

Contribution of MicroRNAs in the Development of Irritable Bowel Syndrome Symptoms

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ABSTRACT

Irritable bowel syndrome (IBS) is a common chronic functional gastrointestinal disorder defined by disturbances in bowel habits and abdominal pain, in the absence of known organic pathology that affects between 5 to 10% of healthy populations. Despite improvements in detection and treatment, the pathogenesis of IBS has not been clarified. Several microRNAs (miRNAs) are involved in the pathogenesis of IBS through increased intestinal permeability, inflammation, and modulation of visceral hyperalgesia, and they may have the potential to be used as biomarkers and therapeutic targets. Here, we have summarized the recent advances about the role of miRNAs in the development of IBS symptoms and the possibility to use them as therapeutic targets to mitigate symptoms in IBS.

Key words: irritable bowel syndrome – microRNAs – intestinal permeability – visceral hyperalgesia – inflammation.

Abbreviations: 5-HT: 5-hydroxytryptamine; AQP: aquaporin; CD: celiac disease; CGN: cingulin; CLDN1: claudin-1; CLDN2: claudin-2; CNR1: cannabinoid receptor 1; FBD: functional bowel disorders; GI: gastrointestinal; GLUL: glutamine synthetase gene; GM-CSF: granulocyte-macrophage colony-stimulating factor; GS: glutamine Synthetase; HTR4: 5-HT receptor 4; HTR7: 5-HT receptor 7; IBD: inflammatory bowel disease; IBS: irritable bowel syndrome; IBS-C: IBS with constipation; IBS-D: IBS with diarrhea; IBS-M: IBS with mixed stool pattern; IL: Interleukin; KD: Knockdown; KO: Knockout; miRNAs: microRNAs; mRNA: messenger RNAs; NCCP: noncardiac chest pain; NFκB: nuclear factor κB; NKRF: NFκB repressing factor; NOD2: nucleotide binding oligomerization domain containing protein 2; NUD: non-unclear dyspepsia; OCLN: occludin; PI3K/AKT: phosphatidylinositol 3-kinase/ protein kinase B signaling pathway; PI-IBS: post-infectious IBS; PKIB: protein kinase inhibitor peptide beta; PRDX1: peroxiredoxin 1; RT-PCR: real time polymerase chain reaction; SERT: serotonin reuptake transporter; TJ: tight junctions; TLR-4: Toll-like receptor 4; TNBS: 2,4,6-trinitrobenzene sulfonic acid; TNF-α: tumor necrosis factor α; TRPV1: transient receptor potential cation channel subfamily V member 1; UC: ulcerative colitis; UTR: untranslated regions; WAS: water avoidance stress; ZO-1: zonula occludens.

INTRODUCTION

Irritable bowel syndrome (IBS) is the most common multifactorial disorder of the gastrointestinal (GI) tract of unknown etiology described by gastroenterologists in which the patients suffer from chronic abdominal pain associated with bloating and change in the bowel motility causing diarrhea/constipation [1]. This condition affects approximately 5% and

10% of healthy people in most geographical regions [2] and symptoms occur more often in patients less than 50 years of age [3]. Irritable bowel syndrome is diagnosed by identifying typical symptoms according to a symptom-based classification system, the Rome IV Criteria [4], and patients are grouped by use of the Bristol Stool Form Scale as IBS with diarrhea (IBS-D), IBS with constipation (IBS-C), and IBS with mixed stool pattern (IBS-M) subtypes [5]. Though the etiology of IBS is not completely understood, many factors are included: motility disturbance, visceral hypersensitivity, altered mucosal and immune function, post-infectious reactivity, brain-gut interactions, altered fecal microbiota, bacterial overgrowth, stress, and idiopathic intestinal inflammation have been all involved in the pathogenesis of IBS [6].

MicroRNAs (miRNAs) are a family of small endogenous non-coding RNA molecules 19 to 25 nucleotides, that regulate gene expression post-transcriptionally, binding through partial sequence homology to the 3'-untranslated regions (UTR) of target messenger RNAs (mRNAs), resulting in decreased stability and repression of translation [7]. A single miRNA can target hundreds of mRNAs and influence the expression of many genes. As a direct consequence, miRNAs regulate many biological processes and have critical roles in cell proliferation, differentiation, and death [8]. Some evidence has shown that alterations in miRNA levels may be related to the pathogenesis of many GI diseases including colorectal and gastric cancer [9], inflammatory bowel disease (IBD) [10], dyspepsia [11], celiac disease (CD) [12] and IBS [13]. Recent papers include relevant information regarding to the clinical aspects of several miRNAs that appear to be important in regulating the expression of genes involved in visceral pain response, barrier functions, intestinal permeability, inflammation and cellular remodeling of GI cells in IBS [14].

In this review, we summarized the current evidence about miRNAs involved in the pathogenesis of IBS and how these miRNAs may be increased intestinal permeability, causing IBS-related symptoms. We also emphasized how the miRNAs modulate inflammation and visceral hyperalgesia in the GI duct of IBS patients (Table I). Finally, we analyzed the possibility to use the regulation of the miRNAs as therapeutic targets to decrease symptoms in IBS.

