

Microbiota Small RNAs in Inflammatory Bowel Disease

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ABSTRACT

MiRNAs are a class of potential gene regulators of critical importance in Inflammatory Bowel Disease (IBD). This review aims to present the connection between gut microbiota, probiotics administration and microRNA (miRNA) expression in IBD. It also brings into question cross-kingdom RNAi (RNA interference). Not only that gut host cells garden the intestinal microbiome via miRNA, but also strong evidence supports the idea that different species of bacteria have an impact on the intestinal immune response by modulating miRNA expression. Cross-kingdom RNAi refers to RNA silencing signals that travel between two unrelated, interacting organisms. RNAs communication between prokaryotes and eukaryotes (bacteria and nematodes) via RNAs transfer has been proved. Some authors also support the idea that non-coding RNAs are being transferred by bacterial pathogens to the host cells as part of the intracellular infection process. Further studies are required in order to clarify whether the mechanism by which bacteria modulate miRNA expression concerns RNAs transfer. These findings may lead to a different approach to IBD therapy in the future.

Key words: bacteria – gastrointestinal microbiome – microRNAs – probiotics – RNA interference.

Abbreviations: AChE: Acetylcholinesterase; AIEC: Adherent-invasive E coli; ATF: Activating transcription factor; Bcl-2: B-cell lymphoma 2; BMDC: Bone marrow-derived dendritic cells; C elegans: Caenorhabditis elegans; CCL: Chemokine C-C motif ligand; CD: Crohn's disease; CDC42: Cell division control protein 42 homolog; CXCL: Chemokine (C-X-C motif) ligand; DC: Dendritic cells; E Coli: Escherichia coli; EcN: E coli Nissle; EPEC: Enteropathogenic E coli; FOXO3: Forkhead box protein O3; GF: Germ-free; IBD: Inflammatory bowel disease; IECs: Intestinal epithelial cells; IGLC: Immunoglobulin Lambda Constant Region; IκB: Inhibitor of NF-Kb; IL: Interleukin; IRGM: Immunity-related GTPase family M protein; L del: Lactobacillus delbrueckii; LGG: Lactobacillus rhamnosus GG; MAPK: Mitogen-activated protein kinases; miRNA: MicroRNA; mRNA: Messenger RNA; MyD88: Myeloid differentiation primary response gene 88; NF-κB: Nuclear factor kappa B; NOD2: Nucleotide-binding oligomerization domain-containing protein 2; PAR: Partitioning defective protein; RhoB: Ras Homolog Family Member B; RISC: RNA induced silencing complex; RNAi: RNA interference; SHH: Sonic hedgehog (gene); SPF: Specific-pathogen-free; SRNA: Small RNA; STAT3: Signal transducer and activator of transcription 3; TGF: Transforming growth factor; Th17: T helper 17 cells; TJ: Tight junction; TLR: Toll like receptor; TNF: Tumor necrosis factor; UC: Ulcerative colitis; Xcv: Xanthomonas campestris pv. Vesicatoria; ZO-2: Zonula occludens-2.

INTRODUCTION

MicroRNAs (miRNAs) are single-stranded non-coding RNA molecules of 19-25 nucleotides discovered in all eukaryotic cells except fungi. MiRNAs are transcribed from intronic, intergenic or exonic DNA as pri-miRNA transcripts. Before being exported in the

cytoplasm, the pri-miRNA is processed into a 70-base pair stem loop precursor miRNA (pre-miRNA). Once in the cytoplasm, the endonuclease Dicer cleaves the pre-miRNA into a small double-stranded RNA duplex, and then a single strand is loaded into the RNA-induced silencing complex (RISC) containing an Argonaute protein, the catalytically active RNase, forming a mature miRNA complex capable of silencing mRNA via 3'untranslated region of target mRNA. mRNA transcripts targeted by miRNAs are either silenced if the base-pair match is imperfect or degraded if there is an identical base-pair match. According to this fact, each miRNA

can target hundreds of mRNAs and a single mRNA may have multiple 3' untranslated region binding sites allowing targeting by multiple miRNAs [1, 2].

Inflammatory bowel diseases (IBD) include Crohn's disease and ulcerative colitis. Inflammatory bowel diseases are chronic, progressive diseases characterized by aberrant immune responses to environmental and gut microbial triggers in possibly genetically susceptible hosts [3]. MiRNA is recognized to play an essential role in the development of the innate and adaptive immune system. There is strong evidence that miRNAs are a class of potential gene regulators of critical importance in IBD, as shown in Table I. It has been

also demonstrated that miRNAs have the possibility to be used as biomarkers and therapeutic targets, especially because miRNA expression changes according to the status of tissue inflammation in IBD patients [4, 5].

Clinical, genetic, and experimental data support the role of gut microbes in initiating and perpetuating intestinal inflammation in IBD [3, 6]. Four broad mechanisms explain the complex relationship between the commensal microbiota and IBD: (i) dysbiosis of conventional microbiota; (ii) induction of intestinal inflammation by pathogens and commensal bacteria; (iii) host genetic defects; and (iv) defective host immune-regulation [3]. Probiotic therapies modify disease symptoms by

