MicroRNA Effects on Intestinal Health and Mechanism of Action: Postprint

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Abstract

The intestine serves as both the primary site for nutrient digestion and absorption in the organism and an innate barrier against intestinal microbial infection, with intestinal health being crucial for normal growth and development. MicroRNAs (miRNAs) are important post-transcriptional regulators of gene expression. This review summarizes research on intestinal miRNA expression profiles, the roles of miRNAs in proliferation, differentiation, and apoptosis of intestinal cells, their functions in regulating nutrient metabolism, intestinal barrier function, and the progression of intestinal-related diseases, as well as intestinal uptake of exogenous miRNAs, aiming to provide a reference for future research in this field.

Full Text

MicroRNAs: Effects on Intestinal Health and Mechanisms of Action

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Abstract

The intestine serves as the primary site for nutrient digestion and absorption while also functioning as a congenital barrier against intestinal microbial infection, making intestinal health critical for normal growth and development.

MicroRNAs (miRNAs) are important post-transcriptional regulators of gene expression. This review synthesizes current research on intestinal miRNA expression patterns, the roles of miRNAs in intestinal cell proliferation, differentiation, and apoptosis, their regulatory functions in nutrient metabolism, intestinal barrier function, and intestinal disease progression, as well as intestinal uptake of exogenous miRNAs, providing a reference for future research in this field.

Keywords: miRNAs; intestine; effects; mechanisms

1. Intestinal miRNA Expression

miRNAs are abundantly expressed in the intestine. Coutinho et al. [2] detected 559 miRNAs in bovine small intestine, with 187 being small intestine-specific. Sharbati et al. [3] identified 332 miRNAs in piglet intestine, including 201 novel intestinal miRNAs. Additionally, significant differences exist in morphology, nutrient digestion/absorption, and microbial colonization across intestinal segments, corresponding to substantial variation in miRNA expression patterns. Moreover, miRNA expression in the intestine exhibits temporal differences during intestinal tissue development [4].

2. miRNAs and Intestinal Cell Proliferation, Differentiation, and Apoptosis

The intestinal mucosal epithelium is a rapidly self-renewing tissue, and intestinal cell proliferation, differentiation, and apoptosis are closely related to intestinal health. Disruption of this process leads to intestinal dysfunction. Dicer1 enzyme is critical for miRNA synthesis; Dicer1 deficiency causes dramatic increases in apoptosis of crypt cells in mouse jejunum and colon, while also significantly increasing cell migration [5]. miR-29b participates in regulating small intestinal mucosal growth, with overexpression causing cell cycle arrest at G1 phase [6]. miR-375 also regulates intestinal endocrine cell development [7]. Collectively, these findings demonstrate that miRNAs play crucial roles in regulating intestinal cell proliferation, differentiation, and apoptosis, thereby maintaining intestinal structural integrity and normal function.

3. miRNAs and Intestinal Nutrient Metabolism

As the primary organ for nutrient digestion and absorption, the intestine relies on miRNAs to regulate these processes. For example, miR-494 participates in intestinal sodium chloride (NaCl) absorption and electrolyte balance regulation [8], while miR-584 regulates nutrient metabolism in neonatal animals by controlling lactoferrin receptor expression in mice [9]. Intestinal luminal nutrients can also influence metabolic pathway regulation by affecting miRNA expression in intestinal tissue. The trace element selenium regulates arachidonic acid metabolism, glutathione metabolism, and oxidative stress pathways in Caco-2

cells through relevant miRNAs [10]. Polyphenols such as quercetin affect intestinal iron absorption and transport by regulating intestinal miRNA expression [11]. Furthermore, miRNAs mediate nutritional regulation of disease processes. High red meat consumption increases colon cancer risk by elevating miR-17-92 expression, whereas resistant starch has the opposite effect [12]. Polyunsaturated fatty acids from walnuts upregulate miRNA expression in mouse colorectal tumor tissues, thereby regulating anti-inflammatory, anti-proliferative, and pro-apoptotic target genes [13]. In summary, miRNAs play important regulatory roles in intestinal nutrient absorption, metabolism, and functional performance, offering novel targets and strategies for nutritional intervention.