PATHOPHYSIOLOGICAL IMPLICATIONS OF MIRNAS REGULATION IN IBS

Role of miRNAs in Intestinal Permeability Dysregulation in IBS

The GI tract has a critical role in many physiologic functions such as digestion, absorption, and metabolism of nutrients from the daily diet. Their components (extracellular and cellular elements) form a selective barrier, which allows fluxes of nutrients, ions, and water, but at the same time, it must regulate the contact with the massive intraluminal dietary antigens and immune system products [15]. However, this barrier is not fully impermeable to macromolecules; the passage of some numbers of dietary antigens molecules and microorganisms promote a homeostatic immune response dominated by immune tolerance to food-derived antigens [16] and the local production of secretory immunoglobulin A, preventing the establishment of pathogenic and commensal microbes into internal compartments [17]. Nonetheless, alterations or defects of the defensive mechanisms of the intestinal barrier can permit excessive input of dietary or microbe-derived molecules to promote an exacerbated immune response, which may contribute to the beginning of many GI disorders, including food allergy, IBD, CD, IBS, and others [15, 18]. Increasing numbers of scientific researchers have focused on the mechanisms of dysregulation of intestinal

Table I. Non-coding RNAs associated with irritable bowel syndrome (IBS)

MiRNAs	Targets	Pathogenesis	miRNA regulation	Reference
miR-16	Claudin-2 (CLDN2)	Intestinal permeability in IBS-D patients	Down	[32]
	Cingulin (CGN)			
	5-Hydroxytryptamine receptor 4 (HTR4)	Visceral hyperalgesia in IBS-D patients	Down	[43]
miR-24	Serotonin reuptake transporter (SERT)	Visceral hyperalgesia in IBS patients and TNBS-induced IBS mouse model	Up	[47]
miR-29	Glutamine synthetase gene (GLUL)	Intestinal permeability in IBS-D patients	Up	[13]
	Claudin-1 (CLDN1)	Intestinal permeability in IBS-D patients and IBS-D rat model	Up	[26]
	Nuclear Factor- κ B-repressing factor (NKRF)			
	Aquaporins (AQP1, AQP3, AQP8)	Intestinal permeability in IBS-D rat model	Up	[28]
	5-Hydroxytryptamine receptor 7 (HTR7)	Visceral hyperalgesia in IBS patients and WAS-induced IBS mice model	Up	[50]
miR-103	5-Hydroxytryptamine receptor 4 (HTR4)	Visceral hyperalgesia in IBS-D patients	Down	[43]
miR-125b	Claudin-2 (CLDN2)	Intestinal permeability in IBS-D patients	Down	[32]
	Cingulin (CGN)			
miR-144	Zonula occludens-1 (ZO1)	Intestinal permeability in IBS-D rat model	Up	[36]
	Occludin (OCLN)			
miR-181c-5p	Interleukin 1A (IL-1 α)	Inflammation in IBS rat model	Up	[76]
miR-199	Transient receptor potential vanilloid 1 (TRPV1)	Visceral hyperalgesia in IBS patients and IBS rat model	Down	[54]
miR-200a	Cannabinoid Receptor 1 (CNR1)	Visceral hyperalgesia in IBS-D rat model.	Up	[59]
	Serotonin reuptake transporter (SERT)			
miR-495	Protein kinase inhibitor peptide beta (PKIB)	Visceral hyperalgesia in IBS mouse model	Down	[66]
miR-510	Peroxisiredoxin 1 (PRDX1)	Inflammation in post-infection IBS patients	Down	[81]

miR: microRNA; IBS: irritable bowel syndrome; IBS-D: IBS diarrhea subtype; TNBS: 2,4,6-trinitrobenzene sulfonic acid; WAS: water avoidance stress.

permeability in IBS. Some of these studies have been related to serum proinflammatory cytokines and anti-flagellin antibodies [19]. Other findings have involved the lower expression of zonula occludens (ZO-1) mRNA, a protein that interacts with occludin (OCLN) and claudins and constitutes tight junctions (TJ) [20]. Emerging evidence has been associated with up or down-regulation of miRNAs with intestinal permeability dysregulation in IBS (Fig. 1).

MicroRNA-29

The miRNA-29 family in humans includes three mature members, miRNA-29a, miRNA-29b, and miRNA-29c [21]. The mature sequences are highly conserved with humans, mice, and rats [22]. Several studies have been shown the importance of miRNA-29 family members in the regulation of biological cell processes such as proliferation, differentiation, and apoptosis in many types of cancer [23]. The first study related to intestinal permeability and miRNAs was carried out by Zhou et al. [13] who demonstrated that a subset of IBS patients with diarrhea-predominant has increased miRNA-29a expression in blood microvesicles, small bowel, and colon tissue compared to healthy controls. The overexpression of miRNA-29a correlates with increased intestinal permeability via downregulation of the glutamine synthetase gene (GLUL) and decreased intestinal glutamine synthetase (GS) activity in IBS-D patients. Glutamine synthetase is an important