Table I. Implication of miRNAs in the pathogenesis of inflammatory bowel diseases

miRNA	mRNA/ pathway target	Roles	Ref.
miR-30C↑	ATG5	Downregulates autophagy	[8]
miR-130a ↑	ATG16L1	Down regulates autophagy	[8]
miR-142-3p ↑	ATG16L1	Targets immune globulin gene IGLC	[9]
miR-93 ↑	ATG16L1	Prevent autophagy-dependent eradication of intracellular bacteria.	[10]
miR-106B ↑	ATG16L1	Prevent autophagy-dependent eradication of intracellular bacteria	[10, 11]
miR-196 ↑	IRGM	Downregulates protective variant of IRGM (c313C)	[12]
miR-29 ↓	IL-12p40 (direct target) IL-12p19 (indirect target via reduction of ATF2)	Contributes to elevated IL-23, therefore contributes to pathogenesis in CD	[13]
miR-10a ↓	IL-12/IL-23p40	Contribute to elevated IL-23, therefore contributes to pathogenesis in CD	[14, 15]
miR-124 ↓	STAT3	Results in proinflammatory response in UC pediatric patients	[16]
miR-21 ↑	RhoB CDC42	Downregulation of miR-21 protects against inflammation and tissue injury.	[17]
miR-146b ↑	siah2	Improves intestinal injury by activating nuclear factor-κB and improving epithelial barrier function.	[18]
miR-192 ↓ miR-495 ↓ miR-512 ↓ miR-671 ↓	NOD 2	Downregulate NOD2 expression, suppress NF-κB activity, and inhibit interleukin-8 and CXCL3 messenger RNA expression.	[19]
miR-200b ↓	TGFb	Inhibits epithelial mesenchymal transition and promotes proliferation of IECs. Ameliorates intestinal fibrosis TGF β1 induced	[20]
miR-126 ↑	IκBa	Down-regulates an inhibitor of NF-κB signaling pathway	[21]
miR-7 ↓	CD98 expression	Regulates the expression of CD98 (which is up-regulated in CD)	[22]
miR-155 ↑	Th17 pathway	Loss of miR-155 results in decreases in T helper type 1/ type17, CD11b+, and CD11c+ cells, which correlated with reduced clinical scores and severity of disease.	[23]
miR-141 ↑	CXCL12β	Downregulates CXCL12β-mediated leukocyte migration	[24]
miR-150 ↑	c-Myb	C-Myb regulates Bcl-2, an anti-apoptotic protein.	[25]
miR-132 ↑	AChE	Potentiates the cholinergic blockade of inflammatory reactions.	[26]
	FOXO3a	Suppresses the level of IκBa leading to enhanced NF-κB signaling.	[27]
miR-122 ↑	NOD 2	Inhibits the activation of NF-κB.	[28]
miR-146a ↑	NUMB SHH signaling	Amplifies inflammatory responses.	[29]
miR-223 ↑	FOXO3a	Suppresses the level of IκBa leading to enhanced NF-κB signaling.	[27,30]

favourably altering the bacterial composition, immune status, and inflammation. The rationale for administering strains of live “beneficial” bacteria for IBD is based largely on the premise of dysbiosis [7]. However, it is not yet known whether dysbiosis is a cause or a consequence in IBD. Nevertheless, it is well known that certain bacteria and strains have protective roles against inflammation in IBD, while others are aggressive.

IMPLICATION OF SMALL RNAs IN IBD

Microbiota dependent miRNAs in IBD

The gastrointestinal tract houses a complex bacterial ecosystem consisting of 10¹⁴ cells in humans; about 10 fold greater than the number of human cells. The intestinal microbiome consists mainly of obligates anaerobes such as *Lactobacillus*, *Bacteroides*, *Bifidobacterium*, *Eubacterium* and *Clostridium*. Facultative anaerobes such as *E. coli* are also present [31].

Dysbiosis confers clear susceptibility to IBD. Not only that the mucosal immune system is influenced by its microbial environment, but there is also growing evidence that the immune system shapes the composition of the intestinal microbiome. There is a bidirectional interaction which achieves homeostasis. There is strong evidence that host cells garden the intestinal microbiome, a fact that is critical for health (Fig. 1). The gardening can be affected by genetic variation, diet, stress, acquired immune deficits [32]. A mechanism by which host cells communicate with bacteria involves miRNAs. Host cells send miRNAs which enter bacteria and regulate bacterial gene expression and growth. MicroRNA mediates inter-species gene regulation which facilitates host control of the gut microbiota [33]. Such stranded miRNAs are normal constituents of murine and human feces [33].

It is also known that microbiota modulates expression of the host genes. There is a correlation between microbial diversity and the host transcriptome, as shown when expression profiles of germ-free (GF) mice were different from specific-pathogen-free (SPF) mice [34]. Using miRNA arrays, comparative

profiling of miRNA expression between GF mice and SPF mice revealed 9 miRNAs that were differentially expressed. This suggests that the microbiota modulates the host microRNA expression, which could in turn regulate the expression of hundreds of host genes [35]. Moreover, when investigating the murine caecal miRNA expression signature, 334 miRNAs were detectable in the caecum of GF and conventional mice, and 16 of them were differentially expressed between the two groups. Again, this study supports the idea that gut bacteria may have an impact on the intestinal gene regulation at the post-transcriptional level by modulating miRNA [36].

Previously it was shown that a number of intestinal barrier genes are post-transcriptionally regulated in Dicer knock-out mice, and therefore, they may depend on miRNAs [37]. These genes may also be targets of microbiota-dependent miRNAs. Taking this data into consideration, the authors support the hypothesis that microbiota can impact the intestinal barrier via miRNAs expression modulation. Also, microbiota-dependent miRNAs may influence gastrointestinal disease, especially at the immune response level. They found that miR-455, which was up-regulated in GF mice, targets *hsf1*, a gene involved in inhibiting the production of pro-inflammatory cytokines and induction cell adhesion molecule [36].

Another study revealed that microbiota interferes with the microRNA response upon oral *Listeria monocytogenes* infection [38]. Comparing miRNA profiles of conventional and GF mice during infection with *Listeria* showed that 5 of 6 miRNAs that seem to increase upon *Listeria* infection were dependent on the presence of microbiota.

