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Maintenance of gut barrier integrity after injury: Trust your gut microRNAs

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Abstract

The gastrointestinal (GI) tract is a highly dynamic structure essential for digestion, nutrient absorption, and providing an interface to prevent gut bacterial translocation. In order to maintain the barrier function, the gut utilizes many defense mechanisms including proliferation, apoptosis, and apical junctional complexes. Disruption of any of these parameters due to injury or disease could negatively impact the intestinal barrier function and homeostasis resulting in increased intestine inflammation, permeability, bacterial dysbiosis, and tissue damage. MicroRNAs are small noncoding RNA sequences that are master regulators of normal cellular homeostasis. These regulatory molecules affect cellular signaling pathways and potentially serve as candidates for providing a mechanism of impaired gut barrier integrity following GI-related pathologic conditions, ethanol exposure, or trauma such as burn injury. MicroRNAs influence cellular apoptosis, proliferation, apical junction complex expression, inflammation, and the microbiome. Due to their widespread functional affiliations, altered expression of microRNAs are associated with many pathologic conditions. This review explores the role of microRNAs in regulation of intestinal barrier integrity. The studies reviewed demonstrate that microRNAs largely impact intestine barrier function and provide insight behind the observed adverse effects following ethanol and burn injury. Furthermore, these studies suggest that microRNAs are excellent

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Authors' Contributions

NLM prepared the first draft which was reviewed and edited by MAC.

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candidates for therapeutic intervention or for biomarkers to manage gut barrier integrity following trauma such as burn injury and other GI-related pathologic conditions.

Summary Sentence:

The studies reviewed in this article focus on microRNAs that largely impact intestine barrier integrity and provide an insight into the mechanism underlying gut barrier disruption in acute injuries.

Keywords

ethanol; inflammation; burn; small RNAs

1 INTRODUCTION

The gastrointestinal (GI) tract performs multiple functions including digestion, nutrient absorption, as well as maintaining a barrier that limits the translocation of bacteria living in the GI tract. Constant exposure to antigens from the diet and commensal bacteria (up to 10^{12} microorganisms per gram of tissue) requires the gut to employ physical and immunologic defense barriers that limit luminal bacteria translocation.[2–7] As shown in Figure 1, the intestine forms a semi-permeable barrier composed of a single layer of columnar epithelial cells that are sealed by tight junction proteins, a semi-permeable protein complex comprised of transmembrane, scaffold, and adaptor proteins.[3,8–10] Although the majority of columnar epithelial cells are absorptive enterocytes, there are other cell types (e.g., goblet cells, M cells, and Paneth cells) that participate in intestinal defense.[2,3,8] Additionally, the intestine contains a mucus barrier composed of mucins secreted by goblet cells, which prevents luminal bacteria from adhering to the epithelial cells.[3] Paneth cells contribute to gut homeostasis by producing a large number of antimicrobial peptides (AMPs). The immune component of the intestine includes a layer of loose connective tissue (lamina propria) beneath the epithelial cells and intestinal lymphoid tissue called Peyer's patches.[2,11] This barrier is tightly regulated by apoptosis, a natural physiologic process resulting in death and removal of unwanted cells,[12–14] and proliferation that counterbalances the constant cell turnover.[15,16]

Together, these components maintain intestinal homeostasis by providing a physical and immunologic barrier. Disruption in any of these barrier functions due to disease, misuse of alcohol, or trauma (e.g., burn injury) may compromise the barrier integrity resulting in increased intestinal permeability.[17,18] Understanding mechanisms by which intestinal barrier components are regulated could provide unique therapeutic strategies to treat pathophysiologic conditions that reduce intestinal barrier integrity.

2 MicroRNAs AND THE INTESTINAL BARRIER

MicroRNAs (miRs) regulate many cellular processes that maintain the intestinal barrier (e.g., apoptosis/proliferation, tight junction proteins, and inflammation; Fig. 1). They are small noncoding RNAs that control gene expression at the post-transcriptional level by