enzyme that catalyzes the production of glutamine from glutamate and ammonia and helps to maintain adequate levels of intestinal glutamine [13]. Glutamine plays a critical role in the growth and function of the GI epithelium, regulates TJ proteins, suppresses pro-inflammatory signaling pathways and protects cells against apoptosis and cellular stresses during normal and pathologic conditions [24]. Decreased plasma and cellular concentrations of glutamine and reduced mucosal GS activity have been described in patients with GI disorders [25]. Subsequently, the same group showed a differential miR-29a/b, nuclear factor κ B (NF κ B) repressing factor (NKRF) and claudin-1 (CLDN1) genes expression in intestinal tissue from IBS-D patients versus IBS-C patients and healthy controls as well 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis animal model [26]. This study showed that silencing miRNA-29 family in knockout (KO) mice (miR-29a/b-/-) restores intestinal permeability and the associated pathologic hallmarks of increased intestinal permeability via up-regulation of CLDN1 and NKRF. CLDN1 is a key TJ protein that interacts with intracellular signaling pathways that regulate and maintains intestinal permeability. Altered expression and cellular distribution of TJ proteins such as CLDN1 or OCLN lead to increased intestinal permeability in IBS-D patients [27]. They observed that IBS-D patients with increased intestinal permeability have significantly decreased gut-NKRF expression by miRNA-29 upregulation expression.

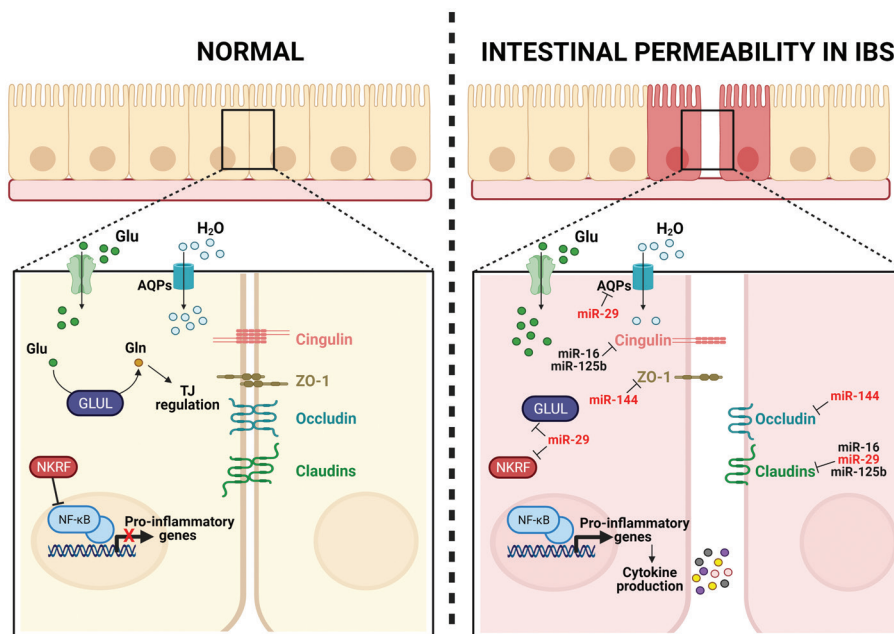


Fig. 1. Role of miRNAs expression on increased intestinal permeability in IBS. Representative scheme about the multiple targets of miRNAs in epithelial cells of patients with IBS. The left side shows a normal structure of TJ proteins in epithelial cells (Cingulin, ZO-1, Occludin and Claudins). The water uptake is mediated by AQP family and GLUL catalyzes the ATP-dependent conversion of Glu to Gln. NKRF interacts with specific negative regulatory elements to mediate transcriptional repression of NF- κ B. The right side shows an increased intestinal permeability induced by up (red) or downregulation (black) miRNA expression. Some miRNAs (miR-16, miR-125b, miR-144 and miR-29) interacts with TJ genes, inhibiting their translation. The overexpression of miR-29 results in deregulation of AQPs, GLUL and NKRF, which causes a decrease of Glu catalyzes, water intake, and increase of inflammatory mediator production. Glu: glutamate; Gln: glutamine; AQP: aquaporins; GLUL: glutamine synthetase; TJ: tight junction; NF- κ B: nuclear factor κ B; NKRF: NF κ B repressing factor; IBS: irritable bowel syndrome.

Another group evaluated the expression change of miRNA-29a in colonic epithelial cells in the IBS-D rat model [28]. They demonstrated that increased intestinal permeability in IBS-D rats is associated with the downregulation of aquaporins (AQPs): AQP1, AQP3, and AQP8 by increased expression of miRNA-29a in colon cells. Aquaporins are highly conserved small transmembrane proteins, which are responsible for the water transport across the cell membrane. AQPs are abundantly expressed in numerous types of cells such as epithelial and endothelial cells [29]. Altered expression of AQPs has been identified as co-factors in the etiopathogenesis of some gastroenteric disorders [30]. These observations led to the hypothesis that modulation of miRNA-29a expression would improve clinical outcomes in IBS-D patients.