As presented above, miR-10a is down-regulated in IBD, and it targets IL-12/IL-23p40 [14]. Also, there is increasing evidence that dysbiosis is implicated in the etiology and pathogenesis of IBD [31]. Xue et al. [14] compared the miR-10a expression between the GF mice and SPF mice. It resulted in decreased expression under SPF conditions compared to that in GF mice. Moreover, bone marrow-derived DCs (BMDC) were stimulated with lysates of *E. coli* and A4 bacteria. They have shown that BMDC miR-10a expression was down-

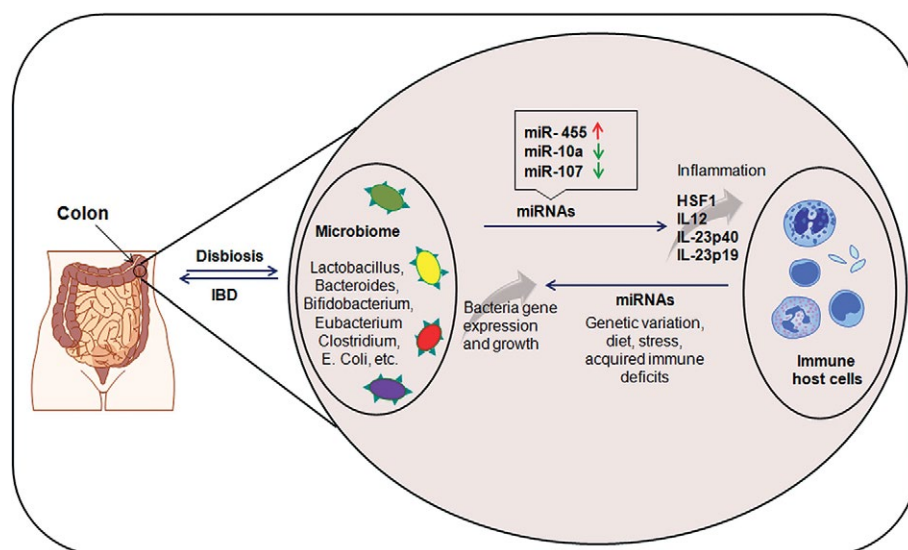


Fig. 1. The role of microbiota dependent miRNAs in Inflammatory Bowel Diseases (IBD). The bidirectional interaction between microbiome and host cells, mediated by miRNAs.

regulated by treatment with *E. coli* and A4 bacteria. Thus, microflora down-regulates miR-10a allowing for expression of IL-12 and IL-23, a fact which might be important in promoting intestinal immune homeostasis by increasing a protective immunity under steady-state or it could contribute to the progression of intestinal inflammation under inflammatory conditions [14].

Similar work was done referring to miR-107. Firstly, it was proven that the inflamed intestine of mice with colitis has lower levels of miR-107 expression and higher levels of IL-23p19; thus more IL-23 produced as compared with normal mice. Secondly, recolonization of the GF mice with normal flora resulted in decreased intestinal miR-107 expression. Moreover, stimulating BMDC with lysates of *E. coli* and A4 bacteria led to down-regulation of miR-107 expression. Certain evidence was presented suggesting that the mechanism by which commensal bacteria negatively regulates miR-107 expression is through interaction of TLR-TLR ligands in a MyD88 and NF- κ B dependent manner. It is still unknown whether this is the sole or the predominant pathway negatively regulating miR-107 *in vivo* or if there might be other pathways involved. The same study demonstrated that miR-107 specifically down-regulates IL-23p19 expression, gene with an expression that seems increased in the inflamed intestinal tissues of mice with colitis. Previously it was demonstrated that miR-10a targets IL-23p40, the other subunit of IL-23 [25]. Therefore, miR-10a and miR-107 could mediate microbiota stimulation of host responses to negatively regulate IL-23. IL-23 production could set up a basic inflammatory environment or contribute to progression of chronic intestinal inflammation [39, 40].

A high prevalence of the adherent-invasive *E. coli* (AIEC) has been shown in the ileal mucosa of CD patients. Adherent-invasive *E. coli* adhere and invade human intestinal epithelial cells (IECs), survive and replicate within macrophages, and produce a proinflammatory response with high secretion of cytokines and chemokines. Thus, AIEC are able to exacerbate intestinal inflammation, especially in a genetically susceptible mouse model. Recent studies found one mechanism by which the host is able to restrain the intracellular replication of AIEC, through autophagy. Regarding this aspect, one study was able to prove that AIEC infection up-regulated the levels of miR-30c and miR-130a in T84 cells and in mouse enterocytes. Up-regulation of these miRNAs seemed to reduce the levels of ATG5 and ATG16L, genes known to be involved in autophagic pathway. This way, through up-regulation of miRNAs, AIEC is responsible for reducing expression of proteins required for autophagy and autophagy response in intestinal epithelial cells, thus conducting to AIEC replication and an increased inflammatory response [8].

Microbiota small RNAs

Similar to eukaryotic cells, myriad of bacterial species harbor small RNAs (sRNAs) that regulate gene expression. Bacterial sRNAs are an emerging class of small regulatory RNAs, 40–500 nt in length, that bind to their mRNA or protein target [41]. Interaction between sRNAs and their specific target can result in positive or negative regulation (Fig. 2). Positive regulation refers to the process where sRNA bind and alter the secondary structure of the mRNA leading to unmasking of a ribosome binding site, the first step in the initiation of