binding to the 3′ untranslated region of their target mRNA resulting in gene silencing.[19,20] As shown in the Figure 1, they undergo a maturation process in order to function as gene silencers. MiRs are transcribed by RNA polymerase II forming a primary miR (pri-miR). The pri-miR undergoes a nuclear cleavage by a microprocessor composed of drosha and its cofactor DiGeorge syndrome critical region gene 8. The nuclear cleavage by the microprocessor forms a 60–70 nucleotide precursor miR (pre-miR), which is exported into the cytoplasm by the ran-GTP dependent exportin-5. In the cytoplasm, the pre-miR is cleaved by dicer and its cofactors trans-activation response RNA-binding protein and protein activator of PKR. The second cleavage forms a 21–24 nucleotide duplex miR consisting of a guide and passenger strand. The passenger strand is usually degraded, whereas the guide strand and an argonaute protein form the miRNA-Induced Silencing Complex (miRISC). The formation of the miRISC permits complementary binding of the seed region of the miR to its target resulting in mRNA degradation or translational repression.[19–21]

MicroRNAs are estimated to target over 60% of all genes and each miR can have multiple target genes.[22,23] Aberrant miR expression can impair normal organ function including the intestinal barrier function. Villin-specific dicer knockout mice exhibited altered intestinal morphology, decreased differentiation, increased intestinal inflammation, and apoptosis. Furthermore, ablation of dicer-1 led to diminished expression and mislocalization of tight junction proteins.[24,25] These observations coincided with diminished barrier integrity providing evidence of the indispensable role of miRs in the intestine.

MicroRNA levels and biogenesis are affected in a number of clinical conditions including trauma/injury, ethanol, and disease.[26–33] Furthermore, as miRs are ubiquitously expressed by cells in both circulation and tissues, their differential expression can be used as diagnostic tools and for possible therapeutic interventions.[31,32,34] MiRs can both be affected and in turn affect cellular process/responses: apoptosis,[35–40] proliferation,[41–47] tight junction protein expression,[29,48–54] ischemia/hypoxia,[26,40,55] inflammation,[26,28,56–64] and the microbiome[65] (Fig. 1) all of which can impact the intestinal barrier function.

3 miRs AND PROLIFERATION AND APOPTOSIS

The intestinal epithelium is one of the most rapidly renewing tissues within the body. The intestine is renewed every 2–3 days in mice and 3–5 days in humans.[16] Intestinal epithelial stem cells develop into transit amplifying progenitor cells and differentiate into various subtypes of intestinal cells. Proliferation occurs in the crypts, allowing for differentiation and migration up the intestinal villi. Proliferation is counterbalanced with the cellular process, apoptosis. Proliferation and apoptosis are tightly regulated in order to maintain the intestinal barrier function. Apoptosis is a natural physiologic process resulting in death and removal of unwanted cells.[12–14] Apoptosis therefore is crucial to enable normal structure and function of the GI tract.[14] Increased levels of apoptosis beyond this normal cellular maintenance however results in an impaired intestinal barrier.[14] It is widely accepted that if the delicate balance between proliferation and apoptosis is disrupted, it can result in increased intestinal permeability. This was clearly described following injury. Burn injury alone or in combination with ethanol decreases intestinal proliferation. Furthermore, ki67 staining was significantly decreased following ethanol and burn injury

compared with sham animals suggesting that the combined insult diminished intestinal epithelial cell proliferation.[66] The balance is further disrupted as ethanol and burn injury increased intestinal apoptosis,[67] which contributes to impaired intestinal barrier function. Understanding the mechanism by which this balance is disrupted can lead to therapeutic strategies to mitigate barrier impairment following trauma as well as in other pathologic conditions that have similar adverse effects. Several studies have illustrated the role of miRs in proliferation[41–47] and intestinal apoptosis.[35–37,39,40,55]

As alterations of cell proliferation is a hallmark of cancer, the majority of work on miRs and proliferation is performed in cancer models. Overexpression of miR-27a and miR-505 influenced cell proliferation and invasion of colonic cancer cells.[46,47] Numerous studies have shown that miR-224 is increased in colorectal cancer patients.[41–45] In vitro studies using HCT-116 and SW-480 colon cancer cell lines demonstrated that up-regulation of miR-224 increased proliferation, and cell migration while promoting cell cycle progression.[41,42,44,45] Aberrant expression of miRs involved in proliferation such as the ones described here could negatively impact barrier integrity. Similarly, miRs have been described to affect apoptosis, the cellular process that counterbalances proliferation in order to maintain the intestinal barrier.[35–39]