MicroRNA-125

MicroRNA-125 is a highly conserved microRNA throughout many different species from nematode to humans. In humans, there are three homologs (hsa-miRNA-125b-1, hsa-miRNA-125b-2 and hsa-miRNA-125a). These miRNAs have been described in the regulation of stem-cell hematopoiesis, inflammation, and some type of cancers [31]. Subsequently, the role of miRNA-125b has been identified in GI disorders such as IBS. Martinez et al. [32] demonstrated that miRNA-16 and miRNA-125b are involved in intestinal permeability dysregulation through the modulation of two TJ proteins, claudin-2 (CLDN2) and cingulin (CGN) gene and protein expression in the jejunal mucosal samples of IBS-D patients versus healthy donors. Downregulation of miRNA-16 and miRNA-125b correlates with increased levels of CGN and CLDN2 proteins in IBS-D samples. In vitro experiments in the human epithelial cell line, colo320 show that overexpression of two miRNAs decreased endogenous levels of CGN and CLDN2 proteins. CGN is a cytosolic protein localized at TJ of vertebrates epithelial and endothelial cells. In the cytoplasm, CGN interacts with several cytoplasmic TJ proteins, including ZO-1, ZO-2, and ZO-3, as well as with actin and myosin [33]. Several studies have evidenced that CGN regulation is dispensable for the structure and function of TJ and plays a role in regulating CLDN2 expression in selected epithelial organs by KO and knockdown (KD) mice [34]. This study provides evidence that the modulation of the intestinal epithelial barrier function in IBS-D involves both transcriptional and post-transcriptional mechanisms, including miRNA-125b and miRNA-16 as master regulators in controlling the expression of specific TJ proteins.

MicroRNA-144

The miRNA-144/451 locus encodes two highly conserved miRNAs: miRNA-144-3p and miRNA-451a [35]. Recently, research has been carried out by Hou et al. [36], following the role of miRNAs in intestinal permeability using an IBS-D rat model. They evaluated the differential expression of miRNAs in colonic epithelial cells of IBS-D rats. The microarray approach revealed 8 up-regulated and 18 down-regulated expressions of miRNAs identified in the IBS-D rat model. Of these, miRNA-144 was markedly up-regulated and resulted in the downregulation of OCLN and ZO1 expression. Additionally, increased intestinal

permeability was enhanced by miRNA-144 up-regulation and attenuated by miRNA-144 down-regulation in IBS-D rat colonic epithelial cells. The TJ proteins are important to create a paracellular barrier in epithelial and endothelial cells protecting them from the external environment. Two different classes of integral membrane proteins constitute the TJ architecture, proteins involving the regulated co-interaction of cytoplasmic adaptor proteins (e.g., ZO1) and integral membrane linker proteins (e.g., OCLN and CLDN). Dysregulations of these TJ proteins have established the pathophysiological hallmark of many GI diseases [37]. These findings suggest a promising therapeutic method to restore healthy intestinal permeability in IBS-D.

Implication of miRNAs Regulation on Visceral Hyperalgesia in IBS

Chronic visceral pain and/or discomfort is a complex and heterologous disorder lasting longer than 3 months after the resolution or in the absence of an injury. Visceral pain is one of the most frequent reasons for patients seeking medical intervention [38]. Usually, the onset of symptoms are related to infections or organic inflammatory conditions of the GI tract (gastroesophageal reflux, peptic ulcer disease, acute and chronic IBD). However, in functional bowel disorders (FBD) such as noncardiac chest pain (NCCP), non-unclear dyspepsia (NUD), and IBS, chronic visceral hyperalgesia persists usually in the absence of detectable mucosal inflammation [39]. The cause of visceral hypersensitivity in FBD patients is unknown; however, scientific evidence established a pathogenic relation between chronic visceral pain with abnormal GI motility, psychological (long term stress), and dietary factors [40] in these conditions. On the other hand, several reports have explored the role of selective miRNAs in visceral hyperalgesia on IBS (Fig. 2).

MicroRNA-15/107 Family

The miRNA-15/107 superfamily contains multiple highly conservative miRNA members, including miRNA-15a, miRNA-15b, miRNA-16, miRNA-103a, miRNA-107, miRNA-195, miRNA-424, miRNA-497, miRNA-503, and miRNA-646 [41]. These miRNAs family regulate gene expression involved in cell division, metabolism, stress response, and angiogenesis in vertebrate species [42]. A report by Wohlfarth et al. [43] demonstrated a single nucleotide polymorphism (SNP) within the 5-hydroxytryptamine [serotonin (5-HT)] receptor 4 (HTR4) in IBS-D patients. This SNP affects a binding site for miRNA-16 and miRNA-103, altering the HTR4 expression. In addition, they have shown that downregulation of miRNA-16 and miRNA-103 in the jejunum of IBS-D patients correlating with symptoms, suggesting that HTR4 might be involved in the development of IBS-D [43]. HTR4 is expressed in the intestinal mucosa, where it is distributed differentially and expressed by several epithelial cell types [44]. This 5-HT receptor has been one of the most widely studied regarding GI function and its regulation and blocking have been used for the treatment of constipation and visceral pain [45]. In conclusion, the regulation of miRNA-16 and miRNA-103 may impact HTR4 receptor levels and functions, predisposing carriers to an IBS-D phenotype.