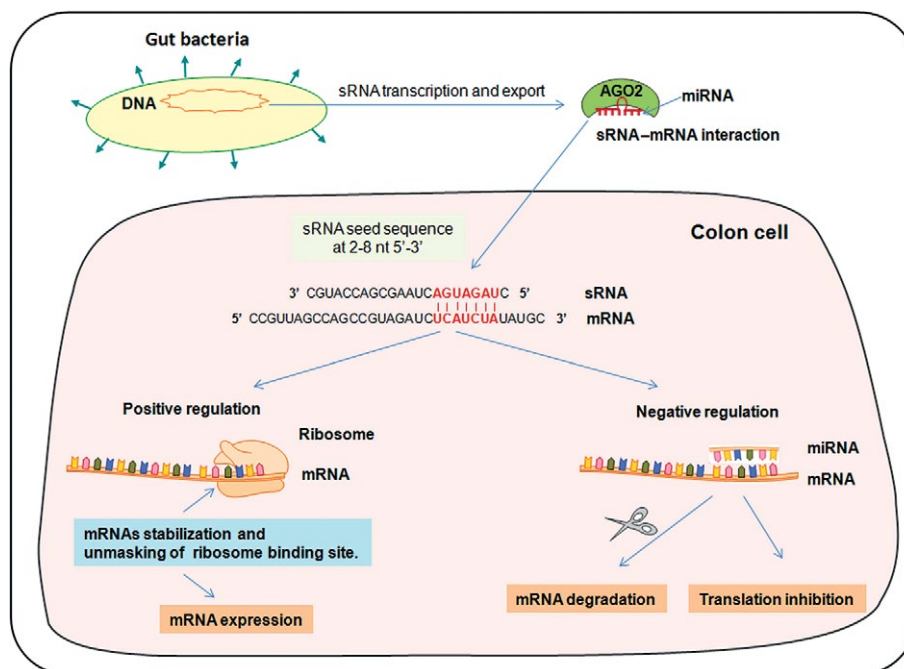


Fig. 2. Interaction between bacteria sRNAs and their mRNAs specific target from the colon cell. After their transcription and export from bacteria, sRNAs enter into the colon cells and based on their seed region complementarities bind to their mRNA targets, producing a positive or negative regulation. Through positive regulation, sRNAs can stabilize mRNAs, unmasking the ribosome binding site, and facilitates gene expression. Through negative regulation, sRNAs leads to altering the function of mRNAs through mRNA degradation and/or their translational inhibition.

translation. There are also studies that demonstrate that sRNA can stabilize mRNA intermediates and full-length mRNAs. Negative regulation refers to the process where sRNA bind to target mRNAs and this interaction leads to the destabilization and subsequent degradation of the mRNA, or it can only inhibit translation by binding to ribosome-binding sites. In addition, sRNA can negatively regulate gene expression by binding and altering the function of post-transcriptionally regulatory proteins. Also, having multiple copies of sRNAs may lead to regulation of expression of the sRNAs themselves [42].

Concerning eukaryotic cells, the seed region is a conserved heptametrical sequence which is mostly situated at positions 2-8 from the miRNA 5'-end. Complementarity between the seed region of a microRNA and the 3'-UTR of its target mRNA is the key determinant in recognition. Therefore, the functional importance of the seed region complementarity as the major determinant of miRNA targeting is well established. However, major role in miRNA-mRNA interaction is attributed to the argonaute protein, too. Interaction between the miRNA seed region and argonaute proteins specifically pre-organize and optimize the conformation of these bases for base pairing with its mRNA target sequence [43].

In prokaryotic cells, the hybridization between sRNAs and their targets are usually dependent on a core interaction of six to eight contiguous bases pairs. Small RNAs are thought to hybridize to well-accessible regions such as hairpin loops or single strand sequence. However, little is known about the mechanisms involved in sRNA-mRNA interaction and further studies are expected to gradually complete our understanding of sRNA-mRNA target interactions [44, 45].

RNA interference (RNAi) refers to non-coding regulatory RNAs that silence genes with complementary sequences. Cross-kingdom RNAi refers to RNA silencing signals that travel between two, often unrelated, interacting organisms. Small RNAs mediate communication between a wide range of species such as humans and *Plasmodium falciparum*, plants and nematodes, fungal pathogens and plants, plants and insects, plants and microbes and bacteria and nematodes. There are numerous studies regarding mammals-parasite and plant-pathogen interaction; however, cross kingdom RNAi does not only refer to eukaryotic cells from different organisms sRNA communication, but it also refers to sRNA transfer from prokaryotes to eukaryotes and from viruses to eukaryotic cells [46, 47].

Liu et al. [48] proved that *E. coli* endogenous sRNAs, OxyS and DsrA could regulate gene expression and physiological conditions of *C. elegans*. In addition, other bacteria such as *Bacillus mycoides* might also utilize its noncoding RNAs to interfere with gene expression in *C. elegans*. The relevant '*C. elegans*-targeting sequences' in both OxyS and DsrA are distinct from the '*E. coli* functional segments' of these RNAs, suggesting that the '*C. elegans*-targeting' effects of these RNAs are probably secondary adaptations. This study supports cross-kingdom RNAi between bacteria and nematodes, and between prokaryotes and eukaryotes, respectively.

It has been shown that the sRNAome of intracellular bacteria is different between *in vitro* conditions and during infection. RNA-seq was used to determine the sRNAome of *Salmonella*, *Yersinia pestis*, *Mycobacterium tuberculosis*, *Listeria*

monocytogenes and the results showed different expression of sRNAs between *in vitro* conditions and *in vivo* infection models [49]. Knocking out of specific sRNAs in *Brucella abortus* resulted in a significant decrease in intracellular survival, and in the plant pathogen *Xanthomonas campestris* pv *vesicatoria* (Xcv) resulted in decreased disease symptoms in infected plants. Similarly, a significant reduction in the lesion diameter was observed in immature pears upon depletion of the sRNAs from the plant pathogen *Erwinia amylovora*. Therefore, the implication of specific sRNA in virulence in pathogens is evident. The real mechanism by which these sRNAs confers virulence in pathogens is not entirely known yet. However, according to the authors, all these observations taken together support the tantalizing idea of some non-coding RNAs being transferred by bacterial pathogens to the host cell as part of the intracellular infection process.

MiRNA IMPLICATION IN PROBIOTICS' ADMINISTRATION

Administration of beneficial bacteria-probiotics is a potential therapeutic option for IBD. Certain mechanisms by which probiotics have beneficial effects consist in competitive exclusion, release of antimicrobial factors, which suppress pathogen growth, enhancement of barrier function and modification of the intestinal immune response [31]. The exact processes by which probiotics have these effects are still unknown. Certainly, they affect signaling pathways that lead to up-regulation of expression of some proteins involved in tight junction stability, mucin secretion, defense enhancement. They also modulate expression of pro- and anti-inflammatory molecules and promote IgA secretion [31]. Also, oral administration of the supernatant of some probiotic bacteria lead to repression of mRNA expression of pro-inflammatory molecules [43, 31]. Another mechanism by which probiotics ameliorate the inflammatory response in mice was proposed in 2004. One study showed that DNA from probiotic bacteria could limit epithelial pro-inflammatory responses *in vitro* as well as *in vivo* [50].