miR-150 was significantly elevated in the dextran sodium sulfate (DSS) model of colitis in mice and in active ulcerative colitis in human colonic tissue. The miR-150 target gene, c-Myb, was reduced in DSS-treated colon tissue, which led to decreased levels of the antiapoptotic protein Bcl-2 resulting in increased apoptosis.[35] This relationship between miR-150 and apoptosis, however, may be cell-type specific. Christensen et al.[36] preformed a TaqMan Human MicroRNA Array and profiled expression of 667 miRs including miR-150 in normal colon mucosa and tissue samples from clinical stage II colorectal cancer patients. The group validated these high-throughput analyses in different colon cancer cell lines. The group found that miR-150 only decreased viability in 1 colorectal cancer cell line suggesting that the apoptotic ability of miR-150 may be cell-type dependent. miR-375 however reduced cell viability by inducing apoptosis. miR-375 indirectly affected apoptosis by regulating its target Yes-associated protein 1 (identified using TarBase6.0) whose downstream targets are antiapoptotic genes BIRC5 and BCL2L1 (also known as Bcl-xl).[36]

In silico analyses (TargetScan and MicroCosm programs) were used to identify sequences matches between the miR and the antiapoptotic protein Mcl-1. miR-29a was increased in a mouse model of DSS-induced ulcerative colitis, which resulted in decreased levels of Mcl-1. In vitro analysis in colonic epithelial cells HT29 showed that miR-29a caused apoptosis, by down-regulation of its target molecule Mcl-1, which activated caspase-3.[37] miR-mediated regulation of Mcl-1 have also been investigated in other studies.[38,39] As mRNAs can have numerous binding sites allowing for binding of different miRs, it is not surprising that multiple miRs (miR-125 and miR-29b) have been shown to reduce Mcl-1 levels.[38,39] Together, these studies suggest that aberrant miR expression in the intestines can directly and indirectly disrupt the balance between proliferation and apoptosis and result in barrier disruption. Thus, miRs provide novel therapeutic targets to mitigate intestinal barrier impairment as well as they can be used as markers for prognosis and predictions for barrier disruption.

4 miRs AND TIGHT JUNCTION PROTEINS

Tight junction proteins play an indispensable role in maintenance of intestinal barrier integrity in epithelial and endothelial cells.[3,10] The claudin family and occludin proteins make up the essential tetra-span transmembrane proteins to function in regulation of paracellular flux between intestinal epithelial cells.[68,69] The adaptor proteins zonula occludens (ZO) anchor occludin and claudin proteins to actin cytoskeleton and adherens junction.[8,10,68,70] This protein complex plays an integral role in maintaining the intestinal barrier. Trauma such as burn injury with or without ethanol exposure negatively impact intestinal tight junction proteins.[67,71–73] Better understanding of cellular regulators such as miRs and how they impact tight junction proteins can help prevent intestinal barrier dysfunction observed following major injury and other disease conditions.

Numerous studies show that altered expression of miRs (miR-21, miR-122a, miR-212, miR-429, and miR-874) can directly and indirectly modulate tight junction proteins and apical junctional complexes resulting in increased intestinal permeability.[29,49–51,54,74] miR-122a has been shown to directly target occludin resulting in degradation of its mRNA that negatively impacted intestinal barrier function.[49,54] Administration of 10^9 CFU/day of probiotics *L. rhamnosus Gorbach-Goldin* per day for 4 weeks reduced miR-122a expression resulting in increased occludin protein levels ablating the detrimental effects of chronic ethanol exposure on intestinal barrier integrity.[54] A similar study investigating the role of ethanol exposure and miRs showed that exposure of Caco-2 cells to 0.1 %–1% ethanol for 3 h resulted in increased expression of miR-212 in a time- and dose-dependent manner, which correlated with decreased ZO-1 expression.[29] Reduced ZO-1 levels are associated with impaired intestinal barrier function.[29,74]

MicroRNAs can also target molecules that indirectly result in reduced tight junction expression. miR-21 was up-regulated in both the colon and serum of patients with ulcerative colitis, which is associated with decreased expression of occludin, ZO-1, and Ras homolog gene family, member B (RhoB).[50] Overexpression of miR-21 in Caco-2 cells resulted in a loss of both occludin, ZO-1, and RhoB protein levels while increasing epithelial permeability. The group used Targetscan and miRanda to predict miR-21 targets and identified that miR-21 targeted RhoB expression. They further found that siRNA-mediated ablation of RhoB resulted in decreased occludin and increased permeability.[50] Similarly, occludin can be indirectly targeted by miR-874. In vitro overexpression of miR-874 resulted in increased paracellular permeability, bacterial translocation, and diminished occludin and claudin-1 levels.[52] These studies establish that miRs could influence intestinal barrier integrity by direct or indirect regulation of tight junction protein expression.