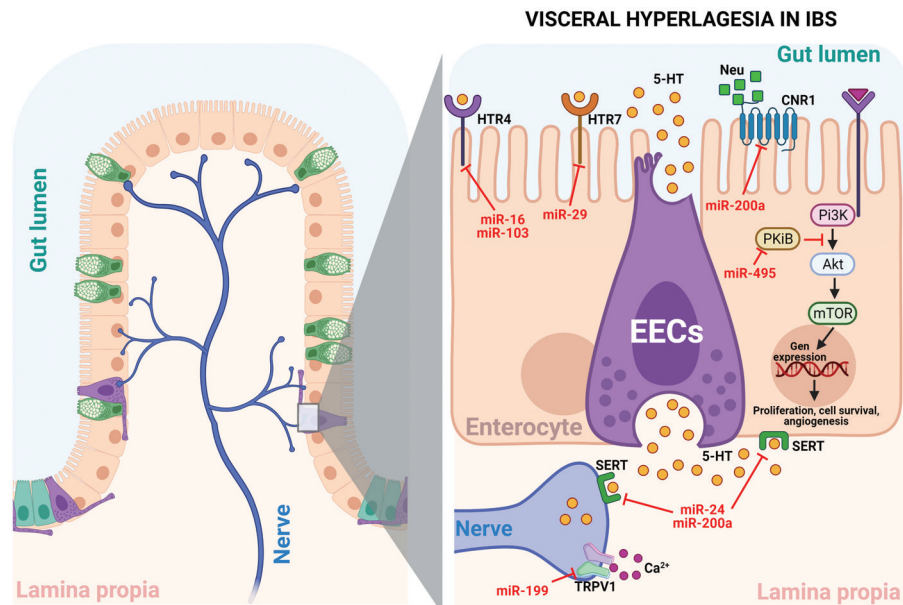


Fig. 2. Regulation of miRNAs expression on visceral hyperalgesia in IBS. Schematic diagram of the differential expression of miRNAs on visceral hyperalgesia in IBS. MiRNA-16 and miRNA-103 are downregulated in the jejunum of IBS-D patients and the overexpression of these miRNAs results in regulation of HTR4 expression. MiRNA-29 expression is upregulated in colon tissues of IBS patients and targeted HTR7, overexpression of HTR7 in miRNA-29 KO mice attenuated visceral hyperalgesia. MiRNA-199 negatively regulated TRPV1 expression in afferent nerve fibers of IBS patients and decreased visceral hyperalgesia and pain. The miRNA-24 and miRNA-200a targeted SERT, leading to a reduction in serotonin uptake. Overexpression of miRNA-495 reduces visceral hyperalgesia through suppression of the PI3K/AKT signaling pathway. MiRNA-200a is overexpressed in colonic tissue of IBS patients and binding at the 3' UTR of CNR1. HTR4: serotonin receptor 4; HTR7: serotonin receptor 7; 5-HT: serotonin; Neu: neurotransmitter; CNR1: cannabinoid receptor 1; EECs: enteroendocrine cells; SERT: serotonin reuptake transporter; Ca²⁺: calcium; PKiB: protein kinase inhibitor peptide beta; Pi3K: phosphatidylinositol 3-kinase; mTOR: mammalian target of rapamycin.

MicroRNA-24

MicroRNA-24 is part of a gene cluster of miRNAs, including the two gene clusters (miR-23a and miR-23b) and three miRNA gene families (miR-23, miR-27, and miR-24) [46]. Recently, the miR-24 expression has been linked to regulating serotonin reuptake transporter (SERT) expression in intestinal epithelium cells of IBS patients and TNBS-induced IBS mice model [47]. Liao et al. [47] demonstrated by Luciferase report assay that miRNA-24 targeted SERT and regulated its expression, which might be implicated in the process of visceral hypersensitivity in IBS. Serotonin reuptake transporter plays a critical role in the uptake and internalization of extracellular 5-HT in the GI tract. Multiple factors such as immunity activation, inflammatory response, gut microbiota, and their relationships have been suggested to regulate SERT expression in post-infectious IBS patients [48]. The study carried out by Coates et al. [49], described a downregulation of SERT expression in ulcerative colitis (UC) and IBS patients, indicating that changes of this 5-HT receptor may contribute directly to the symptoms associated with IBS and other disorders of GI function. This finding suggests that miR-24 is a potential molecular target for the pathogenesis of IBS through regulation of SERT expression.

MicroRNA-29

In addition to the role in increased intestinal permeability, the miRNA-29 expression in visceral hyperalgesia has been described. Zhu et al. [50] have shown that miRNA-29a expression is upregulated in colon tissues of IBS patients and water avoidance stress (WAS)-induced IBS mice. Also, they observed overexpression of 5-HT receptor 7 (HTR7) levels and attenuated visceral hyperalgesia in miRNA-29a KO WAS-induced IBS mice. Additionally, they confirmed that miRNA-29a targeted HTR7 and regulated its expression using Luciferase reporter assay, which might be implicated in the process of visceral hypersensitivity in IBS [50]. 5-Hydroxytryptamine receptor 7 is one of the several different 5-HT receptors. It is expressed abundantly both in peripheral tissues such as smooth muscle and intestine, and in brain regions including the forebrain, hippocampus, hypothalamus, brainstem, and cerebellum [51, 52]. 5-Hydroxytryptamine is a neurotransmitter with a broad range of physiological functions including sleep, mood, cardiovascular function, circadian rhythms, body temperature, food intake, and endocrine regulation [53]. Dysregulation of 5-HT may also be involved in the pathophysiology of several functional GI disorders, such as chronic constipation, IBS, and FD [52]. In summary, this study indicated that abnormal expression of HTR7 by overexpression

of miR-29a might contribute to clinical symptoms such as visceral hyperalgesia in IBS, and HTR7 is a possible candidate for therapeutic interventions.