Another study which supports the idea that probiotics modulate the immune system in the gastrointestinal tract was performed on piglets [51]. They were fed with *Enterococcus faecium*. The results consisted in up-regulation of miR-423-5p and down-regulation of the immune globulin gene IGLC, a target gene of miR-423-5p (Fig. 3).

One more step concerning the mechanism by which probiotics influence the immune response in IBD was made by discovering the effect of heat-inactivated *Lactobacillus rhamnosus* GG (LGG) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (L.del) in human monocyte-derived dendritic cells (DCs). Amongst the changes in the expression of TLR4 and signaling factors such as p38, MAPK and I κ B at transcription level, LGG induced a significant down-regulatory effect on miR-146a expression, a negative regulator of immune response, and up-regulatory effect on miR-155 [52]. In IBD, overexpression of miR-146a leads to down-regulation of NUMB, considered its target, and as a consequence to the activation of Sonic hedgehog (SHH) signaling, including inflammatory genes such as IL-12, TNF- α , IL-6, CCL-5, and

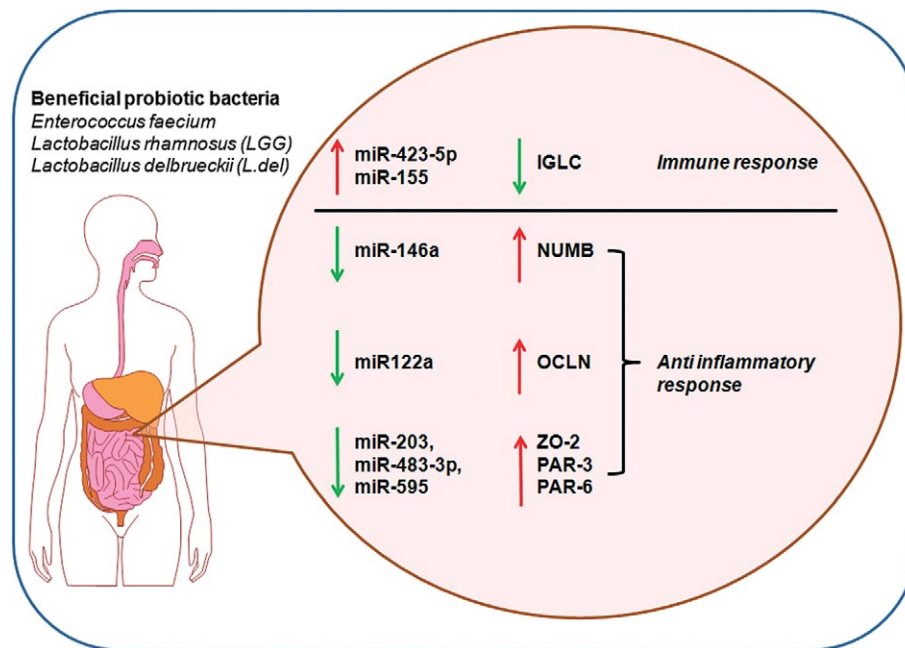


Fig. 3. The positive role of probiotics, inducing anti-inflammatory and immune responses in IBD, based on miRNAs-mRNAs modulation.

CXCL-9 [29]. Therefore, down-regulation of miR-146a might be beneficial in IBD, by decreasing SHH signaling and decreasing expression of some inflammatory genes. On the other hand, miR-155 was up-regulated and, on the contrary, loss of miR-155 correlated with reduced clinical scores and severity of IBD [23].

The previous year, Zhao et al. published a study concerning the effects of the probiotic *Lactobacillus rhamnosus* GG (LGG) in alcoholic liver disease [53]. Chronic ethanol exposure increased the intestinal miR122a expression, which decreased occludin (OCLN) expression leading to increased intestinal permeability. The study concluded that LGGs supplementation functions in intestinal integrity by inhibition of miR122a, leading to OCLN restoration. Also, miR-122a was found to be deregulated in Crohn's disease [28]. It might be interesting to find out whether LGGs supplementation has the same effects in Crohn's disease, too.

Even before that, when comparing miRNA profile of epithelial T84 cells with those of T84 cells that have been co-incubated with *E. coli* Nissle 1912 (EcN) and enteropathogenic *E. coli* strain E2348/69 (EPEC) separately, the results were supportive. The study focused on miR-203, miR-483-3p, and miR-595 which were down regulated by EcN compared to normal and up-regulated by EPEC. *E. coli* Nissle has been known for a long time as a probiotic bacterium that has been used to treat inflammatory intestinal disorders. It resulted that, following infection with EcN, expression of mRNAs for the TJ scaffolding protein ZO-2 and for the regulatory proteins PAR-3 and PAR-6 were found to be up-regulated. Thus, EcN strengthens barrier integrity by up-regulating essential tight junction components. Interestingly, the miRNAs that are down-regulated by this probiotic and up-regulated by the entero-pathogenic *E. coli*, target important regulatory and scaffolding proteins of the TJ complex such as ZO-2, PAR-3, and PAR-6 [54]. It seems that probiotic EcN down-regulates miRNAs that target important

mRNAs, coding for proteins involved in strengthening the barrier integrity.

CONCLUSIONS

In summary, there are beneficial bacteria that regulate the miRNAs known to be dysregulated in IBD, and there are bacteria that have negative effects on the miRNAs known to be involved in the IBD pathogenesis, thus leading to a pro-inflammatory status. This could explain why dysbiosis is linked to IBD.

The mechanism by which bacteria modulates miRNA expression is not yet known. Supposing that there could indeed be an action of bacterial sRNA in eukaryotic cells, the seed region for targeting mRNAs in prokaryotes and eukaryotes contains 6 to 8 nucleotides in both of them. We might assume that there is a possibility that bacteria modulate host genes by sending sRNAs into eukaryotic cells, sRNAs which might interact with eukaryotic mRNA or even miRNA. In any case, this is a novel field of research and numerous studies are needed to truly understand the entire mechanism by which microbiota modulate miRNA expression.