5 miRs AND THE MICROBIOME

The gut microbiota is a relatively constant microbe population comprising over 100 trillion organisms consisting of 1,000 bacterial species.[75,76] Alterations in the gut microbiome can lead to pathologic conditions (inflammatory bowel disease, obesity, and diabetes). In particular, trauma (e.g., burn injury or ethanol intoxication combined with burn injury) has the ability to alter the intestinal microbiome and increase gut bacterial load.[71,77,78]

Studies profiling miR expression utilizing either germ-free or antibiotic-treated mice show that the microbiota composition can influence expression of miRs.[65,79–81] Germ-free mice infected with the foodborne pathogen *Listeria monocytogenes* had reduced ability to clear bacteria in multiple tissues compared to conventional (C57BL6/J) mice suggesting that the microbiota is protective against infection. Furthermore, miRs (miR-143, miR-148a, miR-194, miR-200b, miR-200c, and miR-378) were significantly decreased in conventional mice infected with *Listeria*, which coincided with increased expression in several of their predicted targets (identified by Targetscan).[65] These changes were not observed in germ-free mice infected with *Listeria*, with the exception of miR-378, which was significantly elevated 72 h post infection.[65] Other studies have reported similar findings that host miR expression is altered in response to infections with pathogenic bacteria such as *Helicobacter pylori*.[82,83] As the microbiota/host relationship is so interconnected, it is not surprising that host miRs can also influence the microbiota composition.[25]

The host can influence microbiota composition and growth through intestinal epithelial cell and intestinal epithelial +4 niche stem cells expressing cell derived miRs. These miRs are released via extracellular vesicles into the feces where they can enter bacteria and control bacterial growth and gene expression. It is unclear how the miRs enter bacteria and how the miRs are processed by the bacteria; however, the fecal miRs could be binding to complementary binding sites on bacterial genes. Furthermore, dysregulation of host-derived miRs using mice with an intestinal epithelial cell deficiency in dicer expression resulted in gut microbiota dysbiosis exacerbating DSS-induced colitis.[25] These studies display the essential symbiotic relationship between the host and the microbiota, which in part is shaped by miRs. These studies reveal that the interplay between the host and microbiota is mediated through miRs shaping the intestinal microbiota and the microbiota in turn modifies miR expression. This suggests that the microbiota/miR crosstalk plays an important role in cultivating the gut microbiota composition.

6 miRs ROLE IN ISCHEMIA/HYPOXIA

Ischemia is a major consequence in the intestine following trauma and burn injury, where blood flow is redistributed to more vital organs resulting in hypoxia (diminished oxygen delivery) in the gut.[84–87] Elevated levels of hypoxia-inducible factor (HIF) -1α, a marker of hypoxia, in the gut has been associated with diminished intestinal epithelial and endothelial barrier function.[85,88] HIF-1 (heterodimer composed of HIF-1α and HIF-1β) also plays a role in site repair and healing after hypoxic conditions. As described by Ahluwalia and Tarnawski,[89] elevated HIF-1α stabilization in endothelial cells induces expression of vascular endothelial growth factor leading to reestablishment of the microvascular network allowing for mobilization of blood flow.

Hypoxia/ischemia influences both expression of miRs and miR biogenesis components.[40,52,55,90–94] Intestinal ischemia/reperfusion (I/R) injury leads to intestinal injury through increased inflammation, overproduction of reactive oxygen species, and apoptosis. Using the miRNA target prediction tools miRanda, TargetScan, and PicTar, the group identified miRs that target Sirtuin-1. In particular, miR-34a was significantly up-regulated following I/R. Knockdown of miR-34a was shown to increase Sirtuin-1, which

