified as an activator of Toll like receptor (TLR)-7 in both immune cells and neurons. In line with this finding, an unconventional role of extracellular miRNAs for rapid excitation of nociceptor neurons via TLR7 has been proposed.[98] Here, miRNA-let-7b induces rapid inward currents and action potentials in DRG only in neurons coexpressing TLR7 and transient receptor potential ankyrin 1 (TRPA1) ion channel, and are abolished in mice lacking *Tlr7* or *Trpa1*. Furthermore, let-7b induces TLR7/TRPA1-dependent single-channel activities in DRG neurons and HEK293 cells overexpressing TLR7/TRPA1 and let-7b inhibitor reduces formalin-induced TRPA1 currents and spontaneous pain in vivo system. Therefore, secreted extracellular miRNAs may also serve as novel pain mediators via activating TLR7/TRPA1 in nociceptor neurons.[98]

MicroRNAs in Visceral Pain

After tissue injury, changes in several neurotransmitters, ion channels, and proteins contribute to the development of central and peripheral sensitization, which is considered as the cause of chronic pain.[13] Since miRNAs are the posttranscriptional regulators of protein and gene expression, it is possible that miRNA-mediated deregulation of these molecules both peripherally and centrally also contribute to the visceral hypersensitivity and pain processing in disorders such as interstitial cystitis, IBS and bladder pain syndrome. In a recent study, an increase in miR-29a in colon tissues and blood microvesicles of patients with IBS has been correlated with increased intestinal membrane permeability.[99] Authors further demonstrated that miR-29a interact with complementary binding sites at the 3'UTR of the glutamate ammonia ligase gene and that leads to downregulation of glutamine synthase expression and increased intestinal permeability and eventually results in chronic visceral pain. In another study, increased in expression of several miRNAs namely miR-328, miR-320, miR-449b, and miR-500 was directly correlated with the down regulation of neurokinin-1 (NK1) receptors in the bladder biopsies from BPS patients.[100] Using cell-based models, authors showed that prolonged exposure of NK1 receptors to substance P caused a decrease in NK1 receptor mRNA expression and a concomitant increase of regulatory miRNAs -449b and -500. This modulation of NK1 receptors by miRNAs may be the underlying molecular mechanism of increased urothelial permeability and painful bladder syndrome in BPS patients. Monastyrskaya et al also documented a possible link between miR-199a-5p and the control of urothelial permeability in bladder pain syndrome.[101] In urothelial cells, overexpression of miR-199a demonstrated impairment in tight junction formation resulting in increased epithelial permeability. Differences in miRNAs expression have also been reported in endometrium between patients with and without painful endometriosis.[102] A recent study from our lab established the involvement of miRNA-mediated post-transcriptional regulation of the developing spinal GABAergic system following neonatally-induced cystitis in rats.[103] Cystitis was developed neonatally by intravesicular injection of zymosan into the bladder during postnatal (P) days P14-P16. A significant upregulation of mature miR-181a in the L6-S1 spinal dorsal horns was observed in cystitis rats. The target gene analysis demonstrated multiple complementary binding sites in miR-181a for the $GABA_A$ receptor subunit $GABA_{A\alpha\text{-}1}$. An increase in miR-181a concomitantly resulted in long-term downregulation of spinal $GABA_{A\alpha\text{-}1}$ receptor subunit gene and protein expression in spinal dorsal horns of adults rats following neonatally-induced cystitis. These findings indicate that miRNA-mediated transcriptional deregulation of spinal gene expression may contribute to the visceral sensitization of patients with chronic cystitis. Using bioinformatics, Zhao et al[104] demonstrated that miR-181a indeed carries complementary binding sites for the 3'UTR region of the $GABA_{A\alpha\text{-}1}$ gene. They further verified miR-181, miR-216 and miR-203 as the target miRNAs for $GABA_{A\alpha\text{-}1}$ gene using luciferase reporter assay. We recently investigated the involvement of miRNAs in long-term cortical plasticity following esophageal acid exposure in rats neonatally from P7 to P14 (our unpublished findings). The miRNA expression in cortical synaptoneurosomes was examined using next generation sequencing and quantitative real time PCR in adult rats on P60. Three miRNAs -29b, -34a, and -30a exhibited significant upregulation in acid-treated rats compared to saline-treated controls. This finding strongly suggests the involvement of miRNAs in long-term cortical plasticity following esophageal acid exposure early in life. Here, we review specific miRNAs that have been identified as the regulators of nociceptive activity and highlight their possible association with pain processing in various pain conditions (Table[87,99,100,101,103,105-122]).