These findings may lead to a different approach in IBD therapy, based on the administration of probiotics, or even bacteria derived sRNAs. Understanding the mechanisms involved may allow us to genetically modify probiotics in order to amplify their beneficial effects.

Conflicts of interest: The authors declare that there is no conflict of interest with regard to this work.

Authors' contribution: A.T.F.: study concept and design, literature search and selection, data interpretation; conceived and drafted the manuscript; O.B.: data interpretation, figures and manuscript revision; C.M.: data interpretation, critical comments and manuscript revision;

A.A.: study concept and design, literature search and manuscript revision for important intellectual content. All authors read and agreed with the final version of the manuscript.

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REFERENCES

- Maudet C, Mano M, Eulalio A. MicroRNAs in the interaction between host and bacterial pathogens. *FEBS Lett* 2014; 588: 4140-4147. doi: [10.1016/j.febslet.2014.08.002](https://doi.org/10.1016/j.febslet.2014.08.002)
- Kalla R, Ventham NT, Kennedy NA, et al. MicroRNAs: newplayers in IBD. *Gut* 2015; 64: 504-517. doi: [10.1136/gutjnl-2014-307891](https://doi.org/10.1136/gutjnl-2014-307891)
- Sartor RB, Mazmanian SK. Intestinal Microbes in Inflammatory Bowel Diseases. *Am J Gastroenterol Suppl* 2012; 1:15- 21; doi: [10.1038/ajgsup.2012.4](https://doi.org/10.1038/ajgsup.2012.4)
- Chen WX, Ren LH, Shi RH. Implication of miRNAs for inflammatory bowel disease treatment: Systematic review. *World J Gastrointest Pathophysiol* 2014; 5: 63-70. doi: [10.4291/wjgp.v5.i2.63](https://doi.org/10.4291/wjgp.v5.i2.63)
- Chapman CG, Pekow J. The emerging role of miRNAs in inflammatory bowel disease: a review. *Therap Adv Gastroenterol* 2015; 8: 4-22. doi: [10.1177/1756283X14547360](https://doi.org/10.1177/1756283X14547360)
- Dalal SR, Chang EB. The microbial basis of inflammatory bowel diseases. *J Clin Invest*. 2014; 124: 4190-4196. doi: [10.1172/JCI72330](https://doi.org/10.1172/JCI72330)
- Tamboli CP, Neut C, Desreumaux P, Colombel JF. Dysbiosis in inflammatory bowel disease. *Gut* 2004; 53: 1-4. doi: [10.1136/gut.53.1.1](https://doi.org/10.1136/gut.53.1.1)
- Nguyen HT, Dalmaso G, Müller S, Carrière J, Seibold F, Darfeuille-Michaud A. Crohn's disease-associated adherent invasive *Escherichia coli* modulate levels of micrornas in intestinal epithelial cells to reduce autophagy. *Gastroenterology* 2014; 146: 508-519. doi: [10.1053/j.gastro.2013.10.021](https://doi.org/10.1053/j.gastro.2013.10.021)
- Zhai Z, Wu F, Dong F, et al. Human autophagy gene ATG16L1 is post-transcriptionally regulated by MIR142-3p. *Autophagy* 2014; 10: 468-479. doi: [10.4161/aut.27553](https://doi.org/10.4161/aut.27553)
- Lu C, Chen J, Xu HG, et al. MIR106B and MIR93 prevent removal of bacteria from epithelial cells by disrupting ATG16L1-mediated autophagy. *Gastroenterology* 2014; 146: 188-199. doi: [10.1053/j.gastro.2013.09.006](https://doi.org/10.1053/j.gastro.2013.09.006)
- Zhai Z, Wu F, Chuang AY, Kwon JH. miR-106b fine tunes ATG16L1 expression and autophagic activity in intestinal epithelial HCT116 cells. *Inflamm Bowel Dis* 2013; 19: 2295-2301. doi: [10.1097/MIB.0b013e31829e71cf](https://doi.org/10.1097/MIB.0b013e31829e71cf)
- Brest P, Lapaquette P, Souidi M, et al. A synonymous variant in IRGM alters a binding site for miR-196 and causes deregulation of IRGM-dependent xenophagy in Crohn's disease. *Nat Genet* 2011; 43: 242-245. doi: [10.1038/ng.762](https://doi.org/10.1038/ng.762)
- Brain O, Owens BM, Pichulik T, et al. The intracellular sensor NOD2 induces microRNA-29 expression in human dendritic cells to limit IL-23 release. *Immunity* 2013; 39: 521-536. doi: [10.1016/j.immuni.2013.08.035](https://doi.org/10.1016/j.immuni.2013.08.035)
- Xue X, Feng T, Yao S, et al. Microbiota downregulates dendritic cell expression of miR-10a, which targets IL-12/IL-23p40. *J Immunol* 2011; 187: 5879-5886. doi: [10.4049/jimmunol.1100535](https://doi.org/10.4049/jimmunol.1100535)
- Wu W, He C, Liu C, et al. miR-10a inhibits dendritic cell activation and Th1/Th17 cell immune responses in IBD. *Gut* 2015; 64: 1755-1764. doi: [10.1136/gutjnl-2014-307980](https://doi.org/10.1136/gutjnl-2014-307980)