MicroRNA-199

The miRNA-199a/b family is composed of three members, miRNA-199a1, miRNA-199a2, and miRNA-199b. The biological effect of this miRNA family on the regulation of visceral pain has been demonstrated by Zhou et al. [54]. They used a microarrays assay for miRNA profiling of colonic tissue from IBS-D patients and controls. Of the 460 human miRNAs represented in the microarray assay, they showed that colonic miRNA-199a/b expression was significantly decreased in IBS-D patients compared to controls. The results were verified using a quantitative real time polymerase chain reaction (RT-PCR) assay [54]. Then, they correlated miR-199a/b expression in the colon of patients with IBS-D with the visceral pain scores and revealed an inverse correlation between miRNA-199a expression and visceral pain scores in patients with IBS-D. Additionally, they observed that upregulation of miRNA-199 in animal models decreased visceral pain via inhibition of the transient receptor potential cation channel subfamily V member 1 (TRPV1) signaling. TRPV1 is a nonselective cationic ligand-gated channel located extensively on neuronal cells or nonneuronal cell membranes with high permeability to Ca²⁺ [55]. Emerging evidence has suggested that TRPV1 regulation contributes to GI hyperalgesia in IBS patients. A report by Akbar et al. [56] demonstrated that upregulation of TRPV1 in afferent nerve fibers of IBS patients may contribute to visceral hyperalgesia and pain [56]. In summary, the results shown above suggest that downregulation of TRPV1 expression by miRNAs is a new potential strategy for treating visceral pain in patients with IBS.

MicroRNA-200

The miRNA-200 family consists of five members, which form two clusters located in two different chromosomes. The miRNA-200 family is highly conserved among vertebrate species and highly expressed within epithelial cells [57]. Recently, decreased levels of the miRNA-200 family have been reported in colonic tissue from CD patients with fibrosis [58]. Other biological functions have been described in the regulation of miRNA-200 family expression in the GI tract. A recent report by Hou et al. [59] has evaluated the role of miRNA-200a on visceral hyperalgesia in the IBS-D rat model. The microarray assay revealed differential miRNA expression of distal colon tissue of IBS-D rats (9 upregulated and 15 downregulated). Then, they carried out a quantitative RT-PCR to confirm the high expression of miR-200a into the colonic tissues. Using bioinformatic programs, they showed that miR-200a binding at the 3'UTR of cannabinoid receptor 1 (CNR1) and SERT. Additionally, they observed that CNR1 and SERT were lower expressed in the IBS-D rat model group compared to the control group and overexpression of CNR1 and SERT partially rescued the inhibitory effects of miRNA-200a. The regulation of SERT expression by miRNAs to decrease visceral hyperalgesia in IBS-induced models has been previously elucidated [47], indicating that SERT is an important molecule implicated in the visceral pain in IBS. Cannabinoid receptor 1

is mostly expressed in the nervous system at both central and peripheral sites, including the enteric nervous system [60]. Several studies have demonstrated that activation of CNR1 produces analgesic effects in several experimental pain models, including visceral pain arising from the GI tract [61] and its inhibition expression may enhance visceral sensitivity and susceptibility to UC [62]. In conclusion, miRNA-200a plays a role in visceral hypersensitivity and directly targets CNR1 and SERT, indicating that regulation for miR-200a could be a potential new therapeutic method for reversing visceral hypersensitivity.

MicroRNA-495

MicroRNA-495 is a small non-coding RNA encoded by a gene located on chromosome 14q32.31 and contains a single exon. In humans, miRNA-495 has two mature types, miRNA-495-5p and miRNA-495-3p [63]. MicroRNA-495 has been associated with the regulation of immune response in Crohn's disease by downregulated nucleotide binding oligomerization domain containing protein 2 (NOD2) [64]. The expression of pro-inflammatory [interleukin (IL)-6, IL-8, and Tumor Necrosis Factor (TNF)- α] and anti-inflammatory (IL-10) factors are influenced by NOD2 [65]. A study conducted by Fei et al. [66] provided evidence about the effect of miRNA-495 on visceral sensitivity in IBS. They have shown that the protein kinase inhibitor peptide beta (PKIB) gene is increased in the rectal mucosal tissue of the IBS-D mice model compared to the control group. Additionally, they demonstrated that PKIB is the target gene of miRNA-495. The silencing of the PKIB gene and/or the overexpression of miR-495 could reduce visceral hyperalgesia in the IBS-D mice model through suppression of the phosphatidylinositol 3-kinase (PI3K/AKT) signaling pathway [66]. PI3K/AKT signal transduction pathway is frequently activated in malignance and participates in multiple kinds of cellular processes underlying tumor cell proliferation and migration [67]. Further, activation of this signaling pathway results in the regulation and release of pro-inflammatory such as TNF- α and IL-1 β in IBD [68] and its regulation reduces visceral hyperalgesia and IBS-D symptoms in animal models of IBS [69]. These studies provide relevant information about the PI3K/AKT pathway in GI disorders, indicating that it could be a therapeutic target to reduce visceral pain in IBS patients.