4. miRNAs and Intestinal Barrier Function

Intestinal barrier function encompasses the structural and functional components that prevent toxic and harmful substances from crossing the intestinal mucosa into the body, representing a critical safeguard for maintaining internal homeostasis. Impaired barrier function affects overall health and contributes to various diseases.

4.1. Intestinal Mucosal Epithelial Barrier

The intestinal mucosal epithelial barrier comprises the mucus layer on epithelial cell surfaces and intestinal epithelial cells with their intercellular tight junctions. Barrier disruption increases intestinal permeability and bacterial translocation, triggering inflammatory pathological processes. Numerous studies demonstrate miRNA involvement in regulating intestinal epithelial barrier function. The mucus layer covering intestinal epithelium is secreted by goblet cells, and miRNAs regulate small intestinal epithelial goblet cell differentiation [14]. Mice with small intestine-specific Dicer1 deletion show significantly reduced colonic goblet cell numbers and impaired small intestinal mucosal immunity [15]. Additionally, miRNAs regulate tight junction permeability: they mediate alcohol-induced effects on intestinal epithelial tight junction permeability [16], participate in age-related changes in intestinal permeability [17], and regulate stress-induced intestinal epithelial injury [18].

4.2. Intestinal Immune Barrier

The intestinal mucosal immune system constitutes a vital component of the animal immune system, playing a crucial role in resisting pathogenic microbial invasion and maintaining health. The innate immune system provides the first line of defense against pathogens. miRNAs participate in innate immune cell development: miR-181, miR-150, and miR-15/16 promote natural killer cell development, while miR-483 and miR-583 inhibit it [19]; miR-155 and miR-221 regulate dendritic cell apoptosis [20]. miRNAs also participate in innate immune responses, such as miR-122 regulating inflammatory bowel disease progression by targeting the pattern recognition receptor NOD2 [21]. Moreover,

miRNAs play important roles in adaptive immunity, participating in immune cell maturation and activation: miR-29, miR-155, and miR-17-92 regulate Th1 cell differentiation and function [22], while miR-21 controls Th2 cell differentiation through an intrinsic T-cell pathway [23]. miRNAs also influence immune cell function, as miR-146a constrains intestinal T cell population expansion and reduces immunoglobulin A (IgA) production [24]. Additionally, miRNAs regulate intestinal adaptive immune responses and maintain immune homeostasis by controlling expression of immune-related target genes, such as miR-150 with interleukin-1 receptor kinase [25] and miR-212/132 with interleukin-10 [26]. Abnormal miRNA expression in duodenal tissue of celiac disease patients further confirms their important role in intestinal immune barrier function [27].

4.3. Intestinal Microbial Barrier

The intestine harbors a vast microbial community that coexists with the host, constituting the intestinal microbial barrier. Stable intestinal microecology ensures host health. Commensal bacteria interact with the host to promote intestinal homeostasis, with miRNAs participating in these processes. Intestinal commensal microbes influence intestinal miRNA expression profiles: germ-free mice show abnormal expression of 16 miRNAs in the cecum compared to normal mice [28]. Furthermore, intestinal microbes regulate host intestinal protein-coding genes and miRNA expression during bacterial infection: infection with *Listeria monocytogenes* induces different miRNA expression changes in the intestine of conventional versus germ-free mice [29].

Probiotics are widely used to promote human and animal health, with one potential mechanism being regulation of intestinal miRNA and immune-related gene expression. Feeding piglets *Enterococcus faecium* NCIMA 10415 increases miR-423-5p expression in ileal and jejunal lymphocytes to participate in immune regulation [30]. Chronic alcohol stimulation induces miR-122a expression, leading to intestinal barrier dysfunction, while *Lactobacillus rhamnosus* GG culture supernatant protects intestinal integrity by inhibiting alcohol-induced miR-122a upregulation [31]. Therefore, investigating miRNA regulatory roles in probiotic-intestine interactions is important for probiotic development and utilization.