- Koukous G, Polyarchou C, Kaplan JL, et al. MicroRNA-124 regulates STAT3 expression and is downregulated in colon tissues of pediatric patients with ulcerative colitis. *Gastroenterology* 2013; 145: 842-852. e2. doi: [10.1053/j.gastro.2013.07.001](https://doi.org/10.1053/j.gastro.2013.07.001)
- Shi C, Liang Y, Yang J, et al. MicroRNA-21 knockout improve the survival rate in DSS induced fatal colitis through protecting against inflammation and tissue injury. *PLoS One* 2013; 8: e66814. doi: [10.1371/journal.pone.0066814](https://doi.org/10.1371/journal.pone.0066814)
- Nata T, Fujiya M, Ueno N, et al. MicroRNA-146b improves intestinal injury in mouse colitis by activating nuclear factor- κ B and improving epithelial barrier function. *J Gene Med* 2013; 15: 249-260. doi: [10.1002/jgm.2717](https://doi.org/10.1002/jgm.2717)
- Chuang AY, Chuang JC, Zhai Z, Wu F, Kwon JH. NOD2expression is regulated by microRNAs in colonic epithelial HCT116 cells. *Inflamm Bowel Dis* 2014; 20: 126-135. doi: [10.1097/01.MIB.0000436954.70596.9b](https://doi.org/10.1097/01.MIB.0000436954.70596.9b)
- Chen Y, Ge W, Xu L, et al. MiR-200b is involved in intestinal fibrosis of Crohn's disease. *Int J Mol Med* 2012; 29: 601-606. doi: [10.3892/ijmm.2012.894](https://doi.org/10.3892/ijmm.2012.894)
- Feng X, Wang H, Ye S, et al. Up-regulation of microRNA-126 may contribute to pathogenesis of ulcerative colitis via regulating NF-kappaB inhibitor I κ B α . *PLoS One* 2012; 7: e52782. doi: [10.1371/journal.pone.0052782](https://doi.org/10.1371/journal.pone.0052782)
- Nguyen HT, Dalmaso G, Yan Y, et al. MicroRNA-7 Modulates CD98 expression during intestinal epithelial cell differentiation. *J Biol Chem* 2010; 285: 1479-1489. doi: [10.1074/jbc.M109.057141](https://doi.org/10.1074/jbc.M109.057141)
- Singh UP, Murphy AE, Enos RT, et al. miR-155 deficiency protects mice from experimental colitis by reducing T helper type 1/type 17 responses. *Immunology* 2014; 143: 478-489. doi: [10.1111/imm.12328](https://doi.org/10.1111/imm.12328)
- Huang Z, Shi T, Zhou Q, et al. miR-141Regulates colonic leukocytic trafficking by targeting CXCL12 β during murine colitis and human Crohn's disease. *Gut* 2014; 63: 1247-1257. doi: [10.1136/gutjnl-2012-304213](https://doi.org/10.1136/gutjnl-2012-304213)
- Bian Z, Li L, Cui J, et al. Role of miR-150-targetingc-Myb in colonicepithelial disruption during dextran sulphate sodium-induced murine experimental colitis and human ulcerative colitis. *J Pathol* 2011; 225: 544-553. doi: [10.1002/path.2907](https://doi.org/10.1002/path.2907)
- Maharshak N, Shenhar-Tsarfaty S, Aroyo N, et al. MicroRNA-132 modulates cholinergic signaling and inflammation in human inflammatory bowel disease. *Inflamm Bowel Dis* 2013; 19: 1346-1353. doi: [10.1097/MIB.0b013e318281f47d](https://doi.org/10.1097/MIB.0b013e318281f47d)
- Kim HY, Kwon HY, Ha Thi HT, et al. MicroRNA-132 and microRNA-223 control positive feedback circuit by regulating FOXO3a in inflammatory bowel disease. *J Gastroenterol Hepatol* 2016. doi: [10.1111/jgh.13321](https://doi.org/10.1111/jgh.13321)
- Chen Y, Wang C, Liu Y, et al. miR-122targetsNOD2 to decrease intestinal epithelial cell injury in Crohn's disease. *Biochem Biophys Res Commun* 2013; 438: 133-139. doi: [10.1016/j.bbrc.2013.07.040](https://doi.org/10.1016/j.bbrc.2013.07.040)
- Ghorpade DS, Sinha AY, Holla S, Singh V, Balaji KN. NOD2-nitric oxide-responsive microRNA-146a activates sonic hedgehog signaling to orchestrate inflammatory responses in murine model of inflammatory bowel disease. *J Biol Chem* 2013; 288: 33037-33048. doi: [10.1074/jbc.M113.492496](https://doi.org/10.1074/jbc.M113.492496)
- Wang H, Zhang S, Yu Q, et al. Circulating MicroRNA223 is a New Biomarker for Inflammatory Bowel Disease. *Medicine (Baltimore)*. 2016; 95(5): e2703. doi: [10.1097/MD.0000000000002703](https://doi.org/10.1097/MD.0000000000002703)
- Mikov MM, Stojancevic MP, Bojic GM. Probiotics as a Promising Treatment for Inflammatory Bowel Disease. *Hospital Pharmacology* 2014; 1: 52-60
- Jacobs JP, Braun J. Immune and genetic gardening of the intestinal microbiome. *FEBS Lett* 2014; 588: 4102-4111. doi: [10.1016/j.febslet.2014.02.052](https://doi.org/10.1016/j.febslet.2014.02.052)