Cognitive and Behavioral Components of Pain Mechanism

Brain specific miRNAs are emerging as regulators of cognition, neuronal plasticity, and memory by regulating mRNAs that are associated with synaptic structure and function. A set of specific miRNAs not only control cognition and emotional processes but also neuro-immune communication in the brain.[93,123] Moreover, specific regions in the brain are actively involved in pain perception and behavior in humans and rodents and structural brain changes are associated with sensory and emotional function in long-term neuropathic pain in rodent.[124] The abnormalities in hippocampus volume are observed in human CRPS and in mouse with speared nerve injury. Similar to CRPS in humans, speared nerve injury mice exhibit increased anxiety like behavior and abnormal contextual fear extinction and this is associated with reduced ERK kinase expression, decrease neurogenesis and altered synaptic plasticity.[125] Dopaminergic and glutametergic inputs from amygdala, hippocampus and prefrontal cortex to the nucleus accumbens have been reported to involve in the emotional control circuits and recent human studies documented the role of nucleus accumbens in the emotional aspects of pain processing.[126] Moreover, miRNA-mediated transcriptional deregulation has been linked with the emotional dysfunction and maladaptive responses of nucleus accumbens in chronic pain condition associated with neuropathic pain.[114]

Genetic Polymorphisms and Pain Condition

Altered miRNA expression is usually a consequence of genetic mutation which may also cause loss or gain of function.[127] However, the functional consequences of polymorphisms in miRNA genes and/or their targets, and the mechanisms by which miRNAs regulate pain circuitries and modulate nociception are not yet clearly defined. Several meta-analyses are available of the genetics of pain and associated specific loss or gain of function polymorphisms with altered pain perception.[128] Although, single nucleotide polymorphisms (SNPs) are less common in miRNAs and their target binding sites, several of the SNPs in miRNAs that are bioinformatically predicted to be associated with pathogenesis are also experimentally verified.[129] These SNPs can affect the expression of a large number of genes when the production of the miRNAs is influenced by those particular SNPs. Similarly, SNPs in target sites of miRNAs can either modulate or disrupt existing binding sites and create new binding sites for the miRNAs. These polymorphisms may further influence gene expression and the pathogenic mechanisms in disease development.[130]

Role of MicroRNAs as Novel Biomarkers

Recent studies have suggested that miRNAs are promising biomarkers for a number of disorders including cancer, heart failure, and neurodegenerative disorders. Circulating miRNAs are release in serum and cerebrospinal fluid from cells through an endocytic pathway, and are packed into exosomes vesicles in the circulation. These secreted miRNAs in exosomes and in other microvesicle are protected from endonuclease-dependent degradation and reported to influence cellular microenvironment of immune and endothelial systems.[131] In addition, unlike serum miRNAs, exosome-entrapped miRNAs are capable of crossing the blood brain barrier (BBB).[132] Inflammatory processes are also activated in the spinal cord upon peripheral nerve injury and involved microglia activation and leakage at the blood nerve barrier along the entire neuraxis.[133] The leakage of the blood-nerve barrier or blood-spinal barrier is emerging in pathophysiology of neuropathic pain accompanied with changes in tight junction proteins.[134] Moreover, studies also indicate that a number of neurological diseases are associated with BBB dysfunction resulting in elevated barrier permeability and leakage of molecules such as miRNAs in and out of the brain.[134] Interestingly, miR-132 blood level was reported to alter significantly in patients with CRPS, a disabling chronic neuropathic pain affecting one or more extremities.[85]