reduced I/R-related oxidative damage and apoptosis.[40] In a similar study, miR-682 was identified by TargetScan to target phosphatase and tensin homology (PTEN). miR-682 mediated intestinal I/R injury by targeting PTEN. In vivo overexpression of miR-682 prior to I/R injury significantly reduced apoptosis and other effects of I/R injury.[55] Chassin et al.[26] utilized a mouse model of I/R to show the connection between miR-146a and IRAK1 expression. The group demonstrated that increased IRAK1 due to I/R injury resulted in increased expression of the proinflammatory chemokine CXCL2, apoptosis, and intestinal permeability.[26] In vivo induction of miR-146a via diindolylmethase (DIM), a miR-146a-inducing agent, or administration of miR-146a reduced I/R-mediated inflammation and IRAK1 elevation. Furthermore, exposure of human intestinal tissues ex vivo to hypoxic conditions elevated IRAK1, which was attenuated with DIM treatment and reduced CXCL8 mRNA expression following LPS stimulation.[26] These studies demonstrate that the relationship between hypoxia and inflammation could be influenced by miRs.

7 miRs ROLE IN INFLAMMATION

Intestinal inflammation is a hallmark of intestinal pathology. Numerous disease conditions/injuries (e.g., ulcerative colitis, Crohn's disease, ethanol, and burn injury[67,72,95–98]) are associated with excess inflammation and exhibit modulation of miR biogenesis and/or miR expression. Inflammation in the gut contributes to increased apoptosis, tissue damage, and dismantling tight junction complexes making it a major contributor of increased intestinal permeability.

MicroRNAs can disrupt inflammatory pathways contributing to increased inflammation following disease and trauma. KEGG pathways developed using Gene Set Enrichment Analysis from the dicer mutants identified that ablation of miRs differentially activated pathways with immune pathways making up a third of the differentially expressed genes.[24] Similarly, the expression of miR biogenesis components (drosha and argonaute-2) were diminished following alcohol and burn injury, which correlated with reduced expression of miR-150. Overexpression of miR-150 in young adult mouse colonocytes reduced LPS-mediated inflammation (IL-6 and KC) compared with empty vector controls.[28] Other models have also demonstrated a similar relationship between miR-150 and inflammatory mediators.[34,99–101]

Furthermore, immunomodulatory miRs (miR-21, miR-146a and miR-155) have been shown to be overexpressed in patients with inflammatory bowel disease, *vibrio cholera* infection, and acute intestinal obstruction.[56,58,59,63,64] Knockout of immunomodulatory miR-21 resulted in a reduction in intestinal inflammation (TNF-α and MIP-2) in a model of DSS-induced colitis while improving survival.[58] Several studies have implicated miR-155 and miR-146a as negative feedback regulators of the inflammatory response modulating signal molecules in the NFκB pathway.

miR-155 influenced splenic T cell release of IFN-γ in a murine model following ethanol exposure prior to burn injury. miR-155 was reduced in T cells following ethanol and burn injury compared with sham-injured animals. There was however no difference in ex vivo T cell release of IFN-γ between miR-155 knockout mice and wild-type mice 1 day

following ethanol and burn injury.[102] These studies suggest that the immunomodulatory role of miR-155 in the gut may be cell-type specific. Interestingly, miR-146a and miR-155 are up-regulated in inflammatory bowel disease and *vibrio cholera* infection, suggesting that these miRs are not involved in the hyperinflammatory response associated with the diseases, but may have a role in the resolution of the disease state. Chronic ethanol exposure (Lieber-DeCarli diet for 5 weeks) significantly elevated miR-155 while significantly reducing the AMP, Reg3β. Knockout of miR-155 in the presence of chronic ethanol intoxication, reduced ethanol-mediated increases in serum endotoxins levels, NFκB activation and inflammation (TNF-α) in the small intestine. In contrast, acute ethanol exposure (5 g/kg ethanol in water for 3 days) resulted in elevated AMPs but did not alter miR-155 expression or TNF-α protein levels; however, it significantly increased NFκB activation.[61]

These studies demonstrate that miRs are particularly important in regulating the inflammatory response in the gut. Interestingly, atypical miR expression may be both protective to the gut or contribute to the pathology of the disease. As inflammation is paramount for intestinal barrier dysfunction, more investigation is required to elucidate the relationship between miRs and inflammation.