33. Liu S, da Cunha AP, Rezende RM, et al. The Host Shapes the Gut Microbiota via Fecal MicroRNA. *Cell Host Microbe* 2016; 19: 32-43. doi: [10.1016/j.chom.2015.12.005](https://doi.org/10.1016/j.chom.2015.12.005)
34. El Aidy S, Derrien M, Merrifield CA, et al. Gut bacteria–host metabolic interplay during conventionalisation of the mouse germfree colon. *The ISME J* 2013; 7: 743–755. doi: [10.1038/ismej.2012](https://doi.org/10.1038/ismej.2012)
35. Dalmaso G, Nguyen HTT, Yan Y et al. Microbiota modulate host gene expression via microRNAs. *PLOS One* 2011; 6: e19293 doi: [10.1371/journal.pone.019293](https://doi.org/10.1371/journal.pone.019293)
36. Singh N, Shirdel EA, Waldron L, Zhang RH, Jurisica I, Comelli EM. The murine caecal microRNA signature depends on the presence of the endogenous microbiota. *Int J Biol Sci* 2012; 8: 171-186. doi: [10.7150/ijbs.8.171](https://doi.org/10.7150/ijbs.8.171)
37. McKenna LB, Schug J, Vourekas A, et al. MicroRNAs control intestinal epithelial differentiation, architecture, and barrier function. *Gastroenterology* 2010; 139: 1654-1664.e1. doi: [10.1053/j.gastro.2010.07.040](https://doi.org/10.1053/j.gastro.2010.07.040)
38. Archambaud C, Sismeiro O, Toedling J, et al. The Intestinal Microbiota Interferes with the microRNA Response upon Oral Listeria Infection. *MBio* 2013; 4: e00707-13. doi: [10.1128/mBio.00707-13](https://doi.org/10.1128/mBio.00707-13)
39. Xue X, Cao AT, Cao X, et al. Downregulation of MicroRNA-107 in intestinal CD11c⁺ myeloid cells in response to microbiota and proinflammatory cytokines increases IL-23p19 expression. *Eur J Immunol* 2014; 44: 673-682. doi: [10.1002/eji.201343717](https://doi.org/10.1002/eji.201343717)
40. Masotti A. Interplays between gut microbiota and gene expression regulation by miRNAs. *Front Cell Infect Microbiol* 2012; 2: 137. doi: [10.3389/fcimb.2012.00137](https://doi.org/10.3389/fcimb.2012.00137)
41. Gurung M, Moon DC, Choi CW, et al. Staphylococcus aureus produces membrane-derived vesicles that induce host cell death. *PLoS One* 2011; 6: e27958. doi: [10.1371/journal.pone.0027958](https://doi.org/10.1371/journal.pone.0027958)
42. Caswell CC, Oglesby-Sherrouse AG, Murphy ER. Sibling rivalry: related bacterial small RNAs and their redundant and non-redundant roles. *Front Cell Infect Microbiol* 2014; 4: 151. doi: [10.3389/fcimb.2014.00151](https://doi.org/10.3389/fcimb.2014.00151)
43. Lambert NJ, Gu SG, Zahler AM. The conformation of microRNA seed regions in native microRNPs is prearranged for presentation to mRNA targets. *Nucleic Acids Res* 2011; 39: 4827-4835. doi: [10.1093/nar/gkr077](https://doi.org/10.1093/nar/gkr077)
44. Cao Y, Wu J, Liu Q, et al. sRNATarBase: A comprehensive database of bacterial sRNA targets verified by experiments. *RNA* 2010; 16: 2051-2057. doi: [10.1261/rna.2193110](https://doi.org/10.1261/rna.2193110)
45. Harris JF, Micheva-Viteva S, Li N, Hong-Geller E. Small RNA-mediated regulation of host–pathogen interactions. *Virulence* 2013; 4: 785–795. doi: [10.4161/viru.26119](https://doi.org/10.4161/viru.26119)
46. Weiberg A, Bellinger M, Jin H. Conversations between kingdoms: small RNAs. *Curr Opin Biotechnol* 2015; 32: 207-215. doi: [10.1016/j.copbio.2014.12.025](https://doi.org/10.1016/j.copbio.2014.12.025)
47. Knip M, Constantin ME, Thordal-Christensen H. Trans-kingdom cross-talk: small RNAs on the move. *PLoS Genet* 2014; 10: e1004602. doi: [10.1371/journal.pgen.1004602](https://doi.org/10.1371/journal.pgen.1004602)
48. Liu H, Wang X, Wang HD, et al. Escherichia coli noncoding RNAs can affect gene expression and physiology of Caenorhabditis elegans. *Nat Commun* 2012; 3: 1073. doi: [10.1038/ncomms2071](https://doi.org/10.1038/ncomms2071)
49. Ortega ÁD, Quereda JJ, Pucciarelli MG, García-del Portillo F. Non-coding RNA regulation in pathogenic bacteria located inside eukaryotic cells. *Front Cell Infect Microbiol* 2014; 4: 162. doi: [10.3389/fcimb.2014.00162](https://doi.org/10.3389/fcimb.2014.00162)
50. Jijon H, Backer J, Diaz H, et al. DNA from probiotic bacteria modulates murine and human epithelial and immune function. *Gastroenterology* 2004; 126: 1358-1373. doi: [10.1053/j.gastro.2004.02.003](https://doi.org/10.1053/j.gastro.2004.02.003)
51. Kreuzer-Redmer S, Bekurtz JC, Arends D, et al. Feeding of Enterococcus faecium NCIMB 10415 Leads to Intestinal miRNA-423-5p-Induced Regulation of Immune-Relevant Genes. *Appl Environ Microbiol* 2016; 82: 2263-2269. doi: [10.1128/AEM.04044-15](https://doi.org/10.1128/AEM.04044-15)
52. Giali L, Aumueller E, Elmadfa I, Haslberger AG. Regulation of TLR4, p38MAPkinase, IκB and miRNAs by inactivated strains of lactobacilli in humandendritic cells. *Benef Microbes* 2012; 3: 91-98. doi: [10.3920/BM2011.0052](https://doi.org/10.3920/BM2011.0052)
53. Zhao H, Zhao C, Dong Y, et al. Inhibition of miR122a by Lactobacillus rhamnosus GG culture supernatant increases intestinal occludin expression and protects mice from alcoholic liver disease. *Toxicol Lett* 2015; 234: 194-200. doi: [10.1016/j.toxlet.2015.03.002](https://doi.org/10.1016/j.toxlet.2015.03.002)
54. Veltman K, Hummel S, Cichon C, Sonnenborn U, Schmidt MA. Identification of specific miRNAs targeting proteins of the apical junctional complex that simulate the probiotic effect of E. coli Nissle 1917 on T84 epithelial cells. *Int J Biochem Cell Biol* 2012; 44: 341-349. doi: [10.1016/j.biocel.2011.11.006](https://doi.org/10.1016/j.biocel.2011.11.006)