MicroRNAs as Therapeutic Targets for the Treatment of Chronic Pain

In recent years, several strategies have been put forward to target miRNAs expression in pain management and treatment. In direct strategies, the use of oligonucleotides directly or virus-based constructs to either block the over-expression of miRNAs or to substitute for the loss of expression of miRNAs have been proposed. The indirect strategies involve the use of drugs to modulate miRNA expression by regulating their transcription and processing. For overexpressing miRNAs, miRNA mimics comprising the nucleotide sequences of either pre-miRNAs or mature miRNAs have been developed, whereas, miRNA inhibition utilizes the use of anti-sense oligos (anti-miRNAs),

miRNA sponges, or miRNA decoys.[135,136] MicroRNA decoys enable long-term suppression of miRNA expression by using nuclease resistant complementary RNAs expressed in viral vectors that harbor RNA expression cassettes driven under RNA polymerase II.[136] Antisense oligos function as competitive inhibitors by annealing to the mature miRNA guide strand and inducing degradation or stoichiometric duplex formation. miRNA sponges are designed as longer RNA molecules carrying several miRNA complementary sequences for simultaneously inhibiting several miRNAs.

A major challenge for the delivery of miRNAs into central nervous system is the blood brain barrier, the natural barrier hindering the access of miRNAs to the central nervous system. Some effective approaches for gene silencing in the central nervous system are: lentiviral-vector-mediated delivery; germline transfection and stereotaxic application.[137,138] Another specific approach is to incorporate cholesterol molecules to the sense strand and making it accessible to cells with high density lipoprotein receptors such as oligodendrocytes in the brain.[14] The further advancement in small RNA delivery technique is the development of immunoliposome, the complex of a receptor-directed monoclonal antibody, liposomes and small hairpin RNA expression plasmids. The targeted delivery with immunoliposomes facilitates the conjugated RNA to cross the BBB and reach specific regions in the brain. In the context of chronic pain, intrathecal administration of miR-124 showed an anti-nociceptive effect in both inflammatory and nerve injury-induced pain models. Furthermore, kynast et al showed that the intravenous injection of miR-124 alleviates the nociceptive response to formalin test and identified methyl CpG binding protein 2 (MeCP2) as the target. MeCP2 is an epigenetic modulator of BDNF, one of the major players in inflammatory pain mechanisms.[116] An example of multiple targeting by a single miRNA has been documented for the subunits of voltage-gated calcium channel. MicroRNA-103 simultaneously regulates the expression of three subunits of Cav1.2-comprising L-type calcium channel (Cav1.2-LTC) in an integrative manner. In functional study, it has been shown that miR-103 knockdown in naïve rats result in hypersensitivity to pain. Moreover, in neuropathic pain there was a down regulation of miR-103 and intrathecal application successfully relieved pain, thus identifying miR-103 as a possible therapeutic target in neuropathic chronic pain.[107] However, not much information is available on the application of miRNA-based targeting strategies for the treatment and management of visceral pain.

Conclusion and Future Strategies

The regulatory role of miRNAs in the modulation of pain pathway has been extensively studied in the past decade and accumulating evidence implicates miRNAs as potential targets for the treatment of chronic pain. Several studies have identified differentially expressed miRNAs in specific visceral organs following nociceptive stimuli in experimental animals and also in colon and bladder biopsies from patients with IBS and BPS. However, comprehensive studies elucidating the miRNA-mediated deregulation at the level of viscera as well as the changes at the DRG and spinal levels that innervate visceral organs are still lacking. Future research should focus on answering several important questions such as (1) whether a single gene is regulated by multiple miRNAs or a single miRNA is targeting several genes those are involved in a common downstream signaling process in a tissue specific manner; (2) are there specific miRNAs and their target genes that are differentially expressed in visceral organs such as in bladder and colonic myenteric plexus as well as in DRGs and spinal dorsal horn regions innervating these organs. This information will certainly enable us to delineate the underlying molecular mechanisms of the chronic pelvic neuroplasticity and cross-organ sensitization and facilitates the development of miRNA-based therapeutic strategies for the treatment of visceral pain.

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