Contribution of MicroRNAs in Low-grade Inflammation in IBS

Inflammation is generally defined as a prompt biological response of the immune system that occurs to stress stimulation with outside pathogens such as bacteria, viruses, fungi, and parasites; endogenous signals from post-injury cell damage that results in tissue repair; extreme weather exposition (burns); harmful chemical stimuli intake (drugs, tobacco, and alcohol); allergic proteins; or specific host pathologies when the immune response is directed towards to own tissue components [70]. This complex process involves the recruitment of immune and non-immune components such as leukocytes, macrophages, dendritic cells, cytokines, platelets, and plasma proteins into disturbed tissue [71]. This migration is facilitated by alterations in the local vasculature that lead to vasodilation, increased

vascular permeability, and increased blood flow. Several observations have supported that chronic activation of the immune system in a specific tissue such as the GI tract after infection by invasive pathogens such as *Shigella*, *Salmonella*, and *Clostridioides difficile*, contributes to the pathophysiology and symptom of many intestinal inflammatory diseases include IBD and IBS [72]. In addition, abnormal systemic and mucosal inflammation has been described in a group of patients with IBS. Peripheral blood CD4⁺ and CD8⁺ T cells, as well as lamina propria CD8⁺ T cells, are increased in IBS patients, supporting the hypothesis of IBS patients being a low-grade mucosal inflammation [73]. Recently, various reports have investigated the contribution of miRNAs in the resolution of low-grade inflammation in IBS (Fig. 3).

MicroRNA-181

In the human genome, the miRNA-181 family is composed of four different miRNAs (miRNA-181a/b/c/d). MiRNA-181 family members are distributed in three independent genomic clusters localized to three separate chromosomes, 1, 9, and 19 [74]. Experimental evidence obtained in different laboratories demonstrates that the miRNA-181 family regulates many relevant biological processes such as cell proliferation, apoptosis, autophagy, mitochondrial function, and immune response [75]. A research study carried out by Ji et al. [76] disclosed that silencing the IL1A gene by miR-181c-5p results in decreased low-grade inflammation in colonic tissue of rats with IBS. First, they used microarray analysis to retrieve the genes related to IBS and to predict miRNAs regulating the IL1A gene in the IBS rat model. Subsequently, they carried out

ectopic expression, depletion, and reporter assay experiments to determine the functional role of miRNA-181c-5p in the IBS rat model. Finally, they investigated the expression of pro-inflammatory cytokines such as TNF- α , IL-2, and IL-6 to determine the effects of miRNA-181c-5p and IL1A gene on inflammation in IBS. IL-1 α is constitutively expressed in many cell types in healthy tissues at a steady state, and its expression can be increased in response to growth factors and proinflammatory or stress-associated stimuli [77]. It has been reported that high levels of IL-1 family member cytokine, IL-1 β , modulates the production of other inflammatory cytokines, including IL-8, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), and TNF- α , in cultures of human primary epithelial cells [78]. Recent evidence has been indicated a differential production of proinflammatory cytokines in patients with IBD versus IBS or healthy controls, indicating that IL-6 seems to be the most important pro-inflammatory cytokine in IBD patients, while TNF- α could play a more significant role in IBS pathogenesis [79]. Taken together, the findings of this study provide evidence that miRNA-181c-5p overexpression could downregulate IL1A gene expression and result in an anti-inflammatory effect in IBS, which provides a new therapeutic target for IBS inflammation.

MicroRNA-510

This mature miRNA sequence is 22 nucleotides long and is found in the human X chromosome. MiRNA-510 belongs to the miRNA-506 family that contains 21 miRNAs that are transcribed from five large miRNA clusters region near Slitrk2

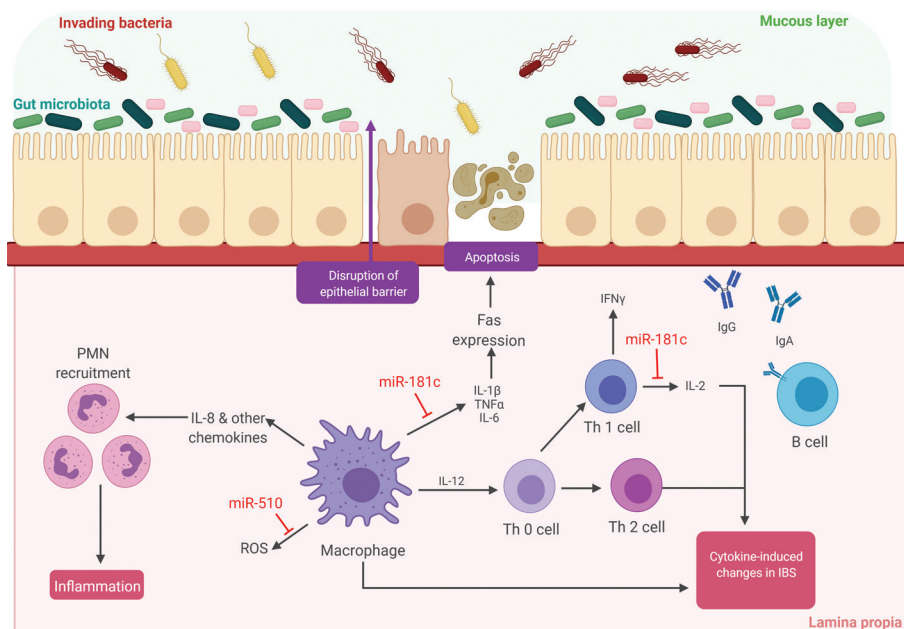


Fig. 3. Role of miRNAs in the regulation of inflammatory processes in IBS

Silencing the IL1A gene by miRNA-181c results in decreased low-grade inflammation in colonic tissue of rats with IBS. Also, the expression of TNF- α , IL-2, and IL-6 was decreased by overexpression of miR-181c. The expression of miR-510 is downregulated and negatively correlated with TNF- α levels in colon tissue in IBS. miR-510 targeted PRDX1 and overexpression of miR-510 significantly suppressed the mRNA and protein expression levels of PRDX1 and ROS production. PMN: polymorphonuclear cells; ROS: reactive oxygen species.