5. miRNAs and Intestinal Diseases

miRNAs participate in inflammatory bowel disease (IBD) progression. For example, miR-125 regulates Crohn' s disease progression [32], while miR-19b reduces intestinal inflammation [33]; miR-29a regulates ulcerative colitis pathogenesis [34], and miR-155 modulates inflammatory phenotypes in intestinal myofibroblasts [35]. These miRNAs show promise as biomarkers for predicting IBD progression and therapeutic targets. Additionally, differential miRNA expression between the two types of IBD can serve as diagnostic biomarkers, including miR-19a, miR-21, miR-31, miR-101, miR-146a, and miR-375 [36]. Colorectal cancer represents a common intestinal malignancy with multiple miRNAs participating in its progression. miR-214 expression correlates with colitis-associated

colon cancer progression [37], while miR-17-92 plays important roles in colon cancer development by targeting multiple pro-angiogenic genes [38]. miRNAs also serve as cancer diagnostic biomarkers: miR-26b is upregulated in ulcerative colitis-associated colon cancer tissues and serum, correlating with disease severity, but downregulated in sporadic colon cancer, making it a potential biomarker to distinguish between these cancer types [39]. In summary, miRNAs play crucial regulatory roles in intestinal disease progression and exhibit differential expression patterns among diseases with similar symptoms, offering new targets and strategies for intestinal disease detection and treatment.

6. Effects of Dietary miRNAs on the Intestine

Diet contains abundant exogenous miRNAs. Studies suggest exogenous miRNAs can be acquired through ingestion, enter recipient cells, regulate target gene expression, and affect cellular function [40]. Milk exosomal miRNAs can enter neonatal organisms through intestinal endocytosis and play important roles in neonatal immune system development [41]. However, the functionality of dietary miRNAs remains controversial. Snow et al. [42] detected minimal plant-derived miRNAs in human serum after apple and banana consumption. Feeding pups milk from miR-30b-overexpressing transgenic mice showed no significant difference in miR-30b levels in blood, small intestine, liver, lung, and kidney compared to pups fed wild-type mouse milk [43]. If exogenous miRNAs can be absorbed through the intestine to regulate target organ gene expression, oral miRNA administration could become a therapeutic approach. Moreover, as the organ directly exposed to exogenous miRNAs, the intestine may be more accessible for miRNA-based therapies than other organs, making this research direction worthy of continued attention.

7. Research Progress on Livestock Intestinal Health-Related miRNAs

In livestock production, intestinal health is a key factor affecting performance. Research on livestock intestinal health-related miRNAs has gained increasing attention. Weaning stress represents a major challenge in piglet production, with significant differential expression of multiple miRNAs in piglet intestine between weaned and suckling states, primarily participating in intestinal metabolism, stress responses, and immune reactions [44]. *Escherichia coli* F18 infection commonly causes diarrhea and edema disease in weaned piglets, and 12 differentially expressed miRNAs in the intestine of sensitive versus resistant piglets may serve as candidate markers for susceptibility [45]. miRNAs also participate in probiotic [30] and mycotoxin [46] regulation of porcine intestinal health. In poultry research, miRNAs are involved in host responses to pathogen infection: chickens inoculated with *Campylobacter jejuni* show significant changes in four cecal miRNAs [47]. Additionally, miRNAs regulate intestinal disease and pathogen susceptibility: two White Leghorn lines with different necrotic enteritis sensitivities show differential expression of 10 intestinal miRNAs [48], and multiple

miRNAs are differentially expressed in the intestine of Marek' s disease-resistant versus susceptible chicken lines, participating in inflammation-related gene regulation [49]. In summary, miRNAs hold important research value for livestock intestinal health regulation, disease diagnosis, and disease-resistant breeding, though current research remains primarily at the screening stage, requiring further investigation into specific mechanisms.

Research on intestinal health-related miRNAs in human and mouse models has established their crucial regulatory roles in intestinal health. However, functional studies of miRNAs in livestock intestine remain limited. As research on miRNA functions in livestock intestinal development, nutrient metabolism, stress responses, and intestinal diseases advances, miRNA-target gene regulatory pathways may become novel targets for livestock health management, providing new strategies for nutritional regulation of intestinal health and disease-resistant breeding research, with significant research implications and application prospects.

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