8 CONCLUSIONS AND FUTURE DIRECTIONS

As demonstrated by the studies reviewed in this article, miRs expression can be altered in pathologic conditions and that they have a significant role in pathology and resolution of disease. These findings suggest that miRs can be used as diagnostic tools. Furthermore, they can also serve as targets for potential therapeutic interventions. Collectively, the findings in this review exemplify the role of miRs in the regulation of intestinal barrier function. These studies demonstrate that miRs can directly and indirectly affect intestinal apoptosis, proliferation, tight junction protein expression, inflammation, and microbiota composition. There are studies that have investigated the effect of ethanol or trauma including burn injury on miR expression in other organ systems. There is however a big gap in research examining how miRs can be influenced or influence ethanol's effects on trauma particularly in the context of burn injury in the gut. Understanding regulation strategies to maintain the epithelial barrier could lead to therapeutic interventions. Furthermore, aberrant miRs expression can be further exploited and used as biomarkers or for therapeutic design.

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Abbreviations:

AMPs	antimicrobial peptides
DIM	diindolylmethase

DSS	dextran sodium sulfate
GI	gastrointestinal
HIF	hypoxia inducible factor
I/R	ischemia/reperfusion
miRISC	miRNA-Induced Silencing Complex
miRs	microRNAs
pre-miR	precursor microRNA
pri-miR	primary microRNA
PTEN	phosphatase and tensin homology
RhoB	Ras homolog gene family member B
ZO	Zonula occludens

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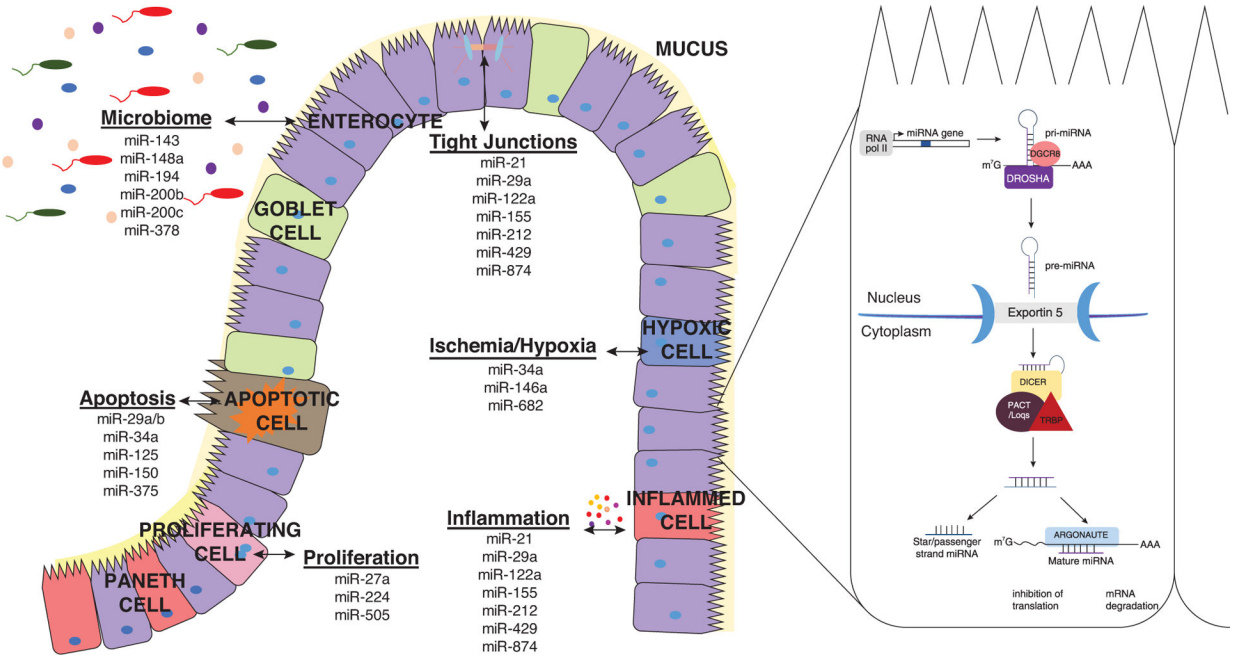

FIGURE 1.

Role of microRNAs in intestinal barrier integrity. Schematic representation of the intestinal barrier. The intestinal barrier can be directly and indirectly affected by cellular regulators, microRNAs. MicroRNAs require a multistep maturation in order to be fully functional. Aberrant expression of microRNAs can in turn affect intestinal barrier integrity by impacting apoptosis, proliferation, tight junction protein expression, ischemia/hypoxia, inflammation, and microbiota composition in the intestine