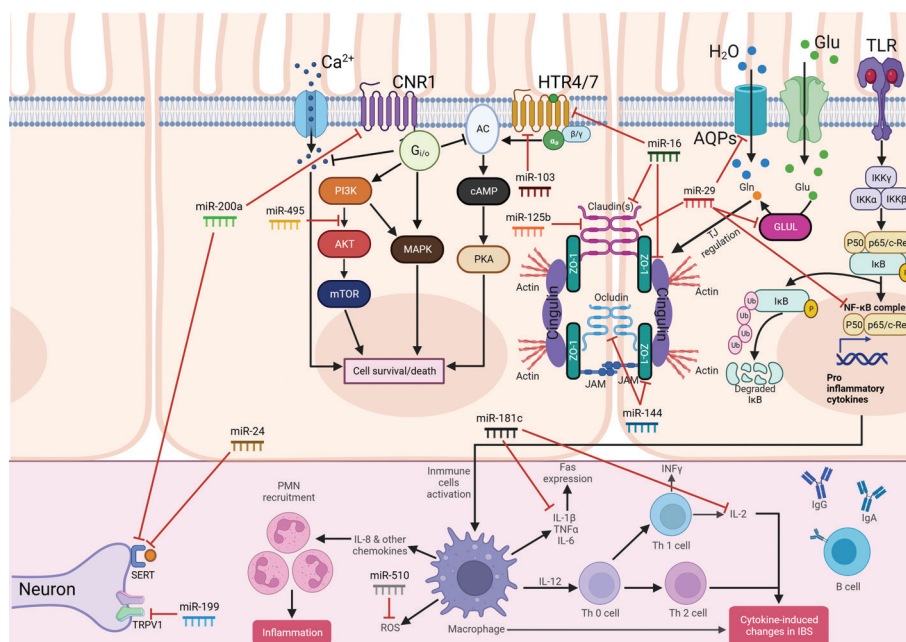


Fig. 4. Mechanistic overview of microRNA (miRNA) role in irritable bowel syndrome (IBS). MiRNAs can act as both suppressive and an enhancer over different targets and inhibit or promote different cellular process involved in the development of IBS symptoms.

and one large miRNA cluster region close to *Fmr1* on the X chromosome [80]. A study conducted by Zhang et al. [81] using quantitative RT-PCR, Western blot, and immunochemistry techniques, revealed that miRNA-510 expression was downregulated and negatively correlated with TNF- α levels, whereas Peroxiredoxin 1 (PRDX1) expression was upregulated in colonic mucosal tissue of post-infectious IBS (PI-IBS) patients. Subsequently, they demonstrated that overexpression of miRNA-510 increased cell viability, decreased apoptosis, and reduced production of proinflammatory cytokines in a colorectal adenocarcinoma cell culture stimulated with LPS. Additionally, they showed that miRNA-510 mimic transfection in cells significantly suppressed the mRNA and protein expression levels of PRDX1. PRDX1, which belongs to the PRDX family, is composed of thiol-specific antioxidant enzymes that reduce H_2O_2 and peroxynitrite [82]. PRDX1 is associated with the mitigation of oxidative damage and severity of colitis activity in UC-associated tumorigenesis patients [83]. Also, PRDX1 interacting with Toll-like receptor 4 (TLR-4), which triggers NF- κ B activation and other signaling pathways to release proinflammatory cytokines [84]. In summary, the findings demonstrate an anti-inflammatory role of miRNA-510 in PI-IBS patients and LPS-induced damage in colorectal adenocarcinoma cell culture and provide an important experimental basis for the development of effective PI-IBS drugs based on the miRNA-510 expression.

CONCLUSIONS

Irritable bowel syndrome is one of the most common disorders of the gut worldwide, defined according to patterns of gastrointestinal symptoms as described by the Rome diagnostic criteria. The development and persistence of IBS symptoms have been acknowledged as multifactorial, making treatment

of the disorder a complicated, clinical endeavor. Approaches are based on the reduction of patient symptomatology, which can be targeted for therapy with a variety of pharmaceutical and nonpharmaceutical agents. However, the current pharmacological management often provides suboptimal relief, resulting in the research for the new therapeutic tools that allow to completely resolve the symptoms in IBS. In this review, we have highlighted recent work in identifying IBS through the dysregulated expressions of miRNAs and the roles that they play in the symptoms of IBS (Fig. 4). MicroRNAs play an important role in the pathogenesis and symptoms development in IBS and they can be considered as novel therapeutic targets to mitigate IBS symptoms. However, further research should be undertaken to promote the clinical application of miRNAs in IBS.

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